NUTRITIONAL BIOCHEMISTRY

M.Sc., FOODS AND NUTRITIONAL SCIENCE,
First Year, Paper - II

Study Material Prepared by:

Dr. J.P. Sharma
M.Sc., Ph.D., FISST

Director

Dr. NAGARAJU BATTU
MBA.,MHRM.,LLM.,MSc.(Psy)MA(Soc).,M.Ed.,M.Phil.,Ph.D

Centre for Distance Eduction
Acharya Nagarjuna University
Nagarjuna Nagar-522510

Ph: 0863-2293299, 2293356, 08645-211023, Cell:98482 85518
0863 - 2346259 (Study Material)
Website : www.anucde.ac.in or www.anucde.info
e-mail : info@ anucde.ac.in

M.Sc (FN) I yr : Nutritional Biochemistry

Edition: 2021

No. of Copies: 59

Published by :

Dr. NAGARAJU BATTU
Director
Centre for Distance Education
Acharya Nagarjuna University

Printed at :
Romith Technologies
Guntur.

FOREWORD

Acharya Nagarjuna University, since its establishment in 1976, has been moving ahead in the path of academic excellence, offering a variety of courses and research contributions. The University achieved recognition as one of the eminent universities in the country by gaining A grade from the NAAC 2016. At present Acharya Nagarjuna University is offering educational opportunities at the UG, PG levels to students of 447 affiliated colleges spread over the two districts of Guntur and Prakasam.

The University had started the Centre for Distance Education in 2003-04 with the aim to bring Higher education within the reach of all. The Centre has been extending services to those who cannot join in colleges, cannot afford the exorbitant fees as regular students, and to housewives desirous of pursuing higher studies to study B.A., B.Com, and B.Sc., Courses at the Degree level and M.A., M.Com., M.Sc, M.B.A. and LL.M. courses at the PG level.

For better understanding by students, self-instruction materials have been prepared by eminent and experienced teachers. The lessons have been prepared with care and expertise. However constructive ideas and scholarly suggestions are welcome from students and teachers. Such ideas will be incorporated for the greater efficacy of the distance mode of education. For clarification of doubts and feedback, Weekly classes and contact classes are arranged at UG and PG levels respectively.

I wish the students who pursue higher education through Centre for Distance Education will not only be personally benefited by improving their qualifications but also strive for nation's growth by being a member in Knowledge society I hope that in the years to come, the Centre for Distance Education will grow in strength by introducing new courses, catering to the needs of people. I congratulate all the Directors, Academic coordinators, Editors, Lesson - Writers, and Academic Counsellors and Non-teaching staff of the Centre who have been extending their services in these endeavours.

Professor Rajasekhar P.
Vice-Chancellor (FAC)
Acharya Nagarjuna University

M.Sc., FOODS AND NUTRITIONAL SCIENCE, (Course Code-139)

Paper - II: NUTRITIONAL BIOCHEMISTRY

SYLLABUS

UNIT I

- Carbohydrate Metabolism: Oxidation of glucose by glycolysis, TCA cycle, HMP pathway, Glycogenolysis and Gluconeogenesis.
- Protein Metabolism: Urea cycle, Biosynthesis of nucleic acids, repair of DNA, protein biosynthesis.
- Fatty Acid Metabolism: Oxidation and biosynthesis of fatty acids, ketonebodies and ketosis, Biosynthesis of lipids and their metabolism, metabolism of bile pigments.
- Metabolic Interrelationship: Carbohydrate, lipid and protein, Inter conversion and metabolic regulations.

UNIT II

- Micronutrient Metabolism: Mineral metabolism, Utilization, factors affecting utilization, Role of minerals in metabolism.

UNIT III

- Vitamin Metabolism: Utilization, Role of vitamins in metabolism.

UNIT IV

- Enzymes: IUB classification, MM equation, Enzyme inhibition, Factors influencing enzymes activity.

UNIT V

- Immunology: Immune system, cell mediated immunity, humeral immunity, Immunoglobulin, Antigen-Antibody interaction and Nutrition, Infection and Immunity interactions.

UNIT VI

- High energy compounds and their role in biochemical and biological energetic, biological oxidation, reduction and Electron transport chain.

CONTENTS

CHAPTER 1 CARBOHYDRATE METABOLISM

NOTES

OBJECTIVES

After going through this chapter, you should be able to:

- understand Glycolysis
- describe pentose phosphate pathway
- define glycogensis
- explain gluconeogenesis.

STRUCTURE

1.1 INTRODUCTION

All living organisms require a continuous supply of energy for carrying out various types of functions. The cell's energy need is met by the breakdown of complex organic molecules, mainly the carbohydrates through cellular respiration. Even the green plants that trap solar energy through photosynthesis and store it as organic compounds depend on respiration for their continuous energy supply.

NOTES

In living organisms storage and breakdown of carbohydrates involve several biochemical reactions. The sum total of these reactions constitutes metabolism of carbohydrates. The whole process of carbohydrate metabolism can be divided into six categories—glycolysis, oxidation of pyruvate, monophosphate pathway, glycogenesis, glycogenolysis and gluconeogenesis.

1.2 GLYCOLYSIS (EMP PATHWAY)

Glycolysis is also called **EMP pathway**, because it was discovered by three German scientists Gustav **Embden, Otto Meyerhof** and **J. Parnas**. Glycolysis is common to both aerobic and anaerobic modes of respiration and is, therefore, called **common pathway**. It is the first step in the breakdown of glucose and is common to all organisms. Glycolysis occurs in the cytosol and results in the breakdown of glucose or similar hexose sugars into two molecules of a three carbon compound—pyruvic acid (pyruvate), releasing some energy (as ATP) and reducing power (as $NADH_2$). In plants, this glucose is derived from sucrose (the end product of photosynthetic carbon reactions) or from storage carbohydrates. Sucrose is converted into glucose and fructose by the enzyme *invertase,* and these two monosaccharides can readily enter the glycolytic pathway. The steps of glycolysis are described below. (Fig. 1.1 to 1.3)

1. Phosphorylation. Glucose is phosphorylated to glucose 6-phosphate by ATP in the presence of *glucokinase* and Mg^{2+} (*kinase* is the enzyme that involves in the formation or utilisation of ATP by removal or addition of a phosphate group).

$$\text{Glucose} + \text{ATP} \xrightarrow[Mg^{2+}]{\textit{Glucokinase}} \text{Glucose 6-phosphate} + \text{ATP}$$

Other types of hexose sugars can also enter into glycolytic pathway after phosphorylation. The disaccharides and polysaccharides enter into glycolysis after their hydrolytic breakdown to hexose sugar by appropriate enzymes.

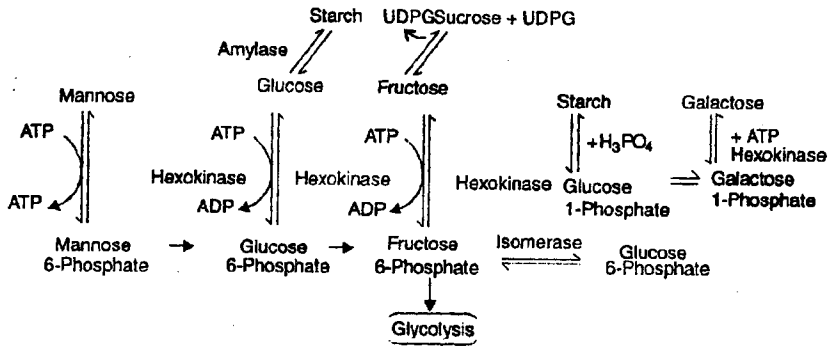


Fig. 1.1. *Schematic conversion of complex carbohydrates before entering into glycolysis.*

NOTES

2. Isomerisation. Glucose 6-phosphate is changed into its isomer fructose 6-phosphate with the help of enzyme *phosphohexose isomerase* (*Isomerase* enzymes usually cause inter-conversion of aldose and ketose sugars.

$$\text{Glucose 6-phosphate} \xrightarrow[\text{Isomerase, } Mg^{++}]{\text{Phosphohexose}} \text{Fructose 6-phosphate}$$

3. Phosphorylation. Fructose 6-phosphate is further phosphorylated by means of ATP in the presence of enzyme *phosphofructo kinase* and Mg^{+2}. The product is fructose 1, 6-diphosphate.

$$\text{Fructose 6-phosphate} + \text{ATP} \xrightarrow[Mg^{++}]{\text{Phosphofructo kinase}} \text{Fructose 1,6-diphosphate} + \text{ADP}$$

4. Splitting or Formation of Glyceraldehyde 3-Phosphate. Fructose 1, 6 diphosphate splits up enzymatically to form one molecule each of 3-carbon compounds, glyceraldehyde 3-phosphate (GAP) or 3-Phosphoglyceraldehyde (PGAL) and dihydroxy acetone 3-phosphate (DHA–P). The enzyme required is *aldolase* (an enzyme that produces or breaks down aldehyde-alcohol compounds). Dihydroxy acetone 3-phosphate is further changed to glyceraldehyde 3-phosphate by enzyme *triose phosphate isomerase* (*phosphotriose isomerase*).

$$\text{Fructose 1,6 diphosphate} \overset{\text{Aldolase}}{\rightleftharpoons} \text{Glyceraldehyde 3-phosphate (GAP)}$$

Phosphoglyceraldehyde (PGAL)

+ Dihydroxyacetone 3-phosphate

$$\text{Dihydroxy acetone 3-phosphate} \overset{\text{Triose phosphate}}{\underset{\text{Isomerase}}{\rightleftharpoons}} \text{Glyceraldehyde 3-phosphate}$$

5. Dehydrogenation and Phosphorylation or Oxidation of Glyceraldehyde 3-Phosphate. In the presence of enzyme *glyceraldehyde phosphate dehydrogenase* (an enzyme that usually removes hydrogen from a compound) glyceraldehyde 3-phosphate loses hydrogen to NAD to form $NADH_2$ and accepts inorganic phosphate to form 1,3 diphosphoglyceric acid.

$$\text{Glyceraldehyde 3-phosphate} + H_3PO_4 + \text{NAD} \overset{\text{Glyceraldehyde}}{\underset{\text{Phosphate dehydrogenase}}{\rightleftharpoons}} 1,3 \text{ diphosphoglyceric acid} + NADH_2$$

6. Dephosphorylation (ATP Formation). One of two phosphates of diphosphoglyceric acid is linked by high energy bond. It can synthesise ATP and form 3-phosphoglyceric acid. The enzyme is *phosphoglyceric acid kinase*. The direct synthesis of ATP from metabolites is called **substrate level phosphorylation.**

1,3 diphosphoglyceric acid + ADP $\underset{\text{Acid Kinase} + Mg^{++}}{\overset{\text{Phosphoglyceric}}{\rightleftharpoons}}$ 3-phosphoglyceric acid + ATP

NOTES

7. Isomerisation. 3-phosphoglyceric acid is changed to its isomer 2-phosphoglyceric acid by enzyme *phosphoglyceratemutase (mutase* is an enzyme which changes the position of a group within the molecule).

3-Phosphoglyceric acid $\xrightarrow{\text{Phosphoglycerate mutase}}$ 2-phosphoglyceric acid

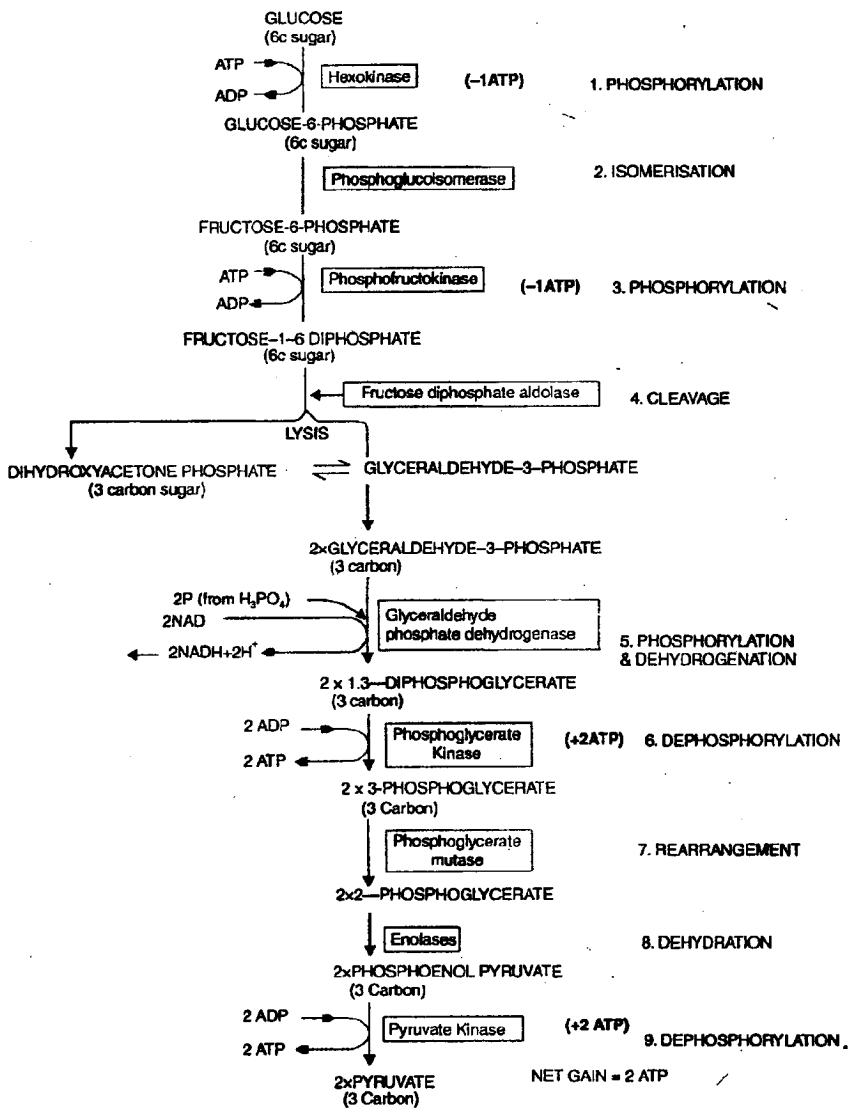


Fig. 1.2. *Different steps of glycolysis.*

NOTES

8. Dehydrogenation (Formation of PEP). Through the agency of enzyme *enolase* (It is an enzyme that causes conversion to or from enol state which has a double bond near an alcoholic group) 2-phosphoglyceric acid is converted to phosphoenol pyruvate (PEP). A molecule of water is removed in the process. Mg^{2+} is required.

$$\text{2-phosphoglyceric acid} \xrightarrow[Mg^{++}]{Enolase} \underset{(PEP)}{\text{Phosphoenol Pyruvate}} + H_2O$$

9. Dephosphorylation (ATP Formation). During this change, the phosphate radical picks up energy. It helps in the formation of ATP by **substrate level phosphorylation** during conversation of phosphoenol to pyruvate acid. The enzyme is *pyruvate kinase.*

$$\underset{(PEP)}{\text{Phosphoenol pyruvate}} + ADP + \xrightarrow[Mg^{++}, K^{+}]{Pyruvate\ kinase} \text{Pyruvic acid} + ATP$$

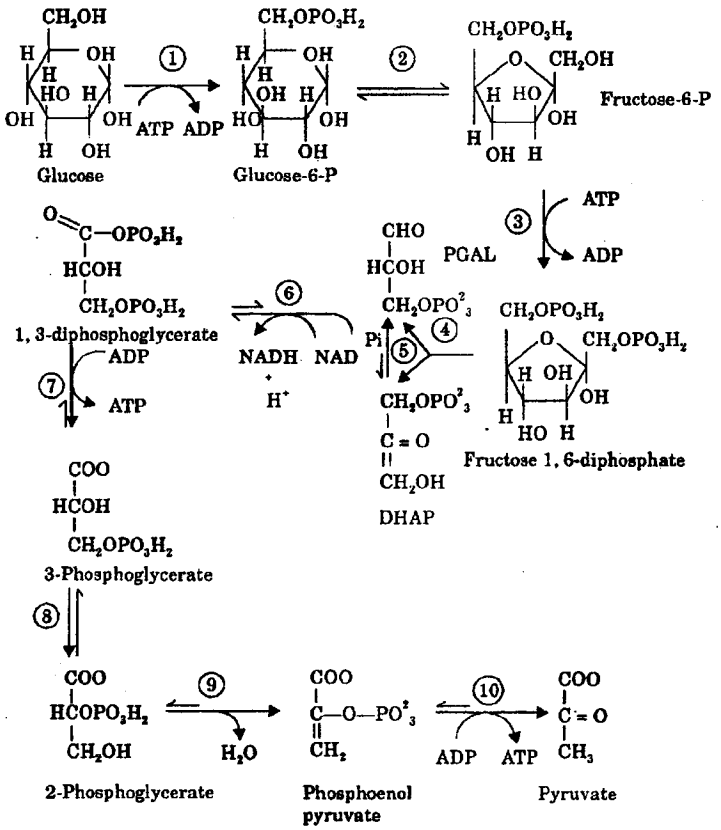


Fig. 1.3. *Reactions of glycolysis.*

NOTES

The various steps involved in glycolysis are summarized in Table 1.1.

Table 1.1. Various Steps Involved in Glycolysis

Steps	*Substrates*	*Enzyme*	*End Products*
1. Phosphorylation (+ Phosphate)	Glucose + ATP (Phosphate acceptor) (Phosphate donor)	**Hexokinase (Cofactor Mg^{2+})**	Glucose-6-Phosphate + ADP
2. Isomerisation	Glucose-6-phosphate	**Phosphogluco-isomerase**	Fructose-6-Phosphate
3. Phosphorylation (+ Phosphate)	Fructose-6-phosphate (Phosphate acceptor) + ATP (phosphate donor)	**Phosphofructo-kinase**	Fructose-1, 6, -Diphosphate + ADP
4. Cleavage	Fructose-1-6,-Diphosphate	**Fructose diphosphate aldolase**	3-Phosphoglyceral-dehyde (3-PGAL) + Dihydroxyacetone phosphate

Both 3-PGAL and dihydroxy-acetone phosphate are interconvertiable in the presence of Triosephosphate isomerase.

3-Phosphoglyceraldehyde $\rightleftharpoons$ Dihydroxy-acetone phosphate

So we consider that in this way 2 molecules of 3-PGAL are produced. Each molecule of 3-PGAL undergoes :

5. Phosphorylation (+ phosphate)	3-PGAL + H_3PO_4 (phosphate acceptor) (phosphate donor)	**Phosphoglycero-kinase**	1, 3 Diphospho-glyceraldehyde + (1, 3-DPGAL)
Dehydrogenation (– 2H)	1. 3-DPGAL + NAD (H-acceptor)	**Glyceraldehyde phosphate dehydrogenase**	1, 3-Diphosphogly-cerate NADH + H^+
6. Dephosphory-lation (–ve Phosphate)	1, 3-DPGA + ADP (Phosphate donor) (Phosphate acceptor)	**Phosphogly-cerate Kinase**	3-PGA + ATP (3-Phosphogly-cerate)
7. Rearrangement – H_2O	3-PGA	**Phosphogly-ceromutase**	2-PGA (2-Phosphoglycerate)
8. Dehydration	2-PGA	**Enolase**	Phosphoenol-pyruvate + H_2O
9. Dephosphory-lation (+ phosphate)	Phosphoenol pyruvate + ADP	**Pyruvate kinase**	Pyruvate + ATP (3C)

NAD = Nicotinamide Adenine Dinucleotide

Net Product of Glycolysis. In glycolysis two molecules of ATP are consumed during double phosphorylation of glucose to form fructose 1, 6-diphosphate. In return four molecules of ATP are produced by substrate level phosphorylation (conversion of 1, 3-diphosphoglycerate to 3-phosphoglycerate and phosphoenol pyruvate to pyruvate) and two molecules of $NADH_2$ are formed at the time of oxidation of glyceraldehyde 3-phosphate to 1, 3-diphosphoglycerate. The whole process may be expressed as under :

$$Glucose + 2NAD^+ + 2ADP + 2H_3PO_4 \longrightarrow 2Pyruvate + 2NADH + 2H^+ + 2ATP.$$

Two molecules of NADH + H^+ on oxidation produce 6 molecules of ATP. Therefore, a net gain of 8 ATP molecules occurs during glycolysis.

1.3 OXIDATION OF PYRUVATE

In anaerobic conditions, pyruvic acid is converted either to ethyl alcohol (alcoholic fermentation) or lactic acid. Under aerobic conditions, pyruvic acid is metabolised to acetyl coenzyme A (Acetyl CoA).

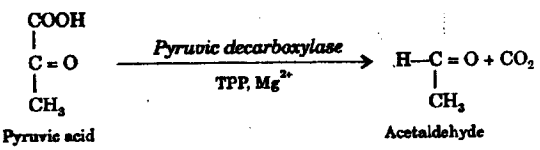
1. Anaerobic Oxidation of Pyruvate

The pyruvate formed at the end of glycolysis is anaerobically broken down to yield various products, depending upon the organism and the type of tissue. The common products are ethyl alcohol and lactic acid. In microorganisms, anaerobic breakdown of carbohydrates is called **fermentation.** Fermentation is named after its product like **alcoholic fermentation, lactic acid fermentation.**

(*i*) *Alcoholic Fermentation*

It occurs in fungi (*e.g., Rhizopus*, yeast) and bacteria. Yeast can respire both aerobically and anaerobically. Anaerobic respiration occurs in sugary solution causing fermentation. If the fungus is not in contact with atmosphere, pyruvate first undergoes decaboxylation (removal of carboxyl group in the form of carbon dioxide) with the help of enzyme *pyruvate decarboxylase, transacetylase*, Mg^{2+} and TPP (thiamine pyrophosphate). This produces acetaldehyde and carbon dioxide from pyruvic acid.

$$\text{Pyruvate} \xrightarrow[Mg^{2+}, TPP]{\textit{Pyruvate decarboxylase}} \text{Acetaldehyde} + CO_2$$



Pyruvic acid — Acetaldehyde

NOTES

Acetaldehyde then accepts hydrogen from $NADH_2$ and is reduced to ethyl alcohol (ethanol) producing oxidised NAD^+ (Fig. 1.4). The process is catalysed by the enzyme *alcohol dehydrogenase.*

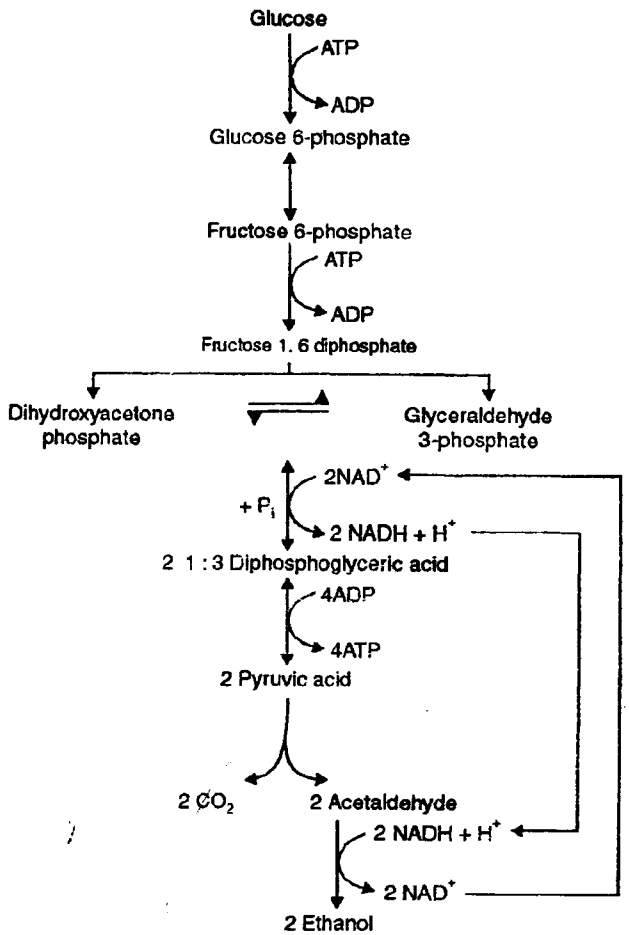


Fig. 1.4. *Pathway of anaerobic respiration (fermentation) in yeast.*

$$\text{Acetaldehyde} + \text{NADH} + \text{H}^+ \xrightarrow{\textit{Alcohol dehydrogenase}} \text{Alcohol} + \text{NAD}^+$$

$$\begin{matrix}\text{CH} = \text{O} \\ | \\ \text{CH}_3\end{matrix} + \text{NADH} + \text{H}^+ \xrightarrow{\textit{Alcohol dehydrogenase}} \begin{matrix}\text{CH}_2\text{OH} \\ | \\ \text{CH}_3\end{matrix} + \text{NAD}^+$$

Accumulation of ethanol by fermentation in a culture of yeast may stop further multiplication and lead to the death of cells. In the presence of oxygen, however, yeast can respire aerobically.

(*ii*) *Lactic Acid Fermentation*

It occurs in lactic acid bacteria (*e.g., Lactobacillus*) and in muscles. Pyruvic acid produced in glycolysis is reduced by $NADH_2$ to form lactic acid. Carbon dioxide is not produced and $NADH_2$ is oxidised to NAD^+.

The reaction is catalysed by the enzyme *lactic dehydrogenase* which requires FMN (Flavin mononucleotide) and Zn^{2+}. NAD^+ is reutilised in glycolysis (Fig. 1.5).

$$\text{Pyruvate} + \text{NADH} + H^+ \xrightarrow[\text{FMN, } Zn^{2+}]{\textit{Lactic dehydrogenase}} \text{Lactic acid} + NAD^+$$

Skeletal muscles usually derive their energy by anaerobic respiration after vigorous exercise, lactic acid accumulates, leading to muscular fatigue. During rest, however, the lactic acid is reconverted to pyruvic acid and is channeled back into the aerobic respiration pathway.

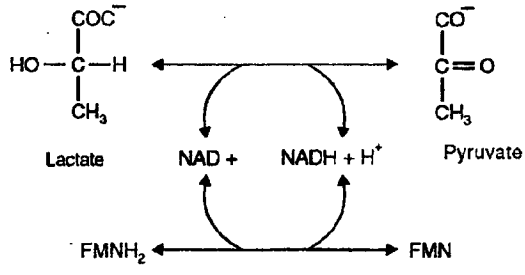


Fig. 1.5. *Pathway of anerobic respiration in muscle cells.*

2. Aerobic Oxidation of Pyruvate

Aerobic oxidation of pyruvate occurs inside mitochondria. In aerobic oxidation, molecular oxygen acts as the ultimate acceptor of electron and protons removed from the substrate. The process is carried out by the enzymes of the matrix and the inner membrane of the mitochondria. Carbon dioxide produced in the process diffuses out of the cell. The aerobic oxidation of pyruvate consists of oxidation of pyruvate to acetyl CoA, Kreb's cycle and terminal oxidation.

Oxidation of Pyruvate of Acetyl CoA

Pyruvic acid generated in the cytosol, is transported to mitochondria, and thus initiates the second phase of respiration. Before pyruvic acid enters Krebs cycle, operative in the mitochondria, one of the three carbon atoms of pyruvic acid is oxidised to carbon dioxide in a reaction called **oxidative decarboxylation.** *i.e.,* pyruvate is first decarboxylated, and then oxidised by the enzyme pyruvate dehydrogenase. The remaining 2-carbon acetate unit is readily accepted by a sulphur containing compound, coenzyme A (CoA), to form acetyl CoA. It is the connecting link between glycolysis and Krebs cycle. During the process, NAD^+ is reduced to NADH. The reaction is summarised below,

$$\text{Pyruvic acid} + \text{CoA} \xrightarrow[Mg^{2+}]{NAD^+ \quad NADH} \text{Acetyl CoA} + CO_2$$

NOTES

During this process, two molecules of NADH are produced, *i.e.*, oxidative decarboxylation of two molecules of pyruvic acid produced during glycolysis. It results in a net gain of 6 ATP molecules (2 NADH × 3 = 6 ATP).

Beta oxidation of fatty acids also produces acetyl CoA as the end product. Acetyl CoA from both the sources acts as **substrate entrant** for Krebs cycle. It is also the **connecting link** between glycolysis and Krebs cycle.

Kreb's Cycle or TCA Cycle

The cycle was discovered by British biochemist **Sir Hans Krebs** (1937). For the pioneer work Hans Krebs was awarded Nobel Prize in 1953. The cycle is also named as **Citric acid cycle** or **Tricarboxylic Acid (TCA) cycle** after the initial product. Krebs cycle is stepwise oxidative and cyclic degradation of acetyl CoA derived from pyruvate. Krebs cycle occurs in mitochondrial matrix and serves as a common oxidative pathway for carbohydrtes, fats and proteins. (Fig. 1.6).

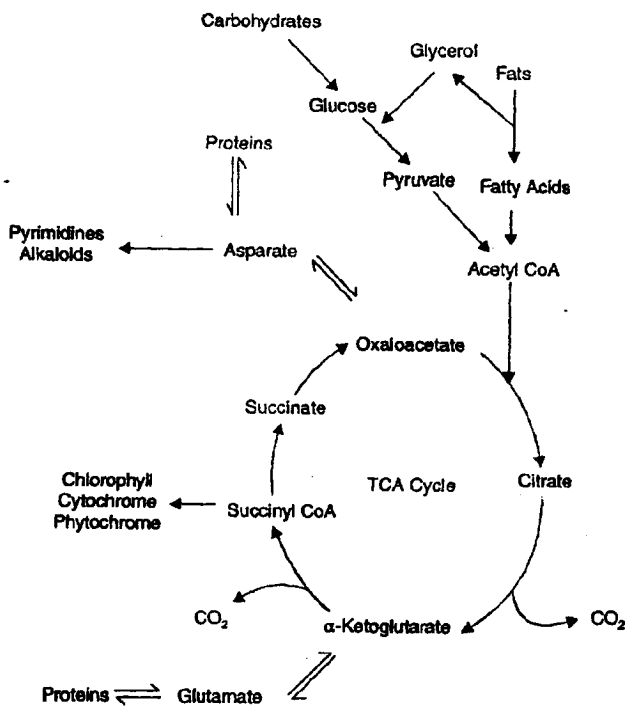


Fig. 1.6. *TCA as common metabolic pathway of carbohydrates, fats and proteins.*

The actual citric acid cycle or Krebs cycle begins when acetyl CoA enters into a reaction to form citric acid. It explains how pyruvate is broken down to CO_2 and water. It also highlighted the concept of cycles in metabolism.

NOTES

The various steps of Krebs cycle (Figs. 1.7 and 1.8) are as follows:

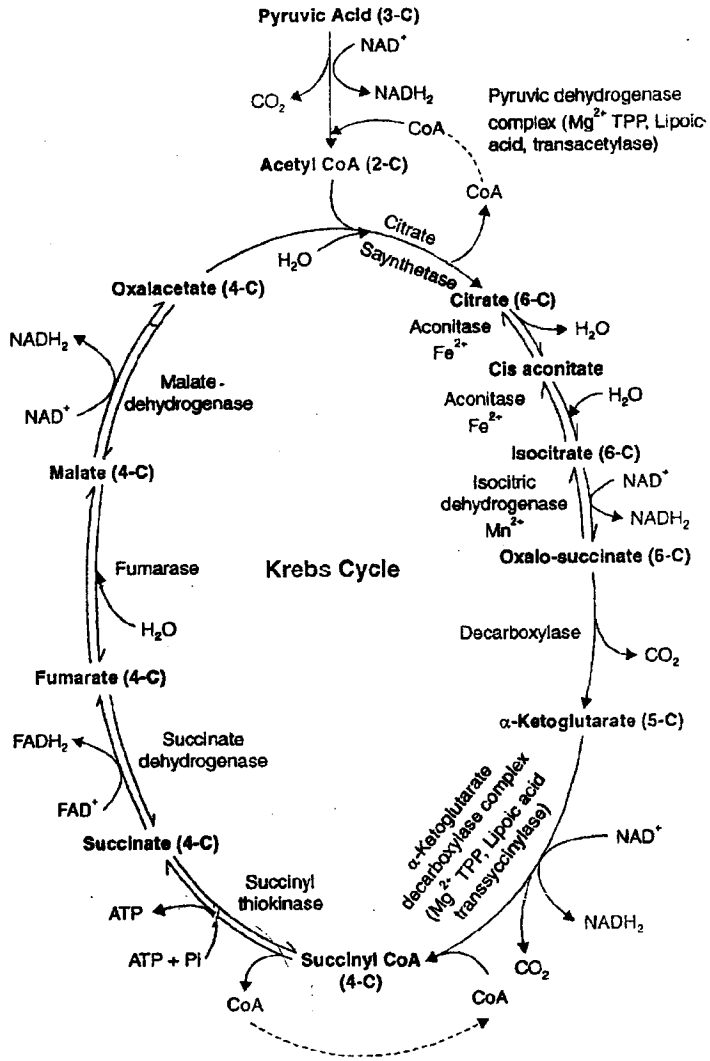


Fig. 1.7. *Schematic representation of Krebs cycle or TCA cycle.*

(*i*) ***Condensation.*** In the first reaction, 2-carbon compound acetyl CoA combines with 4-carbon compound oxaloacetate (OAA) in the presence of condensing enzyme *citrate synthetase* to form a tricarboxylic 6-carbon compound called citric acid and CoA is liberated. Citric acid is the first product of the Krebs cycle.

$$\text{Acetyl CoA + oxaloacetate + } H_2O \xrightarrow{\textit{Citrate Synthetase}} \text{Citrate + CoA}$$

(*ii*) ***Dehydration.*** Citrate undergoes reorganisation in the presence of iron containing enzyme, *aconitase* forming 6-carbon *cis*-aconitate.

$$\text{Citrate} \xrightarrow{\textit{Aconitase}} \textit{cis}\text{-aconitate} + H_2O$$

(*iii*) ***Hydration.*** *cis*-aconitate is further reorganised into 6-carbon isocitrate with the addition of water in the presence of enzyme ***aconitase.***

NOTES

$$\textit{Cis}\text{-aconitate} + H_2O \xrightarrow{\textit{Aconitase}} \text{Isocitrate}$$

(*iv*) ***Dehydrogenation.*** Isocitrate is dehydrogenated to oxalosuccinate in the presence of enzyme *isocitrate dehydrogenase* and Mn^{2+}. A pair of hydrogen atoms is released which is accepted by NAD^+ to form NADH + H^+.

$$\text{Isocitrate} + NAD^+ \xrightarrow[Mn^{2+}]{\textit{Isocitrate Dehydrogenase}} \text{Oxalosuccinate} + NADH + H^+$$

(*v*) ***Decarboxylation.*** Oxalosuccinate is decarboxylated to form a 5-carbon α-ketoglutarate in the presence of enzyme *decarboxylase*, one molecule of carbon dioxide is released in the reaction.

$$\text{Oxalosuccinate} \xrightarrow{\textit{Decarboxylase}} \alpha\text{-ketoglutarate} + CO_2$$

(*vi*) ***Dehydrogenation and Decarboxylation.*** α-ketoglutarate is both dehydrogenated (with the help of NAD^+) and decarboxylated by an enzyme complex *α-ketoglutarate dehydrogenase.* The enzyme complex contains TPP and lipoic acid. The product combines with CoA to form a 4-carbon succinyl CoA.

$$\alpha\text{-ketoglutarate} + CoA + NAD^+ \xrightarrow[TPP,\ Lipoic\ acid,\ Mg^{2+}]{\textit{α-ketoglutarate Dehydrogenase}} \text{Succinyl CoA} + NADH + H^+ + CO_2$$

(*vii*) ***Formation of GTP/ATP.*** Succinyl CoA splits into succinate and CoA in the presence of enzyme *succinyl thiokinase*. The reaction releases sufficient energy to form GTP (in animals) and ATP (in plants).

$$\text{Succinyl CoA} + GDP/ADP + H_3PO_4 \xrightarrow{\textit{Succinylthiokinase}} \text{Succinate} + CoA + GTP/ATP$$

(*viii*) ***Dehydrogenation.*** Succinate undergoes dehydrogenation to form 4-carbon fumarate with the help of enzyme *succinate dehydrogenase* and liberates a pair of hydrogen atom. The latter pass to FAD (Flavin adenine dinucleotide) to form $FADH_2$.

$$\text{Succinate} + FAD \underset{}{\overset{\textit{Succinate dehydrogenase}}{\rightleftharpoons}} \text{Fumarate} + FADH_2$$

(*ix*) ***Hydration.*** Fumarate is changed into 4-carbon malate with the addition of water in the presence of enzyme *fumarase.*

$$\text{Fumarate} + H_2O \overset{\textit{Fumarase}}{\rightleftharpoons} \text{Malate}$$

(*x*) ***Dehydrogenation.*** Malate is dehydrogenated in the presence of enzyme *malate dehydrogenase* to produce 4-carbon oxaloacetate. Hydrogen is accepted by $NAD^+/NADP^+$.

Malate + $NAD^+/NADP^+$ $\xrightleftharpoons{\text{Malate dehydrogenase}}$ oxaloacetate + $NADH/NADPH + H^+$

Oxaloacetate picks up another molecule of activated acetate to repeat the cycle. The summary equation for this phase of respiration may be written as follows:

Pyruvic acid + $4NAD^+$ + FAD + $2H_2O$ + ADP + Pi $\longrightarrow$ $3CO_2$ + 4NADH + $4H^+$ + $FADH_2$ + ATP

NOTES

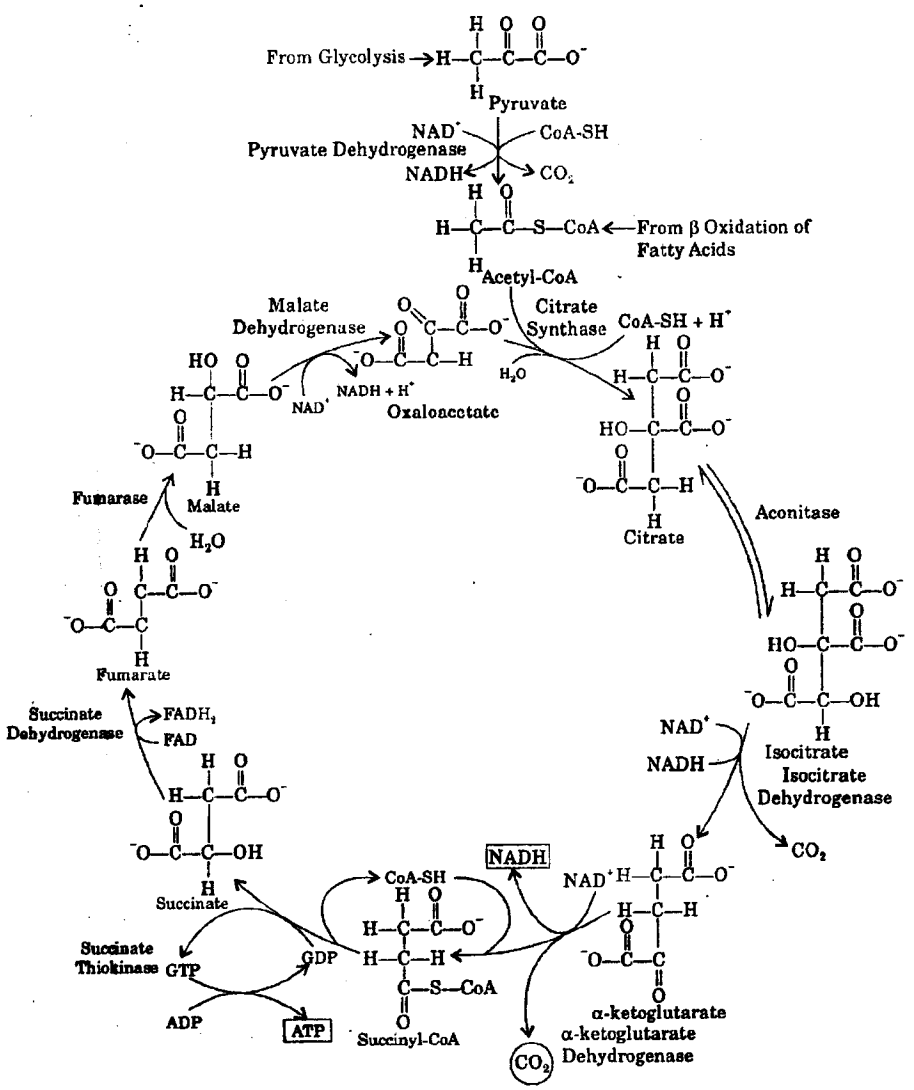


Fig. 1.8. *Kreb's cycle explained with chemical reactions.*

During citric acid cycle, decarboxylation occurs at two steps releasing two molecules of CO_2. In this process 3-molecules of NAD^+ and one molecule

NOTES

of FAD (Flavin adenine dinucleotide) are reduced to produce 3-molecules of NADH and one molecule of $FADH_2$ respectively (Fig. 1.8). These reduced electron carriers pass on the hydrogen atoms to oxygen through electron transport system yielding ATP molecules. 3-molecules of NADH produce 9-molecules of ATP (3NADH × 3 = 9ATP), while the $FADH_2$ gives rise to 2 molecules of ATP (1$FADH_2$ × 2 = 2ATP). In addition, one more ATP molecule is generated during the cycle. Thus, a total of 12 ATP molecules per molecule of pyruvic acid are produced. From each molecule of glucose, a total of 24-molecules of ATP are formed during the citric acid cycle.

Terminal Oxidation

It is the last step of aerobic respiration which involves the passage of both electrons and protons of reduced coenzymes to oxygen.

$$NADH_2/FADH_2 \rightleftharpoons NAD/FAD + 2H^+ + 2e^-$$

$$\tfrac{1}{2}O_2 + 2H^+ + 2e^- \longrightarrow H_2O$$

Terminal oxidation consists of two processes—electron transport and oxidative phosphorylation.

(*a*) ***Electron Transport Chain.*** The inner mitochondrial membrane contains some proteins which act as H^+ ions and electron transporting enzymes. The enzymes are arranged in ordered manner in a specific series called **electron transport chain (ETC) or mitochondrial respiratory chain** or **electron transport system (ETS).** An electron transport chain is a series of enzymes and cytochromes in the inner mitochondrial membrane that take part in the passage of electrons from a substance to its ultimate acceptor. The electron carriers include flavins, iron-sulphur complexes, quinones and cytochromes. Most of them are prosthetic groups of proteins.

The glucose molecule is completely oxidised by the end of the citric acid cycle. But the energy is not released unless NADH and $FADH_2$ are oxidised through the electron transport system. Electrons from NADH produced in the mitochondrial matrix during citric acid cycle are oxidised by an NADH dehydrogenase **(Complex I)** and electrons are than transferred to ubiquinone located within the inner membrane. Ubiquinone also receives reducing equivalents *via* $FADH_2$ that is produced during oxidation of succinate, through the activity of enzyme succinate dehydrogenase **(Complex II),** in the citric acid cycle. The reduced ubiquinone (ubiquinol) is than oxidised with the transfer of electrons to cytochrome C *via* cytochrome bC_1 complex **(Complex III)** in a small protein attached to the outer surface of inner membrane and acts as a mobile carrier for transfer of electrons between complex III and IV. **(Complex IV)** refers to cytochrome *c* oxidise complex containing cytochrome *a* and a_3 and two copper centres (Fig. 1.9).

NOTES

When the electrons pass from one carrier to another *via* complex I to IV in the electron transport chain, they are coupled to ATP synthase **(Complex V)** for the production of ATP from ADP and inorganic phosphate. The number of ATP molecules synthesised depends on nature of the electron donor. Oxidation of one molecule of NADH gives rise to 3 molecules of ATP, while that of one molecule of $FADH_2$, yields 2 molecules of ATP.

At each step of electron transport, the electron acceptor has a higher electron affinity than the electron donor. The energy from such electron transport is utilised in transporting protons (H^+) from the matrix across the inner membrane to its outerside (outer chamber). There are three such sites in the electron transport chain (Fig. 1.10). This creates a higher proton concentration outside the inner membrane (*i.e.*, in the outer chamber or inter mitochondrial space) than in the matrix. The difference in proton concentration on the outer and inner sides of the inner mitochondrial membrane is known as **proton gradient.**

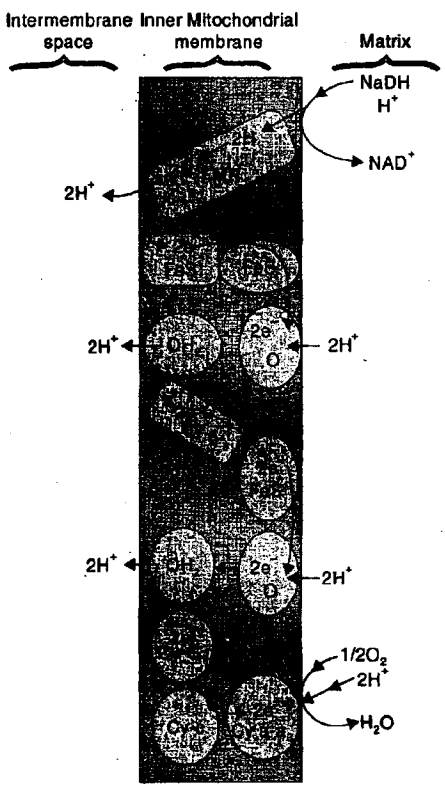


Fig. 1.9. *Mitochondrial electron transport chain.*

NOTES

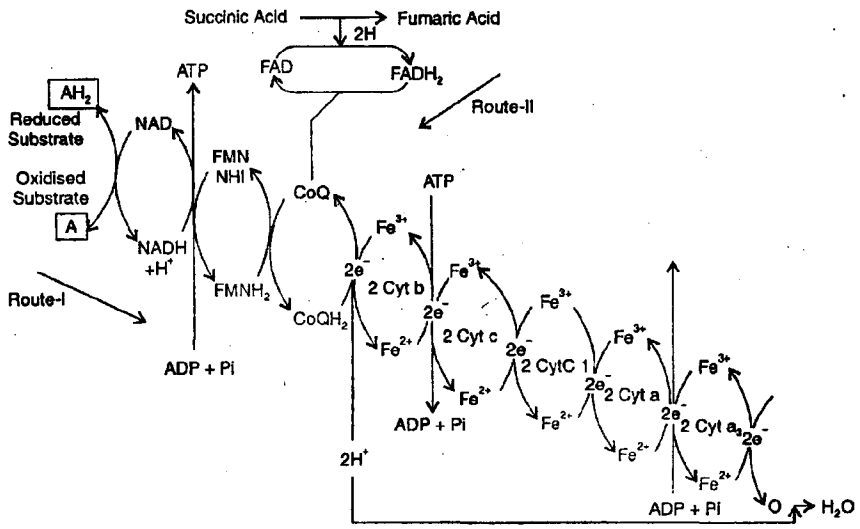


Fig. 1.10. *Schematic representation of mitochondrial respiratory chain or electron transport chain.*

(*b*) ***Oxidative Phosphorylation.*** Oxidative phosphorylation is the synthesis of energy rich ATP molecules with the help of energy liberated by oxidation of reduced coenzymes ($NADH_2$, $FADH_2$) produced during respiration. The enzyme required for their synthesis is called *ATP synthatase*. It is present in F_1 or head piece of F_0–F_1 or elementary particle. The particles are located in the inner mitochondrial membrane. The enzyme *ATP synthatase* becomes active in ATP formation only when there is proton gradient, having higher concentration of protons on the F_0 side (outer side) as compared to F_1 side (inner side).

Because of the higher proton concentration outside the inner membrane, protons return to the matrix down the proton gradient. Just as a flow of water from a higher to a lower level can be utilised to turn a water wheel or a hydroelectric turbine, the energy released by the flow of protons down the gradient is utilised in synthesising ATP. The enzyme *ATP synthatase* synthesises ATP from ADP and inorganic phosphate using the energy from the proton gradient (Chemiosmatic hypothesis of **Peter Mitchell**, 1961; Fig. 1.11).

Transport of two electrons from NADH + H^+ by the electron transport chain simultaneously transfers three pairs of protons to the outer compartment. One high energy ATP bond is produced per pair of protons returning to the matrix through the inner membrane particles. Therefore, oxidation of one molecule of $NADH_2$ produces 3 ATP molecules, while that of $FADH_2$ forms only 2 ATP molecules, as the latter donates its electron further down the chain.

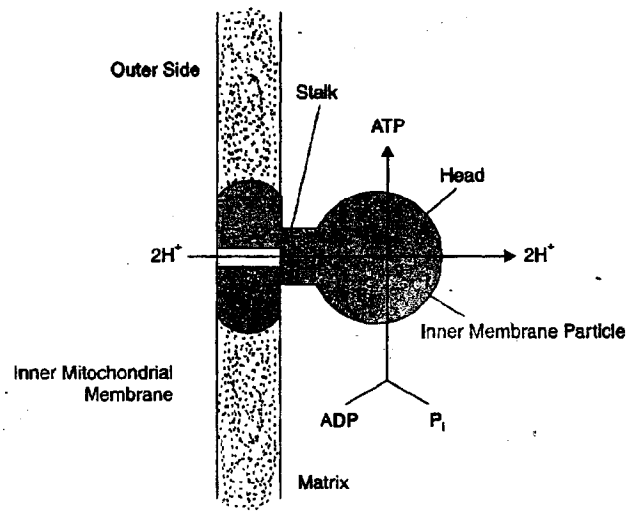


Fig. 1.11. *ATP synthesis by F_0—F_1 particles or inner membrane particles of mitochondria.*

Net gain of ATP. The details of ATP produced per molecule of glucose during aerobic respiration are given in Table 1.2.

Table 1.2. ATP Molecules Produced During Respiration

Stage of Respiration	*Source*	*Number of ATP Molecules Produced*
Glycolysis	Direct	2
	2 molecules of NADH (One molecule of NADH yields 3 molecules of ATP)	6
Pyruvic acid to Acelyl-CoA	2 molecules of NADH	6
Citric acid cycle (Krebs cycle)	—6 NADH	18
	—2 $FADH_2$ ($FADH_2$ produces only 2 molecules of ATP)	4
	—Direct	2
Total yield of ATP molecules		38

There is a net gain of 38 ATP molecules during aerobic respiration of one molecule of glucose. In eukaryotic cells, $NADH_2$ produced in glycolysis are transferred to mitochondria for ATP synthesis. For this, two shuttle systems operate at the inner mitochondrial membrane.

(*i*) $NADH_2 \longrightarrow NAD \longrightarrow NADH_2$ (More efficient shuttle system), and

(*ii*) $NADH_2 \longrightarrow FAD \longrightarrow FADH_2$ (Less efficient shuttle system)

NOTES

The former operates in liver, heart and kidney cells. No energy is spent in this type of transfer. The second method occurs in muscle and nerve cells. It spent 2 ATP molecules in transporting 2 $NADH_2$ to the mitochondria, which lowers the energy level of 2 $NADH_2$ by 2 ATP molecules.

Thus, a total of 38 ATP molecules are produced per molecule of glucose oxidised in aerobic prokaryotes and, heart, liver and kidney cells of eukaryotes. Whereas, the net gain in most of the eukaryotic cells (*i.e.,* muscle and nerve cells) is 36 ATP molecules (as 2ATP molecules are consumed in transporting $NADH_2$ molecules into mitochondria). ATP molecules are transported out of mitochondria to cytoplasm through faciliated diffusion.

1.4 PENTOSE PHOSPHATE PATHWAY (Hexose Monophosphate Shunt)

Although the major pathway for the aerobic respiration of glucose is through glycolysis and the Krebs cycle, there exists an alternate pathway called **Pentose phosphate pathway** (PPP) in both plant and animal cells. This was described by **Warburg** *et. al.* (1935) and **Dickens** (1938). This pathway occurs in presence of oxygen, and involves direct oxidation of glucose 6-phosphate. Therefore, it is also called **direct oxidation pathway** or **hexose monophosphate shunt** (Fig. 1.12). In PPP for every six molecules of glucose, one is completely oxidised to carbon dioxide and water, while the other five are regenerated. Oxidation of glucose is linked to the formation of $NADPH_2$. Glucose is phosphorylated to glucose 6-phosphate by ATP. Glucose 6-phosphate forms the starting point for pentose phosphate pathway. It undergoes two dehydrogenations through a number of intermediates to yield ribulose 5-phosphate, two molecules of NADPH + H^+ and a molecule of carbon dioxide. Complete oxidation of a molecule of glucose produces 12 molecules of NADPH + H^+, which is equivalent to 36 molecules of ATP. Thus, the energy released in the oxidation of glucose via this pathway is almost equal to that of common pathway that liberates 38 ATP molecules per molecule of glucose. Pentose phosphate pathway occurs in cytoplasm but takes the help of mitochondria for producing ATP. In plants cells both common pathway and pentose phosphate pathway of respiration are operative. It appears that common pathway is predominant in the young developing cells, while the pentose phosphate pathway is predominant in mature cells.

NOTES

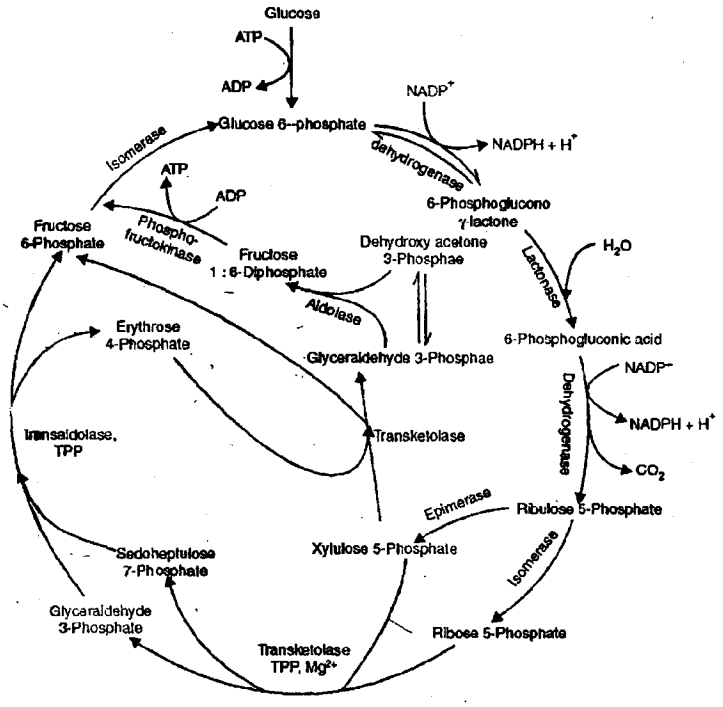


Fig. 1.12. *Pentose phosphate pathway (hexose monophosphate shunt).*

Various steps of pentose phosphate pathway (Fig. 1.13) are described below:

(*i*) The first reaction of the hexose monophosphate shunt (phosphogluconate pathway) is the **enzymatic dehydrogenation** of glucose 6-phosphate to form 6-phosphogluconate. This step is catalyzed by *glucose 6-phosphate dehydrogenase* enzyme. This enzyme is specific for NADP.

(*ii*) Dehydrogenation of carbon-1 of glucose 6-phosphate results in the formation of **6-phosphoglucono-δ-lactone** which is unstable and thus undergoes spontaneous hydrolysis by *gluconolacione hydrolase (lactonase)* to form **6-phosphogluconate.**

(*iii*) The 6-phosphogluconate now undergose **oxidative decarboxylation** by *6-phosphogluconate dehydrogenase* in the presence of Mg^{2+} or Mn^{2+} or Ca^{2+} to form **D-ribulose 5-phosphate** (keto pentose) and carbon dioxide.

(*iv*) The keto pentose is reversibly transformed into **D-xylulose 5-phosphate** by the action of *phosphopentose epimerase* (*ribulose-5-phosphate epimerase*).

(*v*) D-ribulose 5-phosphate may also be converted to form D-ribose 5-phosphate (aldo isomer) by the action of *ribose 5-phosphate*

NOTES

keto isomerase. The D-ribose 5-phosphate can be utilized in the synthesis of pentose-containing nucleotides and RNA. Thus, two molecules of NADPH have been generated so far and also D-ribose is synthesized.

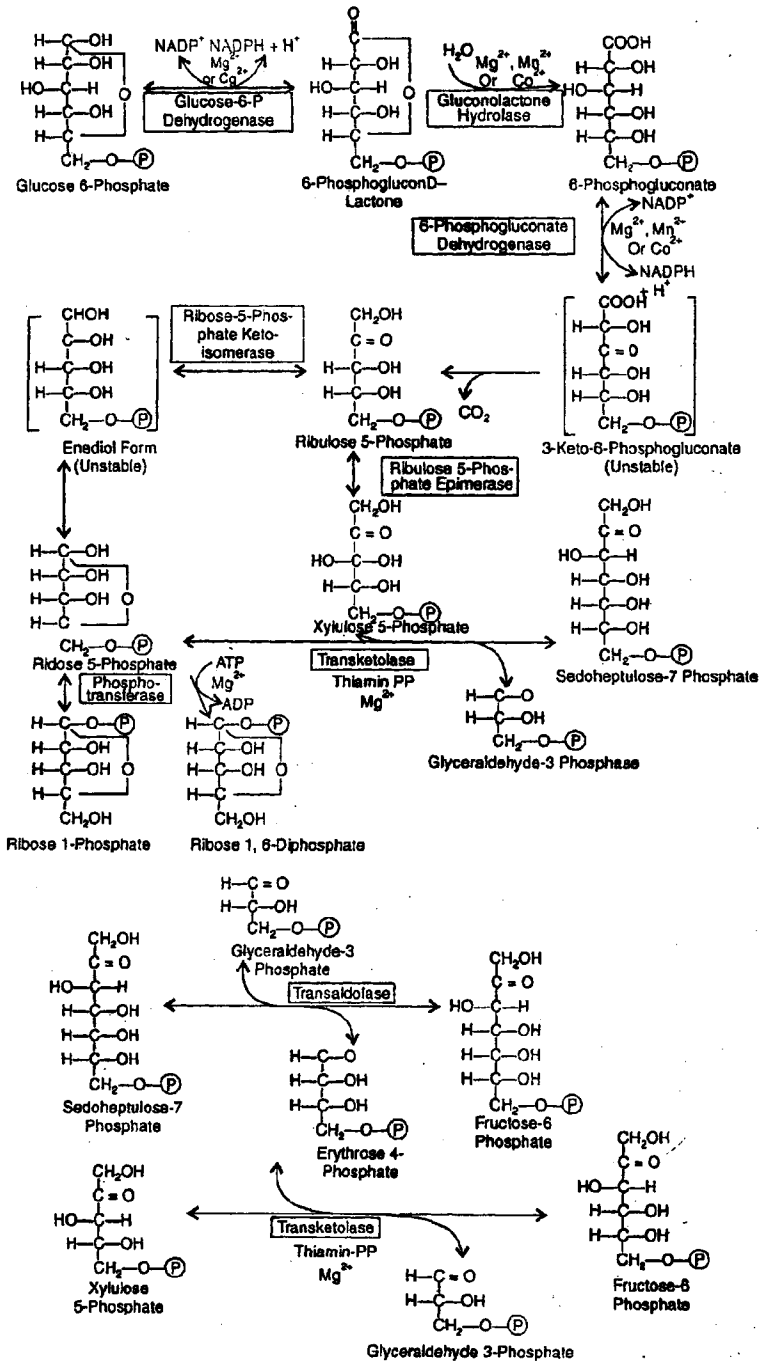


Fig. 1.13. *The hexose monophosphate shunt or pentose phosphate pathway.*

NOTES

The overall reactions can be written as—

Glucose 6-phosphate + $2NADP^+ + H_2O \rightleftharpoons$

D-ribose 5-phosphate + CO_2 + $2NADPH + 2H^+$

In some cases, HMP pathway stops here. However, in other circumstances, it continues further.

(*vi*) Isomeric pentose 5-phosphates so produced can undergo other transformations as well with the help of *transketolase* and *transladolase.*

(*vii*) D-xylulose 5-phosphate and D-ribose 5-phosphate can form seven-carbon keto sugar *i.e.,* **D-seduheptulose 7-phosphate** and a three-carbon sugar *i.e.,* **D-glyceraldehyde 3-phosphate.** This is made possible by enzyme *transketolase* which contains tightly bound thiamine pyrophosphate as coenzyme as well as Mg^{++}. This enzyme, in fact, transfers the glyceraldehyde group from D-xylulose 5-phosphate to D-ribose 5-phosphate. It is important to mention that glyceraldehyde 3-phosphate is also an intermediate of glycolytic pathway thus giving reasons that HMP pathway and glycolytic pathway are linked.

(*viii*) *Transaldolase* is another enzyme which participates in the reactions of HMP pathway. It acts on D-seduheptulose 7-phosphate and D-glyceraldehyde 3-phosphate. In this reaction, dihydroxyocetone group is enzymatically transferred from seduheptulose 7-phosphate to D-glyceraldehyde 3-phosphate. In the process, six-carbon sugar *i.e.,* **D-fructose 6-phosphate** is formed alongwith four carbon sugar *i.e.,* **D-erythrose 4-phosphate.** D-fructose 6-phosphate is another intermediate of glycolytic pathway—another linking point of HMP pathway and glycolytic pathway.

(*ix*) Transketolase can even catalyze the reaction of D-xylulose 5-phosphate and erythrose 4-phosphate to form fructose 6-phosphate and glyceraldehyde 3-phosphate.

Most of the reactions in the HMP pathway are reversible. Thus, the phosphogluconate pathway (HMP pathway) may join the glycolytic pathway or it can alone bring about more complex oxidation of glucose 6-phosphate. The overall reaction can be shown as

6 Glucose 6-phosphate + 12 $NADP^+ \rightarrow$

5 Glucose 6-phosphate + $6CO_2$ + 12 NADPH + $12H^+$ + Pi

After cancelling common factors, we get

Glucose 6-phosphate + 12 $NADP^+ \rightarrow 6CO_2$ + 12 NADPH + $12H^+$ + Pi

(*x*) The two triose phosphate molecules condense to form fructose 1, 6-diphosphate in the presence of *fructose diphosphate aldolase.* This is the reverse step of the one found in EMP pathway. Fructose 1, 6 diphosphate is converted to fructose 6-phosphate by *hexose diphosphatase*—another reverse step of EMP pathway. Finally, fructose 6-phosphate gives rise to glucose 6 phosphate by the enzyme *phosphoglucoisomerase.*

Significance of Pentose Phosphate Pathway

1. Pentose phosphate pathway constitutes an alternate pathway for the breakdown of carbohydrates in respiration.
2. It produces NADPH + H^+ for some synthetic processes.
3. It produces ribose 5-phosphate, which is used in the synthesis of nucleic acid.
4. Erythrose 4-phosphate produced in pentose phosphate pathway is required for the synthesis of lignin, anthocyanin, IAA and a number of other compounds.

1.5 GLYCOGENESIS

Glycogenesis the process of conversion of the excess of glucose into glycogen by liver cells with the help of a pancreatic hormone, insulin. It takes place when the person gets plenty of food. However, in fasting persons, the stored glycogen is changed into glucose and added to the blood stream.

The various steps of glycogenesis are as follows:

(*i*) Glucose is first phosphorylated to form glucose 6-phosphate with the help of enzyme *glucokinase.*

(*ii*) The glucose 6-phosphate so formed is then converted into glucose-1-phosphate. The reaction is catalysed by the enzyme *phosphoglucomutase.*

(*iii*) In the next step glucose 1-phosphate and a nucleoside 5-triphosphate called uridine triphosphate (UTP) form the active nucleotide, *i.e.,* uridine diphosphate glucose (UDPG). The reaction is catalysed by the enzyme *UDPG pyrophosphorylase.*

(*iv*) The next step involves the transfer of glucosyl group of UDPG to the terminal glucose residue at the non-reducing end of an amylose chain to form α-1, 4 glycosidic linkage by the action of enzyme *glycogen synthetose* also called *glucosyl transferase.* This linkage occur between C-1 of the added glucosyl residue and C-1 hydroxyl of the terminal glucose residue of the glycogen chain.

$$(\text{Glucose})_n + \text{UDP-glucose} \longrightarrow (\text{Glucose})_{n+1} + \text{UDP}$$

It is important to note that a pre-existing glycogen molecule (primer) must be present to initiate the reaction. With the continuous addition of glucose residue to the pre-existing primer glycogen molecule, the chain grows.

(*v*) When the chain becomes lengthened to about 6 to 11 glucose residues, a branching enzyme (*amylo 1, 4 → 1, 6 transglycosylase*) catalyses transfer of a terminal oligosaccharide fragment of 6 to 7 glucosyl residues from the end of the main glycogen chain to the C-6 hydroxyl group of a glucose residue of the same or other glycogen chain. This happens in α-1, 6 linkage and thus a branch is formed (Fig. 1.14).

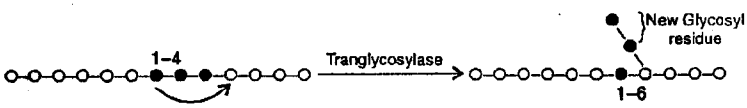


Fig. 1.14. *Action of branching enzyme* (1, 4 → 1, 6 transglycolysase) *on a branch of glycogen.*

The pathway leading to the formation of glycogen from glucose (glycogenesis) and also the one leading to the formation of glucose from glycogen (glycogenolysis) is depicted in Fig. 1.15.

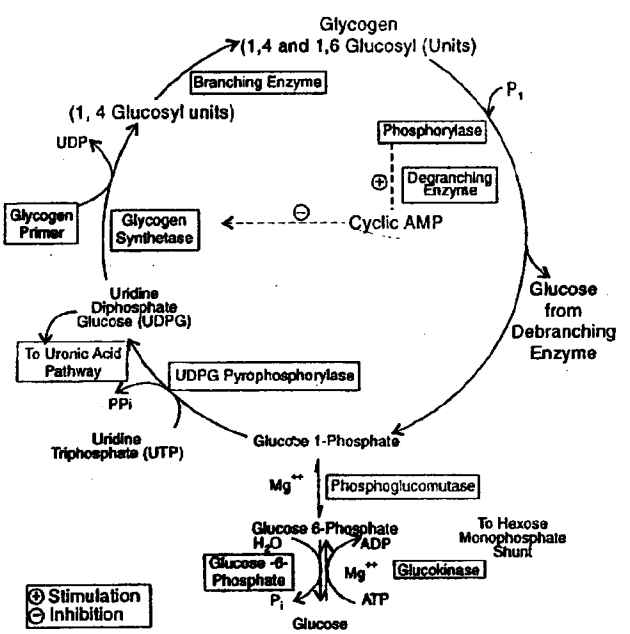


Fig. 1.15. *Showing pathway of glycogenesis and of glycogenolysis in the liver.*

NOTES

1.6 GLYCOGENOLYSIS

Glycogenolysis is the process of conversion of glycogen into glucose by the liver cells with the help of hormone glucagon secreted by the pancreas. The conversion of glycogen (glycogenolysis) is by no means the reversal of enzymatic reactions involved in glycogenesis. It is infact, an entirely different pathway, where various steps are catalysed by different sets of enzymes.

The breakdown of glycogen is initiated by the enzyme *phosphorylase.* This enzyme is specific for the phosphorylytic breaking of 1, 4 linkages of glycogen resulting in the formation of glucose 1-phosphate (Fig. 5.15).

In liver, the enzyme phosphorylase exists both in active and inactive forms. The active enzyme *phosphorylase-a* is converted into inactive *dephosphorylase* by the removal of phosphate (Pi) with the help of enzyme *phosphorylase-phosphatase.* The reactivation of *dephosphorylase* occurs in the presence of ATP and the enzyme *phosphorylase kinase.*

In muscles, *phosphorylase* exists in two forms *i.e., phosphorylase-a* and *phosphorylase-b. Phosphorylase-a* is active only in the absence of 5'-AMP, while. *phosphorylase-b* is active only in the presence of 5'-AMP. The active *phosphorylase-a* is a tetramer, which on hydrolysis, in the presence of *phosphorylase-a-phosphatase* gets converted to a dimer *i.e., phosphorylase-b* (inactive). The enzyme *phosphorylase-b kinase* can combine two dimers of *phosphorylase-b* to an active *phosphorylase-a.*

$$\textit{Phosphorylase-a} + 4H_2O \xrightarrow{\textit{Phosphorylase a-phosphatase}} 2\ \textit{Phosphorylase-b} + 4H_3PO_4$$

$$2\ \underset{\text{(dimer)}}{\textit{Phosphorylase-b}} + 4ATP \xrightarrow[Mg^{2+}]{\textit{Phosphorylase -b kinase}} \underset{\text{(tetramer)}}{\textit{Phosphorylase-a}} + 4\ ADP$$

The hormone epinephrine plays a significant role in the activation of *phosphorylase* (Fig. 1.16).

The various steps of glycogenesis are listed below:

(*i*) The enzyme *phosphorylase* catalyses the removal of 1, 4-glucosyl residues from the outer most chain of the glycogen molecule. This continues till about 4 glucose residues are left on either side of the 1, 6 branch.

(*ii*) The enzyme, *α-1, 6-1, 4-glucan transferase* transfers a trisaccharide unit from one side to other and thereby exposing 1, 6 branch point (Fig. 1.17).

(*iii*) Now the debranching enzyme (*amylo 1, 6-glucosidase*) causes the hydrolytic splitting of the exposed 1, 6-linkage, thereby producing a molecule of glucose in the form of glucose 1-phosphate.

(*iv*) The enzyme *glucose 6-phosphatase* present in liver and kidney removes phosphate (Pi) from glucose 1-phosphate to yield free glucose. This enzyme is not present in muscles, hence, in muscles instead of glucose, lactic acid is formed.

NOTES

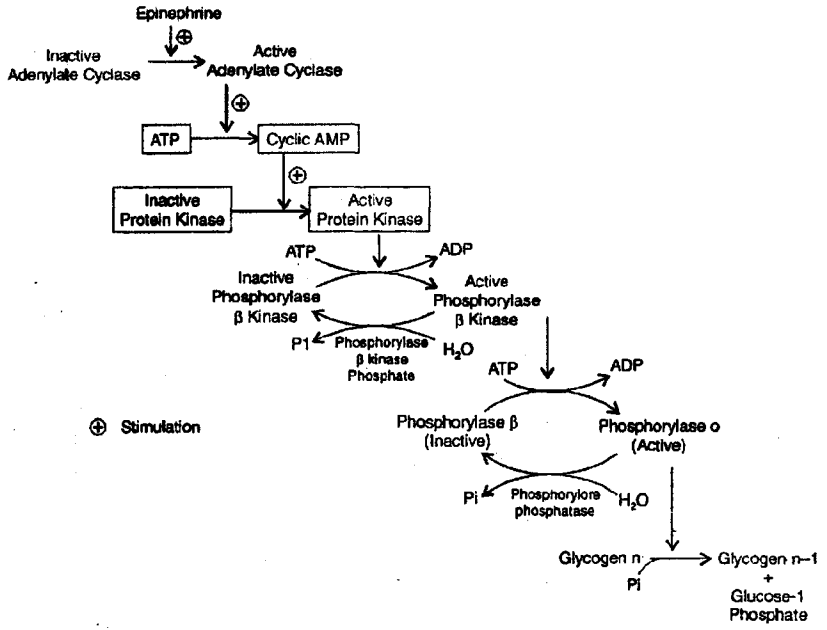


Fig. 1.16. *Role of epinephrine in glycogenolysis.*

In mammals, administration of hormone glucagon or epinephrine results in rise in blood glucose due to conversion of glucogen into glucose (glycogenolysis). It occurs due to the action of hormone, which stimulates the *glycogen phosphorylase* activity. It also simultaneously. depresses the *glycogen synthetase* activity.

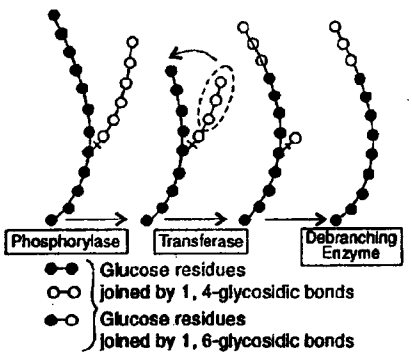


Fig. 1.17. *Various steps in glycogenolysis.*

Blood Sugar

Glucose is the free sugar that circulates in the blood. In human beings, blood sugar level is constantly maintained within narrow limits unless some abnormality develops. Most of the glucose absorbed from the gut

NOTES

after the digestion of food stuffs is passed on to the tissues for oxidation or it is converted into glycogen both in the liver and muscles. If the blood sugar level drops down from the normal, it is maintained by the process of glycogenolysis.

Hormonal Control of Carbohydrate Metabolism

Hormones of pancreas (insulin and glucagon), adrenal medulla (epinephrine), adrenal cortex (adrenocortical hormones) and even of anterior pituitary play significant roles in carbohydrate metabolism. Increase or decrease in the level of blood glucose depends on insulin concentration. Insulin increases the conversion of glucose to glycogen thus lowering the blood glucose level. Hypersecretion of insulin by pancreas will result in very low blood glucose level **(hypoglycemia).** On the contrary, hyposecretion of insulin will result in decreased conversion of glucose to glycogen. In such a situation, blood glucose level increases beyond the normal level. This condition is called **hyperglycemia.** Deficiency of insulin causes a disease **diabetes mellitus.** Epinephrine from adrenal medulla also affects the blood sugar level. It increases the concentration of glucose in the blood by increasing the breakdown of liver and muscle glycogen.

1.7 GLUCONEOGENESIS

Gluconeogenesis literally means the formation of 'new glucose'. New in the sense that it is made from proteins or glycerol of the fats and not from the carbohydrates. Thus, *'it is the formation of glucose or glycogen from non-carbohydrate sources'.* The process occurs chiefly in liver. It also occurs in kidney and skeletal muscles. Gluconeogenesis assumes greater significance in situations when carbohydrates are not therein the regular diet.

The main pathway of gluconeogenesis is essentially the reversal of glycolysis.

The various steps of gluconeogenesis (Fig. 1.18) are as follows:

1. Deamination of Amino Acids

(*i*) The different types of amino acids undergo deamination to form various intermediates of citric acid cycle. These reactions are catalysed by the specific enzyme *deaminases.*

(*ii*) These intermediates of citric acid cycle are converted into malate. The malate can either be converted to oxalo-acetate in the mitochondria which in turn is converted to pyruvate by a special mitochondrial enzyme *pyruvate carboxylase* in the presence of ATP, biotin and carbon dioxide, or it leaves the mitochondria to be converted to oxalo-acetate which then, in the presence of *phospho-enol pyruvate carboxykinase,* is converted to phospho-enol pyruvate releasing out carbon dioxide. The last reaction requires GTP as energy source.

NOTES

(*iii*) Also lactate can also be converted into phospho-enol pyruvate with the help of *lactate dehydrogenase.*

(*iv*) Once phospho-enol pyruvate is formed, it can proceed to the formation of glucose by reversal of EMP pathway. The presence of enzyme *fructose 1, 6-diphosphatase* in the tissues ensures conversion of fructose 1, 6-diphosphate to fructose 6-phosphate. The enzyme *isomerase* convert fructose 6-phosphate to glucose 6-phosphate.

(*v*) The enzyme *glucose 6-phosphatase*, then converts glucose 6-phosphate to glucose. The glucose is then converted to glycogen.

(*vi*) Some amino acids such as leucine, isoleucine and tryphophan on degradation yield acetyl CoA, which is then, converted into citrate and enters into citric acid cycle (Fig. 1.19).

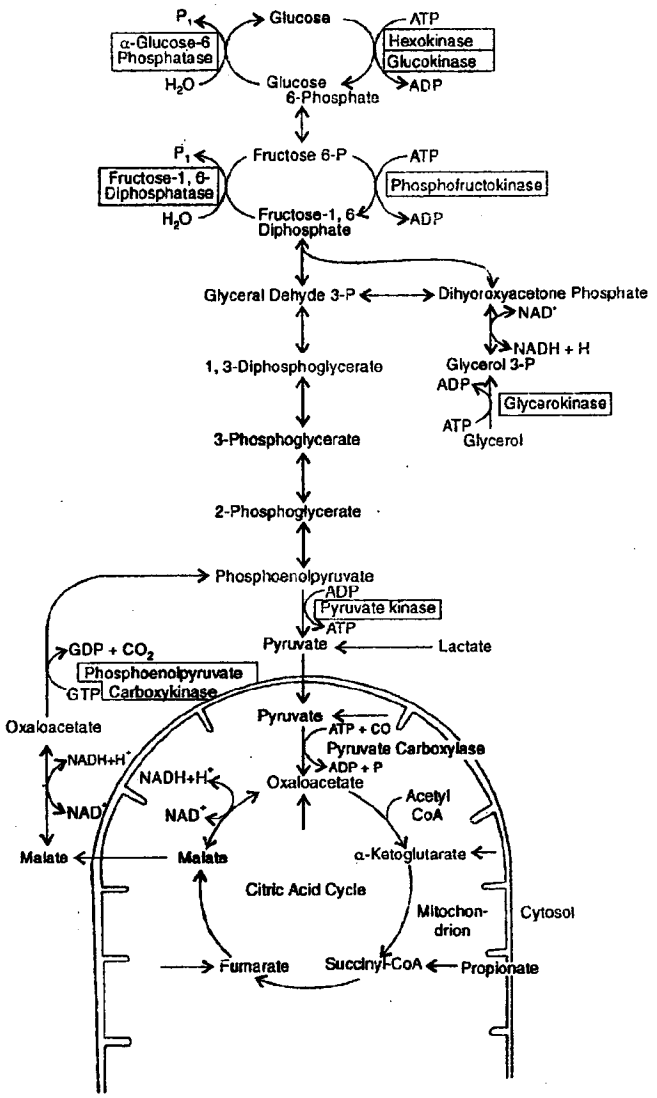


Fig. 1.18. *Major pathways in gluconeogenesis in the liver.*

NOTES

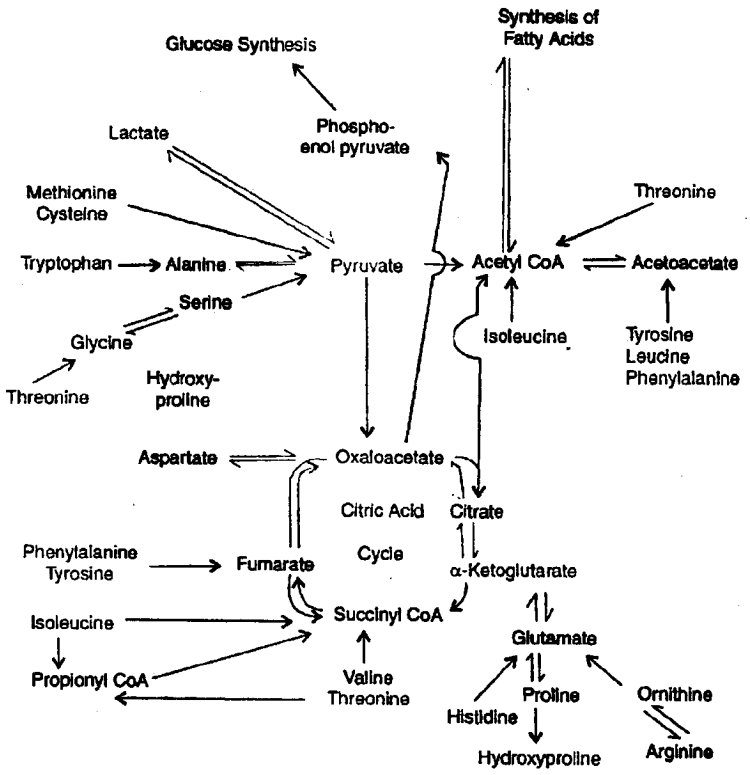


Fig. 1.19. *Interconnections of anabolic and catabolic pathways of citric acid cycle.*

(*vii*) In animals, there is no pathway by which new glucose is formed from carbon atoms of fatty acids. However, plants can synthesize carbohydrates from fatty acids through acetyl CoA, through glyoxylate cycle. Through this cycle acetyl. CoA is converted to succinate.

$$2 \text{ Acetyl CoA} + NAD^+ + 2H_2O \longrightarrow \text{Succinate} + 2CoA + NADH + H^+.$$

(*viii*) Succinate formed in the glyoxylate pathway, forms oxaloacetate, which is then converted into phospho-enol pyruvate and then to glucose through reverse EMP pathway.

(*ix*) Glycerol is another product of the breakdown of stored fats. The enzyme *glycerokinase* converts glycerol to glycerol 3-phosphate in presence of ATP. The glycerol 3-phosphate is then oxidised to dihydroxy acetone phosphate. With the help of *glycerol 3-phosphate dehydrogenase* and NAD^+. The glyceraldehyde 3-phosphate and dihydroxyacetone 3-phosphate are the intermediate of EMP pathway, and hence, are converted into glucose by reserve reactions of EMP pathway.

STUDENT ACTIVITY

1. Give a schematic representation of Kreb's cycle.

2. Give a brief account of glycogenesis.

NOTES

1.8 SUMMARY

- In living organisms storage and breakdown of carbohydrates involve several biochemical reactions. The whole process of carbohydrate metabolism can be divided into six categories : glycolysis, oxidation of pyruvate, pentose monophosphate pathway, glycogenesis, glycogenolysis and gluconeogenesis.
- Glycolysis is the first step of glucose breakdown. It occurs in the cytosol and results in the breakdown of glucose into two molecules of pyruvate releasing some energy as ATP and reducing power as $NADH_2$. The pyruvate formed at the end of glycolysis is anaerobically broken down to ethyl alcohol and carbon dioxide or lactic acid. Lactic acid formation occurs in the skeletal muscles after vigorous exercise due to anaerobic breakdown of pyruvate. Accumulation of lactic acid leads to muscular fatigue. Aerobic oxidation of pyruvate occurs inside mitochondria through Kreb's cycle. Before entering into Kreb's cycle, pyruvate undergoes oxidative decarboxylation to form acetyle CoA. It is the connecting link between glycolysis and Kreb's cycle. Kreb's cycle is stepwise oxidative and cyclic degradation of acetyl CoA derived from pyruvate. During Kreb's cycle 2 molecules of CO_2, 3 molecules of NADH and 1 molecule of $FADH_2$ are formed. In the last step, NADH and $FADH_2$ are oxidised through electron transport chain and produce ATP.
- Although the major pathway for aerobic breakdown of glucose is through glycolysis and Kreb's cycle, there also exists an alternate pathway called pentose phosphate pathway. This pathway occurs in presence of oxygen and involves direct oxidation of glucose 6-phospate.
- Glycogenesis is the process of conversion of the excess of glucose into glycogen by the liver cells and skeletal muscles. Glycogenolysis is the process of conversion of glycogen into glucose by the liver cells. Glycogenesis and glycogenolysis maintain sugar level in the blood. The two processes are regulated by the hormones of pancreas and some other endocrine glands. Gluconeogenesis is the formation of glucose or glycogen from non-carbohydrate sources such as amino acids and fats.

1.9 GLOSSARY

- **Glycolysis:** It is the first step in the breakdown of glucose and is common to all organisms and is also called EMP pathway.

- **Acetyl CoA:** It is the connecting link between glycolysis and krebs cycle.
- **Krebs Cycle:** Krebs cycle is stepwise oxidative and cyclic degradation of acetyl-CoA derived from pyruvate. It occurs in mitochondrial matrix and serves as a common oxidative pathway for carbohydrtes, fats and proteins.
- **Glycogenesis:** The process of conversion of the excess of glucose into glycogen by liver cells with the help of a pancreatic hormone, insulin.
- **Glycogenolysis:** It is the process of conversion of glycogen into glucose by the liver cells with the help of hormone glucagon secreted by the pancreas.

1.10 REVIEW QUESTIONS

I. Very Short Answer Type Questions:

1. What does the term glycolysis mean?
2. Why is glycolysis called common-pathway?
3. What are the products of glycolysis?
4. Why does strenuous exercise cause muscular fatigue?
5. Why is Kreb's cycle is also called TCA cycle?
6. What is terminal oxidation?
7. What is the terminal oxygen acceptor in ETC?
8. What is the role of F_0—F_1 particles in the formation of ATP?
9. How many ATP molecules are produced per molecule of glucose during aerobic respiration in different types of cells?
10. Define the following:

    (*i*) Glycogenesis (*ii*) Glycogenolysis (*iii*) Gluconeogenesis.

II. Short Answer Type Questions:

1. What is electron transport chain? What is its role in terminal oxidation?
2. Explain, the process of oxidative phosphorylation.
3. What is the significance of pentose phosphate pathway?
4. Give the schematic representation of glycolysis.
5. Write explanatory notes on:

    (*i*) Glycolysis (*ii*) Fermentation.
6. Explain the main steps of glycogenolysis.
7. Write brief notes on:

    (*i*) Pentose phosphate pathway (*ii*) Gluconeogenesis.

NOTES

III. Long Answer Type Questions:

1. Enumerate the steps involved in the aerobic breakdown of pyruvate.
2. Describe Kreb's cycle and its role in the living organisms.
3. Describe the electron transport chain. How is it involved in the development of proton gradient?
4. Where does glycolysis occur in the cell? Explain its different steps.
5. Enumerate the main steps glycogenesis.

1.11 FURTHER READINGS

- **Outlines of Biochemistry;** Conn E.E., Stumpf P.K., **Bruening** G., Doi R.H.; Wiley India (P) Ltd, New Delhi, 2007.
- **Essentials of Food and Nutrition;** Swaminathan M; Ganesh Madras, India; 1985.
- **Introduction to Nutrition;** Fleck Henrietta; MacMillan, New York, 1981.

CHAPTER 2 PROTEIN METABOLISM

NOTES

OBJECTIVES

After going through this chapter, you should be able to:

- explain amino acids metabolism
- describe biosynthesis of nucleic acids
- define nucleotides
- summarize protein synthesis
- know about genetic code.

STRUCTURE

2.1 INTRODUCTION

Proteins are the most abundant organic compounds and constitute a major part of the body dry weight (10–12 kg in adults). They perform a

NOTES

wide variety of structural and dynamic functions being components of cell organelles, enzymes, hormones, clotting factors, receptors etc. About half of the body protein (predominantly collagen is present in the supportive tissues like skeletal and connective tissues. The remaining half is intracellular.

Proteins are nitrogen containing macromolecules consisting of amino acids as the repeating units. Thus, proteins are highly organised linear polymers of amino acids. Of the 20 amino acids found in proteins about a dozen can be synthesized by the body called non-essential amino acids. The remaining have to be provided in the diet and are called essential amino acids.

Each one of the 20 naturally occurring amino acids, undergoes its own metabolism and performs specific functions. Thus, protein metabolism is more appropriately learnt as metabolism of amino acids.

2.2 AMINO ACIDS METABOLISM

Amino acid metabolism includes synthesis and degradation of amino acids.

Synthesis of Amino Acids

Amino acids are synthesised from keto organic acids produced during the respiratory breakdown of sugars, through the process of amination at α-carbon of keto group. Direct amination is found in case of glutamic acid and possibly also aspartic acid and alanine. In others amination occurs through transfer of amino group of one amino acid to another organic acid. A few amino acids are produced by transformation of others. Twenty different amino acids are synthesised by involving 20 different multienzyme sequences. The pathways of biosynthesis of amino acids are quite different from those employed in their degradation. The synthesis of the protein amino acids from the intermediates of glucose metabolism is depicted in Fig. 2.1.

1. Reductive Amination. Ammonia is produced through reduction of nitrate. It combines with a keto acid in the presence of a hydrogenase and reduced coenzyme.

$$\alpha\text{-ketoglutaric acid} + NH_3 + NADPH_2/NADH_2 \xrightarrow{Dehydrogenase} \text{Glutamic acid} + NADP/NAD + H_2O$$

$$\text{Oxaloacetic acid} + NH_3 + NADPH_2/NADH_2 \xrightarrow{Dehydrogenase} \text{Aspartic acid} + NADP/NAD + H_2O$$

2. Catalytic Amidation. Ammonia initially combines with glutamic acid to form amide glutamine in the presence of ATP and enzyme glutamine synthetase. Glutamine then reacts with α-ketoglutaric acid in the presence of reduced coenzyme and glutaminase to form two molecules of glutamic

acid. One molecule of the latter can combine again with ammonia to continue the process.

$$\text{Glutamic acid} + NH_3 + ATP \xrightarrow{\textit{Glutamine Synthetase, } Mg^{++}} \text{Glutamine} + ADP + Pi$$

$$\text{Glutamine} + \alpha\text{-ketoglutaric acid} + NADP_2/NADH_2 \longrightarrow 2 \text{ Glutamic acid} + NADP/NAD$$

3. Transamination. An amino acid transfers its amino group to another keto acid so that there is an exchange of $CH(NH_2)$ with $>C = O$. A transaminase enzyme having coenzyme pyridoxal phosphate is required. Usually amino group is donated by glutamic acid.

$$\text{Glutamic acid} + \text{Pyruvic acid} \xrightleftharpoons{\textit{Glutamic-Alanine Transaminase}} \alpha\text{-ketoglutaric acid} + \text{Alanine.}$$

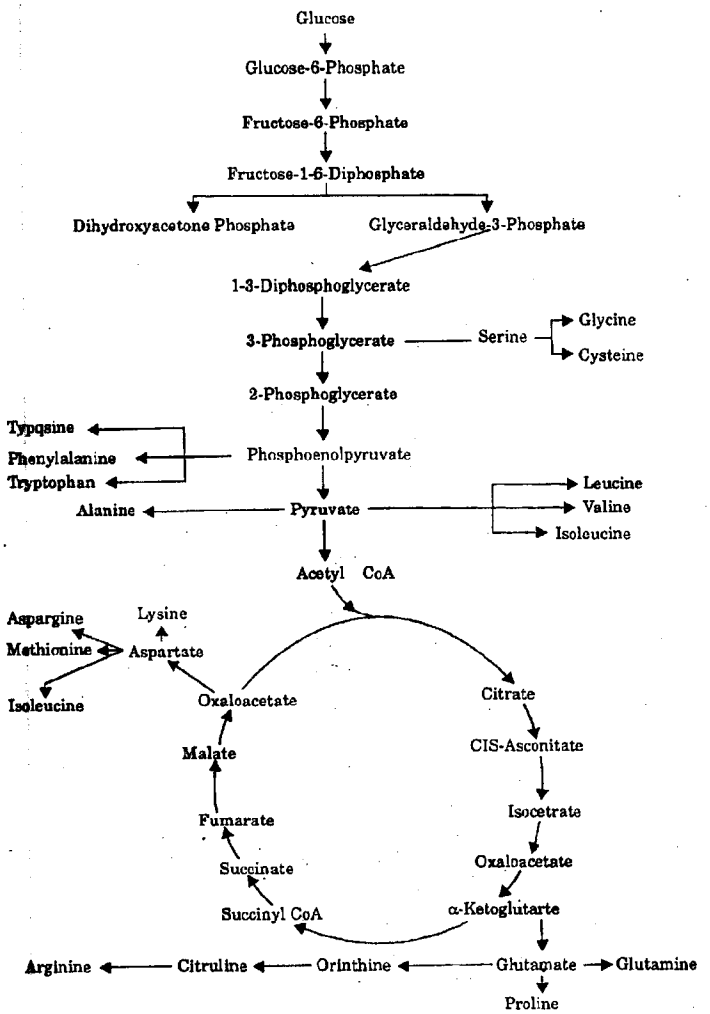


Fig. 2.1. *Biosynthesis of amino acids from the intermediates of glycolysis and Krebs cycle. The amino acids citrulline and ornithine do not occur in proteins.*

NOTES

4. Transformations. (*i*) Hydroxyproline is produced by oxidation of proline. (*ii*) Homoserine is formed from aspartic acid with the loss of oxygen an gain of hydrogen. (*iii*) Serine is a product of condensation of two glycine molecules.

Animals cannot synthesise seven of the protein amino acids. For them, they are called essential amino acids. These acids must be present in their diet, *viz.*, leucine, isoleucine, valine, trytophan, phenylalanine, lysine and methionine.

Catabolism of Amino Acids

Besides synthesis of proteins, amino acids also serves as sources of carbon and nitrogen, when required. The first step in their catabolism is the removal of $—NH_2$ group and formation of corresponding keto acid. In the process ammonia is released, which is quickly converted into urea or is incorporated in some other amino acid.

$$\underset{\text{Amino acid}}{R—CH—NH_2COOH} \longrightarrow \underset{\text{Keto acid}}{RCOCOOH} + \underset{\text{Ammonia}}{NH_3} \longrightarrow \text{Urea}$$

The removal of $—NH_2$ group takes place in two ways : transamination and deamination.

1. Transamination

Transamination is the most important mechanism of the conversion of amino acid into keto acid. Its importance was shown for the first time by Russian workers **Braunstein** and **Bychkou** in 1939. In the process, the amino group is transferred to recipient keto acid which is converted to amino acid.

$$R_1–CH(NH_2)COOH + R_2COCOOH \rightleftharpoons R_1COCOOH + R_2CH(NH_2)COOH$$

The enzyme *transaminases* or *amino transferases* catalyzing this type of reactions, are common in biological systems. Coenzyme for such reactions is pyridoxal phosphate, a derivative of vitamine B_6. Most of the amino acids may act as donor amino acids. The recipients, however, are mostly ketoglutarate, oxalacetate or pyruvate and produce glutamate, asparate and alanine, respectively. Threonine and lysine probably do not participate in transamination.

2. Deamination

Amino group of the amino acids may be removed either by oxidative or by non-oxidative deamination. One example of oxidative deamination is deamination of glutamate by *glutamate dehydrogenase.*

$$\text{Glutamate} + NAD^+ + H_2O \rightleftharpoons NH_3 + \alpha\text{-ketoglutarate} + NADH + H^+$$

This reaction is reversible and the electron is accepted by either NAD^+ or NADP. A group of flavin-containing enzymes (amino acid-oxidases)

also catalyze oxidative deamination. In this case H_2 is transferred to O_2 and H_2O_2 is produced.

Non-oxidative deamination is catalyzed by *amino acid-ammonialyase.* In this process, NH_3 is eliminated with the formation of a double bond in the remaining carbon-skeleton. In bacteria and plants, asparate is deaminated to fumarate by the enzyme *aspartase (aspartase-ammonia-lyase).*

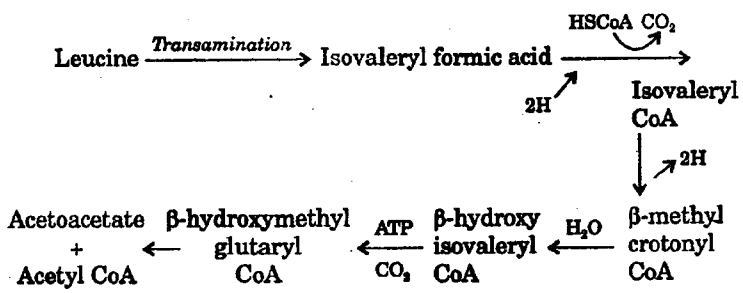
$$COOHCH(NH_2)CH_2COOH \rightleftharpoons HOOC-\underset{\displaystyle H}{\overset{\displaystyle H}{C}}=\underset{\displaystyle H}{\overset{\displaystyle H}{C}}-COOH + NH_3$$

Fumarate

Similarly, phenylalanine can be deaminated to cinnamate. Deamination of histidine is more common in mammals.

Fate of Deaminated Amino Acids

Acids produced from deamination or transamination of amino acids are channelled to several metabolic routes. Keto acids are eventually oxidised to CO_2 and H_2O through acetoacetate and acetyl CoA. Acetoacetate is one of the chemical constituent of the "ketone bodies" appearing in the pathological conditions of urine. Thus, the amino acids which give rise to acetoacetate during their metabolism are called **ketogenic.** For example, leucine is first deaminated to isovaleryl formic acid which reacts with coenzyme-A to yield a series of compounds ultimately producing acetoacetate and acetyl CoA



Some amino acids are deaminated to produce acids which are broken down $_{10}C_4$ dicarboxylic acid or pyruvate and then converted to carbohydrate. For example, alanine is deaminated to pyruvate which can be utilized for the synthesis of glucose or glycogen. These amino acids are called *glucogenic* or antiketogenic amino acids. Examples of other glucogenic amino acids are glycine, serine asparate, glutamate, valine, histidine, arginine, proline, threonine, tryptophan, methionine and serine. Catabolism of arginine is represented in Fig. 2.2.

NOTES

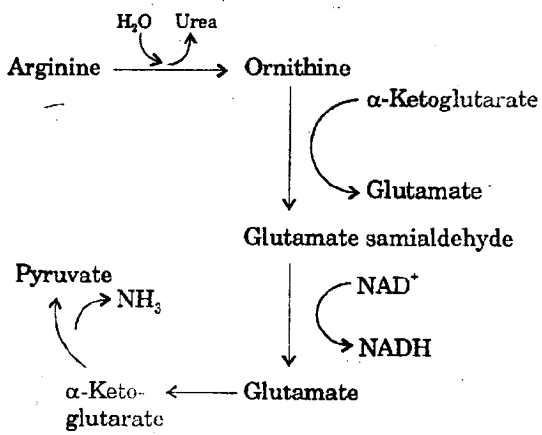
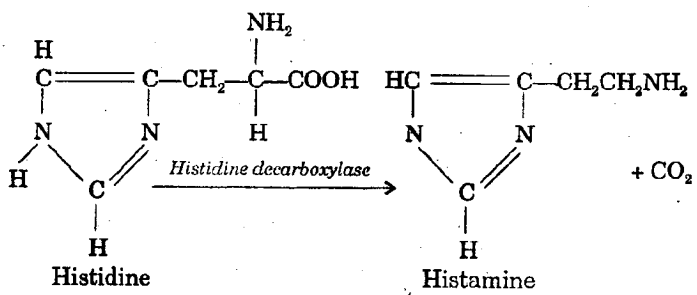


Fig. 2.2. *Catabolism of arginine.*

Decarboxylation of Amino Acids

The enzyme *amino acid decarboxylases* catalyse the removal of CO_2 from the carboxyl group of amino acids. All *decarboxylases* seem to require pyridoxal phosphate as coenzyme. Thus, histidine may be decarboxylated to histamine by *histidine decarboxylase.*



Similarly, 3, 4-dihydroxyphenylanine is decarboxylated to dopamine, tryptopan to tyrptamine, tyrosine to tyramine, glutamate to amino butyrate, and so on. This type of amines are called **biogenic amines.** Many of these amines have strong pharmacological effects and others are important as precursors of hormones or as coenzymes.

2.3 UREA CYCLE (ORNITHINE CYCLE)

This cycle was first discovered by *H.A. Krebs* and *K. Henseleit* and hence, it is also called **Krebs-Henseleit cycle.**

When production of ammonia exceeds beyond a certain level, it becomes toxic. Therefore, it is quickly converted into a less toxic substance, urea, which is then eliminated from the body through urine. The conversion of ammonia is a cyclic process and occurs in liver (Fig. 2.3). Urea cycle completes in five steps involving five distinct enzymes. The first two enzymes are present in mitochondria, while the rest are present in the cytosol.

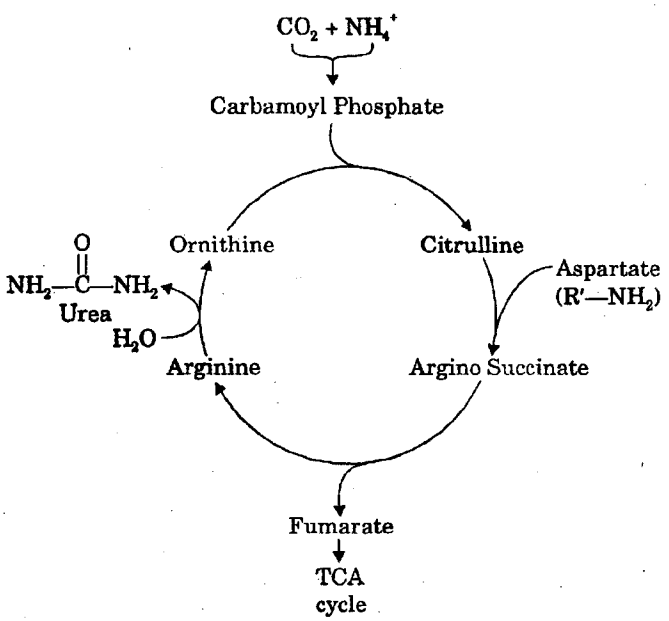


Fig. 2.3. *Urea cycle.*

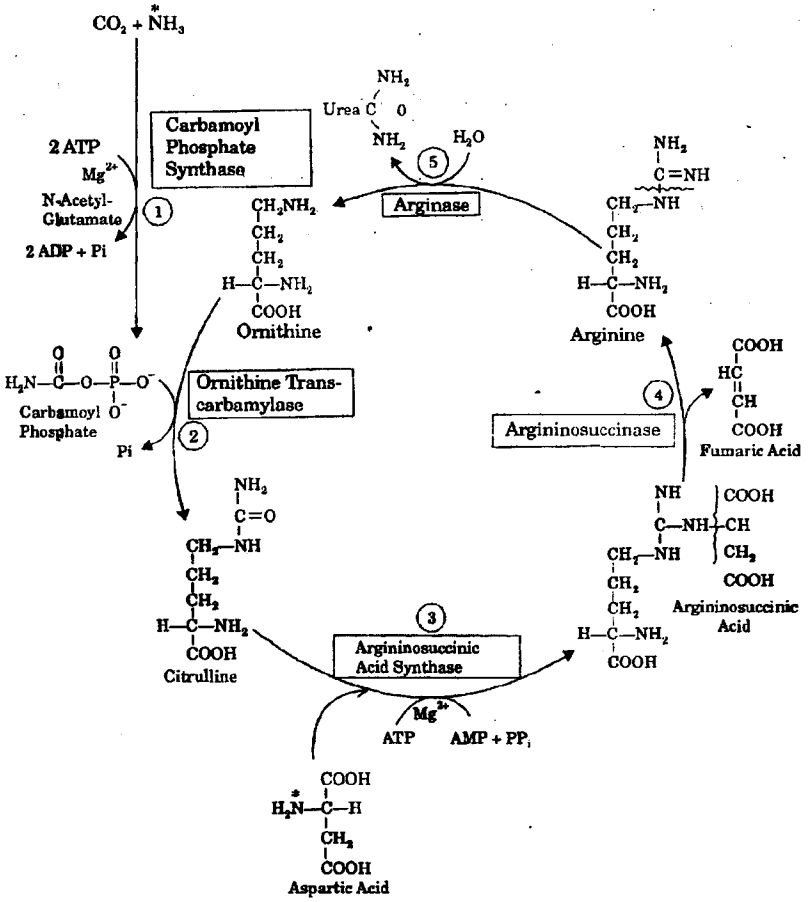


Fig. 2.4. *Chemical reactions of urea cycle.*

(*i*) Enzyme *Carbamoyl Phosphate Synthase I* (CPSI) of mitochondria catalyses the condensation of ammonia and CO_2 to form carbamoyl phopshate, utilising two ATPs.

(*ii*) Citrulline is synthesized from carbamoyl phosphate and ornithine (regenerated in the urea cycle). The reaction is catalysed by *ornithine trans-carbamoylase*. Ornithine and citrulline are basic amino acids.

(*iii*) Citrulline combines with aspartate to form argino succinate. The reaction is catalysed by the enzyme *arginosuccinate synthase* and utilizes ATP.

(*iv*) Argino succinate cleaves into arginine and fumarate in presence of the enzyme *arginosuccinase*. The fumarate produced here provides a link with krebs cycle gluconeogenesis, etc.

(*v*) The arginine splits to form ornithine and urea with the help of enzyme *arginase*. The ornithine enters into mitochondria for its reuse in urea cycle. The chemical reactions of urea cycle are depicted in Fig. 2.4.

2.4 BIOSYNTHESIS OF NUCLEIC ACIDS

Chemistry of Nucleic Acids

Nucleic acids are polymeres of nucleotides, and hence, may be called polynucleotide sequences. When nucleic acids are hydrolysed completely, yields heterocyclic nitrogenous bases, five carbon sugars and phosphoric acid molecules, while partial hydrolysis yields nucleotide residues only (Fig. 2.5).

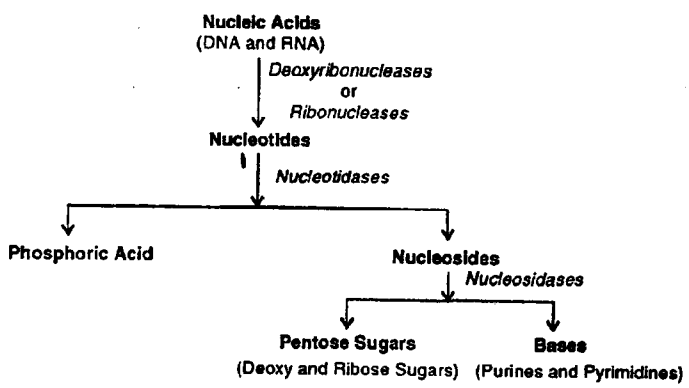


Fig. 2.5. *Hydrolysis of nucleic acids.*

NOTES

2.5 NUCLEOTIDES

They are the building blocks (monomers) of nucleic acids. They also participate in energy transfer system in the cell.

Nucleotides are compounds of carbon, hydrogen, oxygen, nitrogen and phosphorus. Each nucleotide is made up of three small chemicals—a pentose sugar, a cyclic nitrogenous base and one to three phosphate groups.

Sugars

The sugar molecule in a nucleotide may be ribose ($C_5H_{10}O_5$) or deoxyribose ($C_5H_{10}O_4$). Both sugars are in furanose or pentagon state with one oxygen and four carbon atoms. The fifth carbon along with CH_2OH occurs outside the ring. Ribose molecule differs from deoxyribose molecule in having a hydroxyl (—OH) group instead of hydrogen at carbon 2 (Fig. 2.6). Deoxyribose sugar is found in the nucleotides forming DNA, while ribose occurs in the nucleotides forming RNA and a number of other compounds like AMP, ATP, NAD, FAD etc.

Nitrogenous Bases

The nitrogen bases occurring in nucleotides are heterocyclic compounds. They are of two types—**purines** and **pyrimidines.** Pyrimidines are six membered rings, while purines are nine membered double rings (Fig. 2.6). Purines are of two types—**Adenine (A)** and **Guanine (G),** while pyrimidines are of three types—**Thymine (T), Cytosine (C)** and **Uracil (U).**

Differences between Purines and Pyrimidines

Purines	*Pyrimidines*
1. Purines are larger than pyrimidine molecules.	1. Pyrimidines are smaller than purine molecules.
2. They are nine membered molecules with double rings.	2. They are six membered molecules with single ring.
3. A purine contains four nitrogen atoms at 1′, 3′ and 9′ positions.	3. A pyrimidine has nitrogen atoms at two places, 1′ and 3′ positions.
Examples. Adenine (A) and Guanine (G)	**Examples.** Thymine (T), Cytosine (C)

The nitrogen base joins to the sugar molecule at its carbon atom 1′ by a **glycosidic bond** by one of its nitrogen atoms (usually 3 in pyrimidines and 9 in purines). A combination of the two is called **nucleoside.** For example, a combination of adenine-ribose or adenine-deoxyribose are nucleosides. A nucleoside with ribose sugar is known as **ribonucleoside** or simply **riboside** and a nucleoside with deoxyribose sugar is termed as **deoxyribonucleoside** or simply **deoxyriboside.** Thymine forms

NOTES

nucleoside with only deoxyribose sugar, uracil, similarly, produces nucleoside with ribose sugar. Other nitrogen bases form nucleoside with both ribose and deoxyribose sugars. The various nucleosides are **adenosine** (adenine + ribose), **deoxyadenosine** (adenine + deoxyribose), **guanosine** (guanine + ribose), **deoxyguanosine** (guanine + deoxyribose), **cytidine** (cytosine + ribose), **deoxycytidine** (cytosine + deoxyribosome), **deoxythymidine** (thymine + deoxyribose) and **uradine** (uracil + ribose).

Phosphoric Acid

Nucleotides are phosphoric acid esters of nucleosides. Phosphate combines with the sugar of the nucleoside at its 5′ carbon atom by an ester bond to form a **nucleotide** or **nucleoside monophosphate** (Fig. 2.6). Both

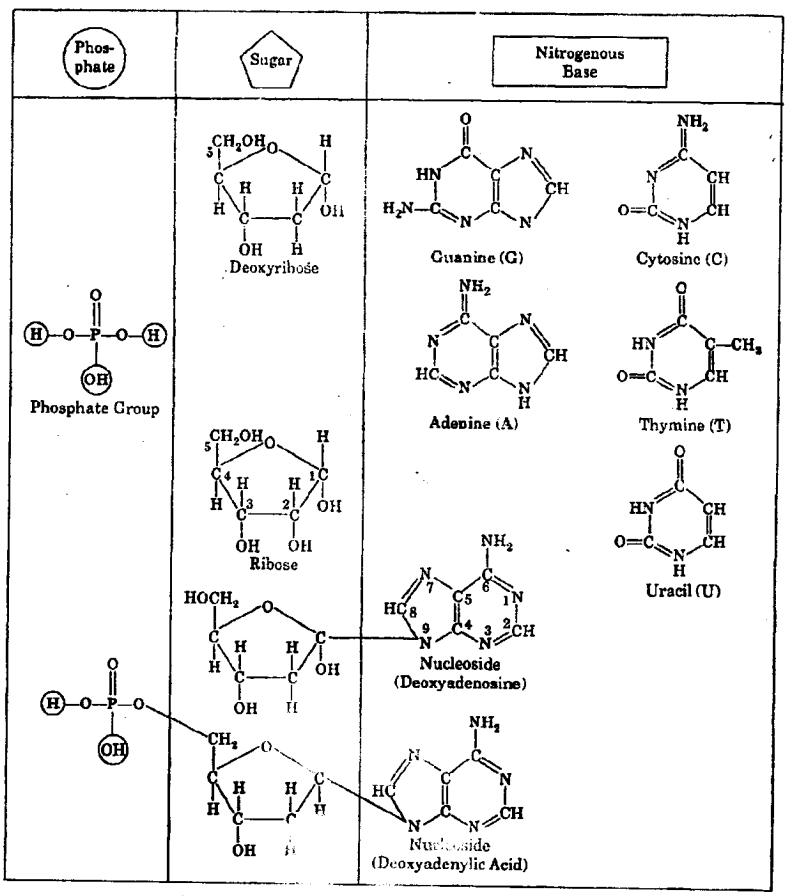


Fig. 2.6. *Building blocks of nucleic acids.*

glycosidic and ester bonds are formed by condensation reactions involving elimination of water. A nucleotide having ribose sugar is called **ribonucleotide** or simply **ribotide,** and a nucleotide containing deoxyribose is termed as **deoxyribonucletide** or simply **deoxyribotide.** The different nucleotides are adenylic acid or Adenosine Monophosphate (AMP), deoxyadenylic Acid or Deoxyadenosine Monophosphate (dAMP), guanylic acid or Guanosine

Monophosphate (GMP), deoxyguanylic Acid or Deoxyguanosine Monophosphate (dGMP). Cytidylic acid or Cytidine Monophosphate (CMP), deoxycitidylic acid or Deoxycytidine Monophosphate (dCMP), deoxythymidylic acid or deoxythymidine monophosphate (dTMP) and uridylic acid or Uridine Monophosphate (UMP). The free occurring nucleotides may have upto three phosphate groups.

Differences between Nucleoside and Nucleotide

Nucleoside	*Nucleotide*
1. Nucleoside is a compound formed by the combination of a nitrogen base and a pentose sugar.	1. Nucleotide is a compound formed by the combination of a nitrogen base, a pentose sugar and phosphate.
2. It is slightly basic in nature.	2. It is acidic in nature.
3. A nucleoside is a component of a nucleotide.	3. A nucleotide may be a component of nucleic acids, energy carriers or coenzymes.

Structure of Polynucleotide Chain (DNA or RNA)

Nucleotides are basic units of nucleic acids. A nucleotide has three components : a nitrogenous base, a pentose sugar (ribose in case of RNA and deoxyribose for DNA), and a phosphate group. There are two types of nitrogenous bases ; **Purines** (Adenine and Guanine) and **Pyrimidines** (Cytosine, Uracil and Thymine). Cytosine is present both in DNA and RNA. Thymine is present in DNA, but RNA possesses uracil in place of thymine.

A nitrogenous base is linked to the pentose sugar through a **N-glycosidic linkage** to form a **nucleoside.** Depending upon the type of pentose sugar, nucleosides are differentiated into **ribonucleosides** and **deoxyribonucleosides.** Uracil produces nucleoside with only ribose sugars. Thymine, similarly forms nucleoside with only deoxyribose sugar. Other nitrogen bases produce nucleosides with both ribose and deoxyribose sugars. The different nucleosides are **adenosine** (adenine + ribose), **deoxyadenosine** (adenine + deoxyribose), **guanosine** (guanine + ribose), **deoxyguanosine** (guanine + deoxyribose), **uridine** (uracil + ribose), **deoxythymidine** (thymine + deoxyribose), **cytidine** (cytosine + ribose), **deoxycytidine** (cytosine + deoxyribose).

When a phosphate group is linked to 5′—OH of a nucleoside through **phosphoester linkage**, a corresponding nucleotide (**ribonucleotide** or **deoxyribonucleotide**, depending upon the type of sugar present) is formed. Two nucleotides are linked through 3′–5′ phosphodiester linkage to form a dinucleotide. More nucleotides can be joined in such a manner to form a polynucleotide chain. A polymer, thus, formed has at one end a free phosphate moiety at 5′ end of the sugar, which is referred to as **5′ end** of polynucleotide chain. Whereas, at the other end of the polymer, the sugar has a free 3′—OH group. Which is termed as **3′ end** of the

NOTES

polynucleotide chain. The sugars and phosphates form the backbone of the polynucleotide chain. The nitrogenous bases linked to sugar moiety project from the backbone (Fig. 2.7). In RNA, every nucleotide residue has an additional —OH group present of 2′-position in the ribose.

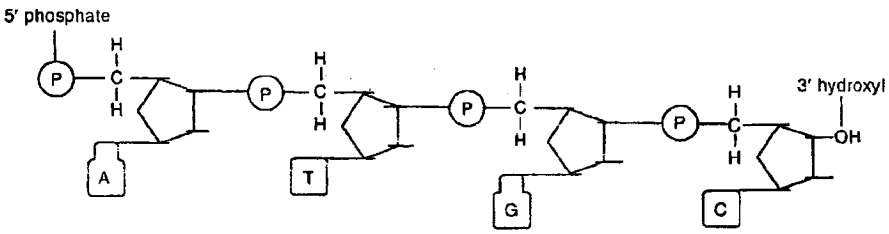


Fig. 2.7. *A polynucleotide chain.*

Synthesis of Pyrimidine Nucleotides

The various steps (Fig. 2.8) in the synthesis of pyrimidine nucleotides are described below.

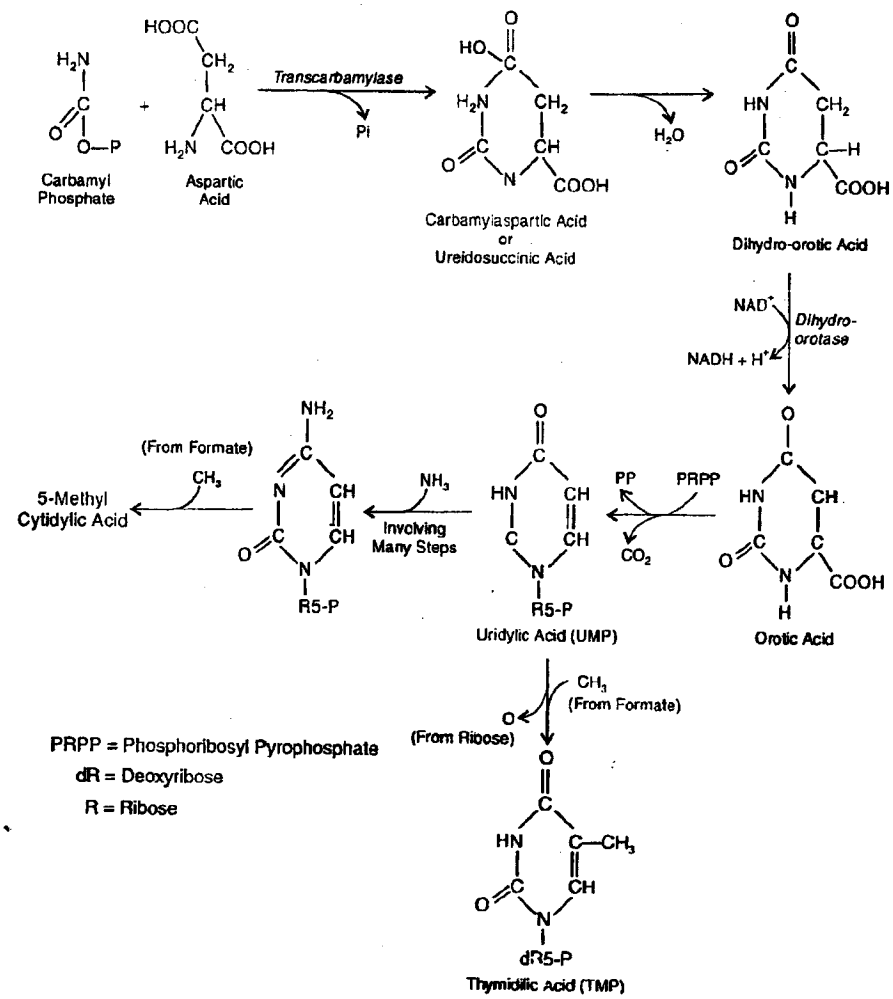


Fig. 2.8. *Biosynthesis of pyrimidine nucleotides.*

1. Formation of Carbamyl Aspartate. Carbamyl phosphate is first formed from CO_2 and NH_3 by the action of *carbamyl phosphate synthase II.* The reaction is similar to the first step in urea synthesis. But, the liver enzyme is present in the mitochondria and uses free ammonia and requires *n*-acetylglutamate as a cofactor. The enzyme in other tissues in cytoplasmic and can utilize only the amide nitrogen of glutamine as the source for $—NH_2$.

$$\text{Glutamine} + CO_2 + 2ATP \longrightarrow \underset{\text{Carbamyl phosphate}}{NH_2COP} + \text{Glutamate} + 2ADP + P$$

The carbamyl phosphate now combines with aspartate to form carbamyl aspartate. This is a committed step in pyrimidine biosynthesis.

2. Formation of Dihydro-orotic Acid. The carbamyl aspartate loses a molecule of water to form dihydro-orotic acid.

3. Formation of Uridylic Acid. On removal of 2 hydrogens, orotic acid is formed. The orotic acid is then decarboxylated and combined with phosphorylated ribose to form uridine S-phosphate (uridylic acid or UMP). UMP can be phosphorylated further by ATP to form UDP and UTP.

4. Formation of Cytidilic Acid. Uracil is the pyrimidine base first synthesized. It can be aminated in position C-4 to form the cytosine derivatives. The amino group of glutamine contributes the NH_2 to the C-4 to form cytidilic acid or CMP.

5. Formation of Thymine. The uracil moiety can be methylated in position C-5 in presence of serine, tetrahydrofolic acid, ATP and Mg^{2+} to form thymine.

Synthesis of Purine Nucleotides

The purine ring is also synthesized along with the nucleotide. First of all inosinic acid (containing hypoxanthine as the purine base) is formed. The various steps of synthesis of purine ring are given below.

1. Formation of 5-phosphoribosyl 1-pyrophosphate (PRPP). Ribose 5-phosphate is converted to PRPP by the enzyme *ribose 5-phosphate phosphokinase.* It transfers a pyrophosphate from ATP to ribose 5-phosphate. This is a key enzyme in the synthesis of the nucleotides and its activity is regulated by the levels of Pi (inorganic phosphate), Mg^{2+} and ADP.

2. Formation of 5-phosphoribosyl, 1-amine. The pyrophosphate in position C-1 is replaced by amino ($—NH_2$) group from glutamine to form 5-phosphoribosyl 1-amine.

3. Formation of Glycinamide Ribotide. Glycine combines with the 5-phosphoribosyl 1-amine to form glycinamide ribotide.

4. Formation of Formyl Glycinamide Ribotide. A formyl group is added to the glycinamide ribotide to the N at position 7 in the ring to form formyl glycinamide ribotide. This step requires tetrahydrofolic acid.

NOTES

5. Formation of Formyl-glycinamidine Ribotide. Now —NH_2 is added at position 3 from glutamine. It closes the ring between C-8 and N-9 to form formyl-glycinamidine ribotide.

6. Formation of 5-amino-4-imidazole-carboxamide-ribotide. Carbon dioxide (from CO_2 pool of the body) and NH_2 from aspartic acid are added as carbamate to C-5 to form the C-6 and N-1 and the compound formed is 5-amino-4-imidazole-carboxamide-ribotide. This step requires biotin.

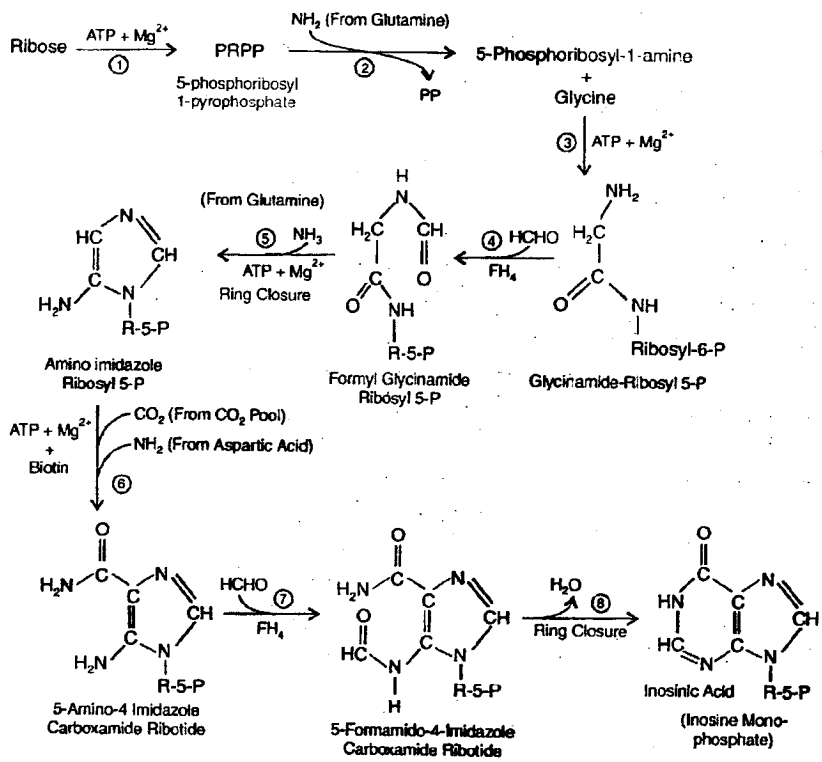
7. Formation of 5-formamido-4-imidazole-carboxamide Ibotide. A formyl group is now added to the amino group of N_3. This step requires tetrahydrofolic acid. The intermediate formed is 5-formamido-4-imidazole-carboxamide-ribotide. The formyl C will be the future C-2 of the purine ring.

8. Formation of Inosine Monophosphate (IMP). Ring closure now occurs between N-1 and C-2 form Inosine Monophosphate (IMP) or inosinic acid.

9. Formation of Adenine. Inosine is converted to adenine by taking an amino group at C-6 from aspartic acid.

10. Formation of Guanine. Inosine can be converted to guanine by oxidation of C-2 to C = 0 and later amination from glutamine to form C-NH_2. The reactions 9 and 10 occur while still in the nucleotide (as inosinic acid). Hence the product formed in step 9 is adenylic acid (AMP) and in step 10, it is guanylic acid (GMP).

The synthetic process is summarised in Fig. 2.9.



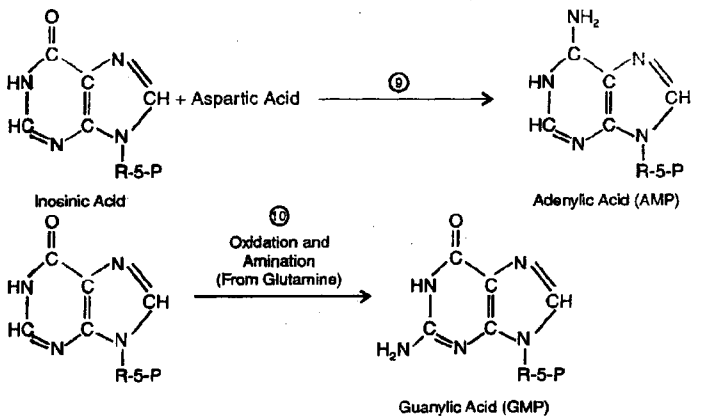


Fig. 2.9. *Biosynthesis of Purine nucleotides.*

2.6 REPLICATION OF DNA

Watson and Crick proposed that DNA replication is **semiconservative**. It is a type of replication in which one strand of daughter duplex is derived from the parent, while the other strand is formed a new. The two strands of a DNA molecule separate and each strand serves as a template (model or guide) for the formation of a new but complementary strand. Thus, the new or daughter DNA molecules formed would be made of one old or parental strand and another newly formed complementary strand. This method of formation of new daughter DNA molecules is said to be semiconservative (Fig. 2.10).

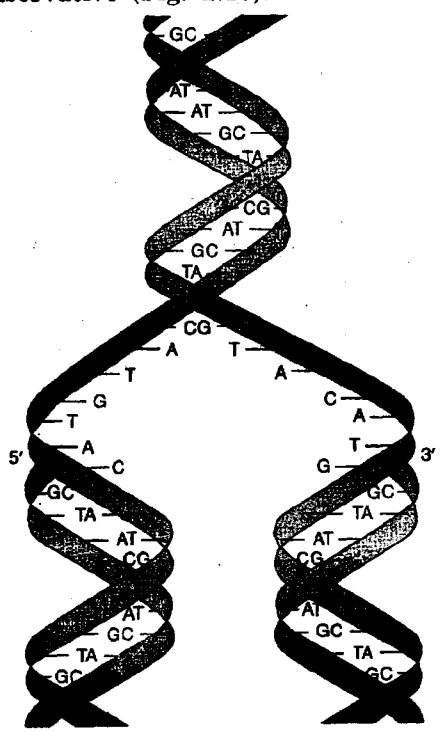


Fig. 2.10. *Watson-Crick model for semiconservative DNA replication.*

NOTES

The Machinery and the Enzymes of DNA Replication

The process of replication is a complex, multi-step process which involves the participation of over a dozen of enzymes and other protein factors. These enzymes are highly efficient enzymes as they have to catalyze polymerization of a large number of nucleotides in a very short time. For instance, in *E. coli* that has only 4.6×10^6 bp, the process of replication completes within 38 minutes. That means the average rate of polymerisation has to be approximately 2000 bp per second. In human beings where diploid content is 6.6×10^9 bp, the process of replication completes in 9 hours 15 minutes. Not only these enzymes to be fast, but they should catalyse the replication with high degree of accuracy. Any mistake during replication would result into mutations. The process of DNA replication involves following steps.

1. Origin of Replication. Replication of DNA begins at a specific point called **origin of replication** or **Ori.** In most bacteria and viruses, there is only one origin of replication and moves bidirectionally. In eukaryotes, with giant DNA molecule, there can be several origins of replication, and these eventually merge with each other once replication is under process. Thus, a prokaryotic DNA acts as a single replicating unit or **replicon**, while a eukaryotic DNA molecule has several replicating segments or replicons.

2. Activation of Deoxyribonucleotides. The deoxyribonucleotide monophosphates-AMP (Adenosine Monophosphate), GMP (Guanosine Monophosphate), CMP (Cytidine Monophosphate) and TMP (Thymidine Monophosphate) occur freely in the nucleoplasm. They serve as raw material in DNA synthesis. They are first phosphorylated and changed into active forms with the utilization of ATP. The reactions are catalysed by enzyme phosphorylase. The phosphorylated nucleotides are ATP (Adenosine Triphosphate), GTP (Guanosine Triphosphate), CTP (Cytidine Triphosphate), and TPP (Thymidine Triphosphate).

3. Unwinding of DNA Helix. The two strands of DNA molecule separate, before each serves as a template for the synthesis of a new strand. It is not easy to separate the interwind strands of a long DNA molecule. It is like separating two interwind ropes by pulling them apart with force. But the moment you stop applying force, the two strands will automatically interwine with each other. At the same time, if you cut partially separated strands, the tension is relieved and the two strands will not wind back together.

Enzymes **helicases** and **topoisomerases** almost work at the same principle, while unwinding the DNA molecule. Enzymes helicases (unwindases) unwind the DNA helix and separate the two strands, while the enzymes topoisomerases (DNA gyrases) can break and reseal one strand of DNA

to facilitate uncoiling. The separated strands are stabilized by **helix destabilizing proteins.** The whole of DNA does not open at one stretch, but the separation of the two strands proceeds slowly from one end to another. It results in the formation of Y-shaped structure called **replication fork.**

4. Formation of RNA Primer. RNA primer is a short segment of RNA which is synthesized at the 5′ end of DNA before replication begins. The enzyme **primase** catalyses the polymerization of RNA building blocks (A, U, G, C) into primer. It is like putting a knot at the end of thread before you make a string of pearls. Formation of RNA primer constitutes the initiation phase of DNA synthesis because without the presence of RNA primer, DNA polymerase cannot initiate the synthesis of a new DNA strand. Later, the primer RNA, is removed and the gap is filled with deoxyribonucleotide to make DNA strand continuous.

5. Base Pairing. The two separated strands in the replication fork function as templates. The deoxyribonucleoside triphosphates come to lie opposite the appropriate nitrogen bases of both the exposed DNA templates according to base pairing rule *i.e.,* ATP opposite T, GTP opposite C, TPP opposite A and CTP opposite G. The two extra phosphate present on the deoxyribonucleotides set free with the help of enzyme **pyrophosphatase** and energy is released. This energy is utilized in the formation of hydrogen bonds between the free nucleotides and nitrogen bases of the templates.

Deoxyribonucleotide triphosphate $\xrightarrow{Pyrophosphatase}$ Deoxyribonucleotide monophosphate + 2 Pi + Energy.

6. Elongation of New Strands. The adjacent deoxyribonucleoside monophosphates attached to two template DNA strands get linked to form replicated DNA strands. The process is catalysed by the enzyme **DNA polymerase III** and requires the presence of Mg^{2+}, ATP (or GTP) and TPP. DNA polymerase III along with other DNA polymerases (I and II) has the capability to elongate an existing DNA strand but cannot initiate the synthesis. All the three DNA polymerases can polymerise nucleotides only in 5′ → 3′ direction bacause they add them at the 3′ end of the sugar molecule. The two strands of DNA run in antiparallel directions, therefore, the two templates provide different ends for replication. Replication over the two templates thus proceed in opposite directions. One strand is formed continuously because its 3′ end is open for elongation. It is called **leading strand**. On the other strand, short DNA segments are formed in the 5′ → 3′ direction due to exposure of a small stretch

NOTES

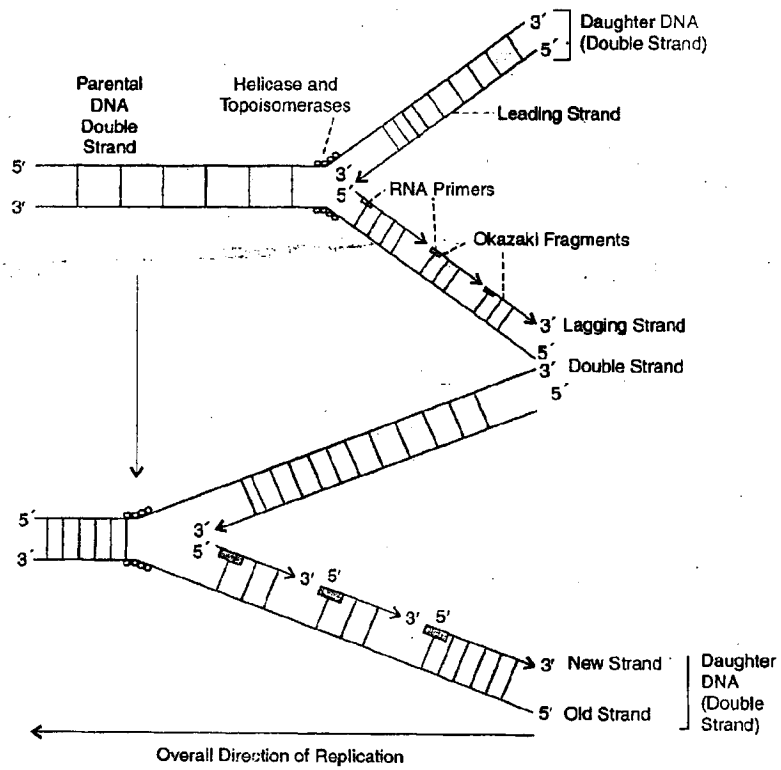


Fig. 2.11. *Continuous and discontinuous synthesis of DNA. One new strand referred to as the leading strand is formed in the 5′ → 3′ direction in a continuous fashion following the movement of fork. The other new strand is formed in small pieces, (referred to as okazaki fragments) and then joined together. The small pieces also grow is 5′ → 3′ direction but the over all direction of the lagging strand in 3′ → 5′.*

of template at one time. The short segments of replicated DNA are called **okazaki fragments or okazaki segments** (named after Japanese scientist, **Okazaki**, who discovered them in 1968). Each of them has 3000–4000 nucleotides. An RNA primer is required every time a new okazaki fragment is to be formed. Such a process is also referred to as semidiscontinuous replication. The RNA primers are then removed and the gaps are filled by DNA synthesis. Both steps are performed by DNA polymerase I. The okazaki fragments are later joined up by the enzyme **DNA ligase (DNA synthetase)**. This strand is called **lagging strand** (Fig. 2.11). This mechanism, where leading strand is synthesized continuously and lagging strand is synthesized discontinuously, is called **semidiscontinuous replication.**

7. Proofreading and DNA-repair. The specificity of base pairing ensures accurate replication. However, sometimes a wrong base may get in. The frequency is one in ten thousand. Bacterial DNA polymerase is able to sense the same. It go back, removes the wrong base and allows the addition of proper base before it proceeds forward.

There is also a repair mechanism for abnormal base pairs formed in the DNA due to mutation, escaping the proofreading mechanism. The damaged region of the DNA strand is corrected with the help of enzymes called **repair enzymes.** Enzyme **endonuclease** causes a break near the region of repair. **DNA polymerase I** and **III** remove the mismatched or abnormal nucleotides and synthesises a correct replacement by using the intact strand as template. The newly formed segment is sealed by **DNA ligase.** Proofreading and repair mechanisms ensures formation of identcal DNA molecules during replication.

2.7 PROTEIN SYNTHESIS

Proteins are synthesised in the cytoplasm. The basic mechanism of protein synthesis is that DNA makes RNA, which in turn makes protein. The central dogma of protein synthesis in expressed as follows:

$$\text{DNA} \xrightarrow{\textit{Transcription}} \text{RNA} \xrightarrow{\textit{Translation}} \text{Protein}$$

Thus, protein synthesis consists of two main events:

1. Transcription, and
2. Translation.

2.8 TRANSCRIPTION

The process of copying genetic information from one strand of the DNA into RNA is called transcription. The process of transcription is also governed by the principle of complementarity. However, here, adanosine forms base pair with uracil instead of thymine. During transcription only a segment of DNA and only one of its strand is copied into RNA. It is because, if both strands are copied, they would code for RNA molecule with different sequences (because complementarity does not mean identical). Consequently, the sequences of amino acids in proteins synthesized by the two strands of a DNA segment would be different. This would complicate the genetic information transfer machinery. Secondly, if two RNA molecules produced simultaneously, they would be complementary to each other and would form a double stranded RNA. This would prevent RNA from being translated into protein, and the process of transcription would be of no use.

Transcription Unit

A transcription unit consists of three regions in DNA. There are— 1. A promoter, 2. The structural gene and 3. A terminator.

The two strands of the DNA in the structural gene forms a transcription unit. As the two strands of DNA have opposite polarity and the DNA-

NOTES

dependent RNA polymerase also catalyze the polymerization in only one direction, *i.e.*, 5′ → 3′. Therefore, the DNA strand that has the polarity 3′ → 5′ acts as a template. It is called **'template strand'.** The other strand which has the polarity 5′ → 3′ and the sequence same as RNA (except thymine at the place of uracil), is displayed during transcription. Strangely, this strand (which does not code for anything) is termed as **'coding strand'.** All the reference point, while defining a transcription unit is made with coding strand. This can be explained by taking a hypothetical sequence from a transcription unit.

3′-ATGCATGCATGCATGCATGCATGC-5′ Template Strand

5′-TACGTACGTACGTACGTACGTACG-3′ Coding Strand

The sequence of RNA transcribed from above DNA will be as under

5′-UACGUACGUACGUACGUACGUACG-3′

The promoter and the terminator regions of a transcription unit are located at either ends of the structural genes. The promoter region is present toward 5′ end (upstream), whereas the termitor region is located toward 3′ end (down stream) of the structural gene (the reference of 5′ end and 3′ end is made with respect to the polarity of coding strand). The terminator region defines the end of the process of transcription. The presence of a promoter in a transcription unit defines the template and coding strands (Fig. 2.12). The switching of promoter with terminator could reverse the definition of coding and template strands. A number of regulatory sequences may be present further upstream or downstream to the promoter.

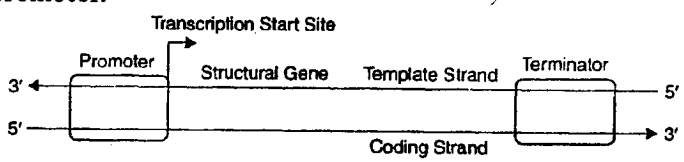


Fig. 2.12. *Schematic structure of a transcription unit.*

Transcription Unit and the Gene

The term 'gene' was coined by **Johannsen** (1909) to replace the term of mendelian factor. According to him *'gene is an elementary unit of inheritance which can be assigned to a particular character.'* The work of **Morgan** on crossing over and recombination suggested that *'gene is a segment of chromosome which can be separated through crossing over and is, therefore a unit of recombination'*. The work on mutation suggested that *'gene is the smallest segment of chromosome which can undergo mutation'*. **Benzer** proposed the terms **muton** for the unit of mutation and **recon** for the unit of recombination. **Beadle** and **Tatum** recognised a gene to be *'a unit of hereditary material that contains information for the synthesis of an enzyme'*. Meanwhile it was found that hereditary material of chromosomes

is DNA. **Yanofsky** *et. al.*, (1965) observed that a gene has information for a single polypeptide. Therefore, gene is often considered as a segment of DNA consisting of a number of deoxyribonucleotide pairs which specifies the synthesis of a single polypeptide. However, DNA not only contains information for the synthesis of polypeptides, through mRNA but for the synthesis of other RNAs (tRNA or rRNA) as well. Therefore, the term gene, nowadays being replaced by **cistron**. Cistron can be defined as a segment of DNA capable of directing the synthesis of a polypeptide chain or a RNA molecule.

While defining a cistron as segment of DNA coding for a polypeptide, the structural gene in a transcription unit could be said as **monocistronic** (mostly in eukaryotes) or **polycistronic** (mostly in prokaryotes such as bacteria). In eukaryotes, the monocistronic structural gene has two types of interspersed coding and non-coding segments called **exons** and **introns** respectively. Such a gene is called **split gene (Roberts** and **Sharp,** 1993). Exons are essential regions of a gene which on transcription form part of mRNA that code for different regions of protein. Introns are non-essential regions of the gene which are also called **junk DNA** or **spacer DNA** or **Intervening Sequence (IVS).**

During the synthesis of mRNA, the junk DNA or introns also get transcribed. Thus a long strand of mRNA is synthesised initially. It contains both unwanted base sequences (transcribed from introns) alternated with useful base seqences (transcribed from exons). Such a strand of mRNA is called **heterogeneous nuclear RNA** (hnRNA) or **pre-messenger RNA.** The unwanted base sequences are now removed by enzymes from hnRNA. The remaining useful base sequences coding for amino acids joint together. This processing of mRNA is known as **splicing.** Thus, the processed mRNA and the polypeptide it codes for are collinear even in the eukaryotes, although the genes are split. The processed mRNA passes out of the nucleus and joins the ribosomes. Here, it is expressed through the synthesis of a polypeptide.

The introns or intervening sequences do not appear in mature or processed RNA. The split gene arrangement further complicates the definition of a gene in terms of a DNA segment. The inheritance of a character is also affected by promoter and regulatory sequences of a structural gene. Hence, sometime the regulatory sequences are loosely defined as **regulatory genes,** even though these sequences do not code of any RNA or protein.

Process of Transcription

The process of transcription involves following steps (Fig. 2.13):

(*i*) *Initiation.* On specific signal, the RNA polymerase binds to promoter and initiates transcription. The promoter region in the cistron is recognised by sigma (σ) subunit of the enzyme RNA polymerase in prokaryotes and by many transcription factors in eukaryotes.

NOTES

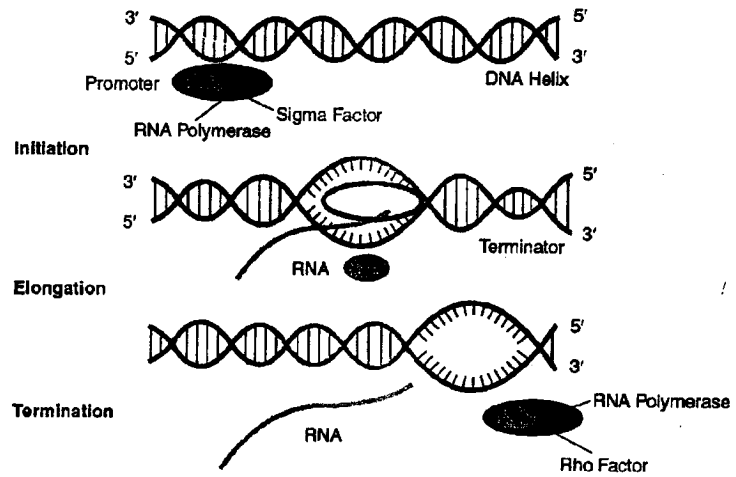


Fig. 2.13. *Process of transcription in bacteria.*

(*ii*) *Elongation*. The RNA polymerase uses ribonucleoside triphosphates as substrate and polymerizes in a template dependent manner following the rule of complementarity. RNA polymerase moves along the transcription unit and causes unwinding and opening of the helix and continues the elongation of RNA. The sigma (σ) factor of the RNA polymerase separates on the initiation of RNA chain. The remaining part of the RNA polymerase, referred as core enzyme moves along the DNA template causing elongation of RNA chain at the rate of some 30 nucleotides per second. Elongation takes place in 5′–3′ direction.

(*iii*) *Termination*. Once the RNA polymerase reaches to the terminator, the nascent RNA fall off and also the enzyme. A terminator factor—Rho (ρ) factor is required for the termination of RNA chain. Unlike the situation in prokaryotes, transcription in eukaryotes occurs within the nucleus and mRNA moves out of the nucleus into the cytoplasm for translation. In eukaryotes, there are two additional complexities :

1. There are at least three RNA polymerases in the nucleus (in addition to the RNA polymerase found in the organelles). There are RNA polymerase I, II and III. The RNA polymerase I transcribes rRNAs (28*s*, 18*s* and 5.8*s*), whereas RNA polymerase III is responsible for transcription of tRNA, RNA, 5*s* rRNA and *sn* RNAs (small nuclear RNAs). The RNA polymerase II transcribes precursor of mRNA (pre-mRNA) or hnRNA (heterogenous nuclear RNA). Thus, there is a division of labour in the functioning of the three types of RNA polymerase.
2. The second complexity is that the primary transcript contains two types of segments, the non-coding **introns** or **intervening sequences** and the coding **exons.** The primary eukaryotic mRNA transcript is much longer and is non-functional. Hence,

it is subjected to a process called **'splicing'**, where the introns are removed and exons are joined in a definite order. Hn RNA (primary mRNA transcript) undergo two additional processing called as **'capping' and 'tailing'.** In capping an unusual nucleotide, **methyl guanosine triphosphate** ($^{m}G_{ppp}$) is added to the 5′ end of hn RNA. In 'tailing' adenylate residues (200–300) are added (polyadenylation) at 3′-end to hn RNA in a template independent manner (*i.e.*, without a template). The fully processed hnRNA is now called as mRNA, that is transported out of the nucleus for translation (Fig. 2.14).

Recently the significance of complexity in eukaryotic gene is being studied. It is believed that the split gene arrangement probably represent an ancient feature of the genome. The presence of introns is reminiscent of antiquity, and the process of splicing represents the dominance of 'RNA world'. As such the RNA and RNA dependent processes have assumed more importance in the living system.

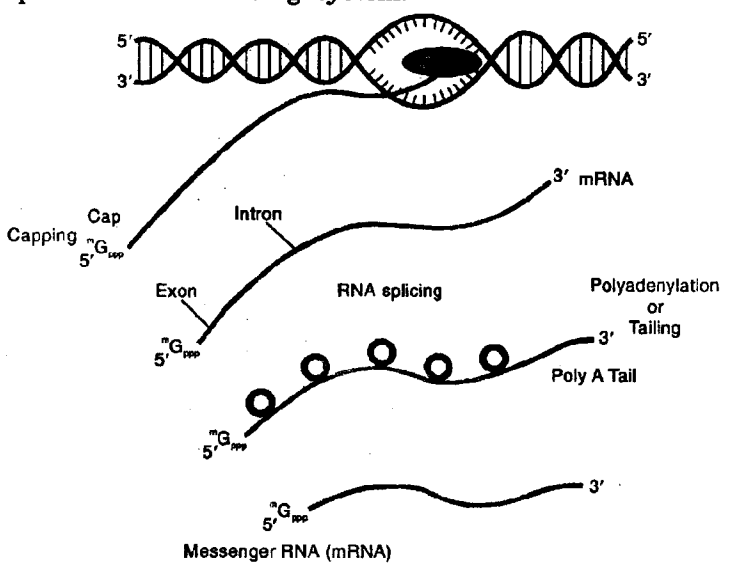


Fig. 2.14. *Process of transcription in Eukaryotes.*

2.9 GENETIC CODE

The process of translation requires transfer of genetic information from a polymer of nucleotides to a polymer of amino acids. But, neither there exists any complementarity between nucleotides and amino acids, nor could be drawn theoretically. However, there were enough evidences which support the view that change in nucleic acids (genetic material) were responsible for change in amino acids in proteins. This led to the proposition of a genetic code that could direct the sequence of amino

NOTES

acids during synthesis of proteins. *'The relationship between the sequence of amino acids in a polypeptide and nucleotide sequence of DNA or mRNA is called* **genetic code'**. The process by which the information coded in mRNA is decoded into a polypeptide is one of the exciting discoveries of biology. It is often referred to as deciphering the genetic code.

It was **George Gamow** (1954), a physicist, who argued that since there are only 4 bases and if they have to code for 20 amino acids, the code should constitute a combination of bases. In order to code for all the 20 amino acids, he suggested that the code should be made up of three nucleotides (**triplet code**). The permutation and combination of three nucleotides 4^3 (4 × 4 × 4) would generate 64 codons. Proving that codon was triplet (*i.e.*, three nucleotides) was quite challanging task. But the chemical method developed by **Har Govind Khurana** for synthesizing RNA molecules with defined combinations of bases (homopolymers and copolymers), and **Marshall Nirenberg's** cell free system for protein synthesis finally helped the genetic code to be deciphered. The discovery of enzyme-polynucleotide phosphosylase, by **Severo Ochoa** was also helpful to polymerise RNA with defined sequences in template independent manner (*i.e.*, enzymatic synthesis of RNA without a template). Finally a checker board for genetic code (Fig. 2.15) was prepared which is as follows.

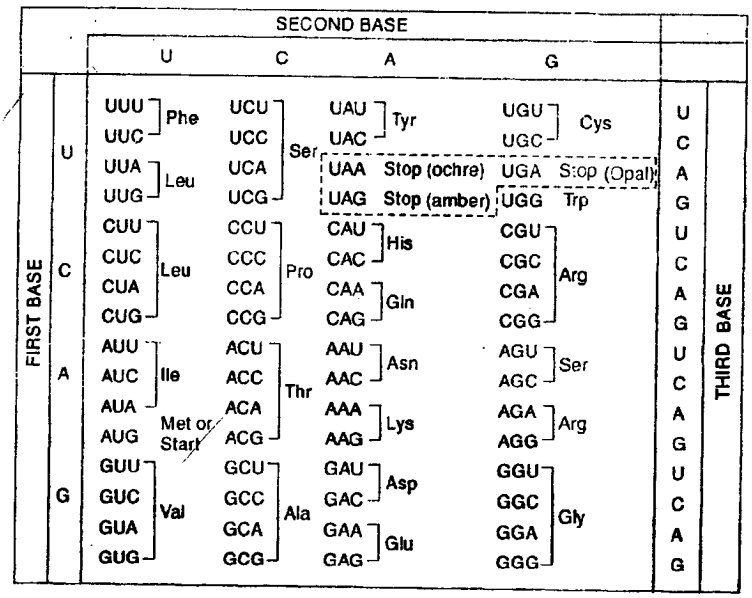


Fig. 2.15. *Genetic code. The first, second and third bases as read from 5¢ to 3¢ direction constitute the triplet code in RNA. The codon AUG specifies methionine and is usually the starting point for protein synthesis. The word 'stop' indicates codons serving as signals to terminate protein synthesis. For each amino acid more than one codon have been identified. It would be clear from the Figure that while the first and second bases remain the same for a particular amino acid, the third base can be different.*

NOTES

Characteristics of Genetic Code

1. Triplet Code. The genetic code is a triplet code. Three adjacent nitrogen bases constitute a codon, which specifies the placement of one amino acid in a polypeptide.

2. Non Overlapping. The adjacent codons do not overlap. Each single base is a part of only one codon.

3. Commaless. The genetic code is continuous and does not possess pauses after triplets. Reading of the code begins at a fixed point and continues three nucleotides at a time, without a pause till the terminator codon.

4. Universality. The genetic code is universal, *i.e.*, a given codon in the DNA and mRNA specifies the same amino acid in the protein synthesizing systems of all organisms.

5. Non-sense or Terminator Codons. Three of the 64 codons, namely UAA (ochre), UAG (amber) and UGA (opal) do not specify any amino acid but signal the end of the message. They are called **non-sense** or **terminator codons.** Either of these stops synthesis of the polypeptide chain.

6. Initiation or Start Codons. The codons AUG and GUG code for methionine and valine respectively, also act as start signal (dual functions). They initiate the synthesis of polypeptide chain, hence are called **initiate** or **start codons.**

7. Degeneracy. The genetic codons are degenerate codons *i.e.*, they lack specificity and one amino acid often has more than one code triplet, only methionine and tryptophan have single triplet codons. All other amino acid are specified by 2–6 codons. Such codons are called degenerate codons. In degenerate codons the first two nitrogen bases are similar, while the third one is different. The third nitrogen base has no effect on coding, and it permit interaction with the anticodon on the corresponding tRNA. This position on the codon is known as the **wobble position.**

8. Colinearity. DNA is a linear polynucleotide chain and a protein is a linear polypeptide chain. The sequence of amino acids in a polypeptide chain corresponds to the sequence of nucleotide bases in the DNA (gene) that code for it. Change in specific codon in DNA produce a change of amino acid in the corresponding position in the polypeptide. The gene and the polypeptide it codes for are said to be **colinear.**

9. Gene Polypeptide Parity. A gene or a portion of DNA called **cistron** specifies the synthesis of a particular polypeptide. It means that the genetic system should have as many cistrons (genes) as the types of polypeptides found in the organism.

If the sequence of nucleotide in an mRNA is known, the sequence of amino acids coded by it can be predicted by taking the help of checker board of genetic code.

NOTES

Let us find out the sequence of amino acids for the transcript of an mRNA given below.

AUG UUU UUC UUC UUU UUU UUC

The sequence of amino acids coded by the above transcript will be—
Met-Phe-Phe-Phe-Phe-Phe-Phe

Similarly, the sequence of nucleotides in an mRNA transcript can be predicted, if the sequence of amino acids in a polypeptide chain is known.

Exceptions in Genetic Code

1. Overlapping Genes. Certain bacteria and animal viruses have a few overlapping genes. In $\phi \times 174$ virus, three genes E, B and K overlap other genes. Nucleotide sequence at the beginning of E gene is contained within gene D. Likewise gene K overlaps with gene A and C. A similar condition is found in SV-40.

2. Mitochondrial Genes. The codons AGG and AGA which code for arginine, act as stop signals in human mitochondria. UGA, a termination codon codes for tryptophan while the codon AUA (for isoleucine) denotes methionine in human mitochondria.

3. Different Codons. In *Paramecium* and other ciliates termination codons UAA and UGA code for glutamine.

DNA, The Master Copy

DNA is called the master copy of genetic information, because it is not directly involved in the formation of polypeptide. DNA is carefully preserved in the nucleus and is used in the formation of the **working copies** in the form of mRNA molecules. The latter pass out to the ribosome in the cytoplasm to guide the synthesis of polypeptide.

2.10 MUTATION AND GENETIC CODE

During DNA replication, the fidelity (*i.e.,* correct base pairing) maintained and any error that might occur due to wrong base pairing, is corrected through proofreading and repair mechanisms. Still, sometimes base changes occur in DNA due to environmental mutagens such as UV rays, X-rays and certain chemicals. Such changes called mutations are reflected in the structure and functions of proteins.

The mutations that affect protein structure and functions belong to three categories:

(*i*) gene or point mutation, which involve addition, deletion or replacement of a base pair in a gene.

(*ii*) addition or deletion of DNA chunks during crossing over and recombination under special conditions. and

(*iii*) jumping genes which shuffle their position from one chromosome to another due to similarity of DNA sequence flanking them.

The effect of all these mutations are reflected in the proteins. This is because the mRNA derived from gene will lack a base or a segment or will have an altered base. The abnormal mRNA will introduce different amino acids in the polypeptide synthesized with its altered message. If a single base is altered in the gene, only one codon in mRNA will change and this will change a single amino acid in the polypeptide. If the mutation, involves loss or addition of a single base or a segment of DNA, then the entire reading frame will change from the site of mutation. This will result in a protein with a new set of amino acids. This is called **frameshift mutation.**

Let us understand, the effect of point mutations that insests or deletes a base in a structural gene. Consider the following statement consisting of words each having three letters like genetic code.

RAM HAS RED CAP

If we insert a letter 'B' in between 'HAS' and 'RED' and rearrange the statement, it would read as follows :

RAM HAS BRE DCA P

Similarly if we insert now two letters at the same place, say BI'. Now it would read,

RAM HAS BIR EDC AP

Now we insert three letters together, say BIG, the statement would read

RAM HAS BIG RED CAP

The same exercise can be repeated, by deleting the letters R, E, and D, one by one and rearranging the statement to make a triplet word.

RAM HAS EDC AP

RAM HAS DCA P

RAM HAS CAP

In some instances, mutation may change a normal codon to a non-sense codon, causing termination of the polypeptide synthesis. This will form an incomplete polypeptide.

There are also some cases in which a single base change may not lead to a change in amino acid. This happens when the mutation takes place in the third base of the triplet codon, which still permits interaction with the anticodon on the corresponding tRNA. This phenomenon is called **wobble hypothesis** of crick. It states that the first two bases of the *t*RNA anticodon undergo hydrogen bonding specifically with the first two bases of the mRNA codon but the third base can undergo

NOTES

unusual base pairing *i.e.*, it can "wobble". The third position on the codon is, therefore, called **wobble position.** The mutation that does not cause any change in the proteins is called **silent mutation.**

The disease **sickle cell anaemia** in human beings is caused due to a single base change in β chain of haemoglobin at the position 6, where amino acid glutamic acid is replaced by valine. **Thalassemia** is another haemoglobin based genetic disorder. One type of Thalassemia is caused by frame shift mutation in haemoglobin β chain. Cancers are often observed to be related to chromosomal delection and translocation.

Role of tRNA as an Adapter Molecule

Francis Crick (1961), who contributed significantly in deciphering the genetic code, suggested that there has to be a mechanism to read the code and also to link it to the amino acids, because amino acids have no structural uniqueness to read the code uniquely. He postulated an adapter molecule that would on one hand read the code and on other hand would bind to specific amino acids. It was found to be tRNA, which acts as an **adapter molecule.** tRNA has an 'anticodon loop' that has bases complementary to the code, and it also has an 'amino acid accepter end' to which it binds to amino acids. tRNAs are specific for each amino acids (Fig. 2.16). For initiation, there is another specific tRNA that is referred to as initiator tRNA. There are no tRNAs for stop codons.

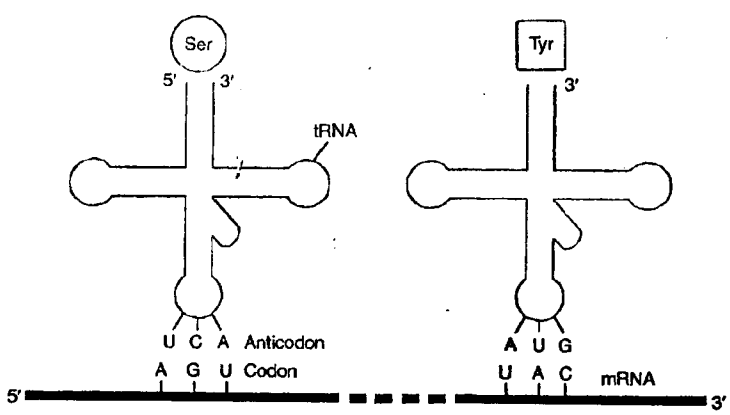


Fig. 2.16. *tRNA—the adapter molecule.*

2.11 TRANSLATION (mRNA TO PROTEIN OR PROTEIN SYNTHESIS)

Translation refers to the process of polymerisation of amino acids to form a polypeptide. The order and sequence of amino acids are defined by the sequence of mRNA. The amino acids are jointed by a bond known as **peptide bond.**

The synthesis of protein is a complex process and involves following steps:

NOTES

1. Activation of Amino Acids. There are some 20 different kinds of amino acids which constitute building blocks or monomers of proteins. Though all types of proteins are formed from the same amino acids but the number and the arrangement of amino acids in the proteins provide specificity to the latter.

Amino acids react with ATP to form **amino acid-AMP-complex** and inorganic phosphates. The reaction is catalysed by a specific amino acid activating enzyme called *amino acyl-tRNA synthetase* in presence of Mg^{2+}. The amino acid AMP complex remains temporarily associated with the enzyme. The amino acid AMP enzyme complex is called an **activated amino acid.** It reacts with tRNA specific for the amino acid to form amino acyl tRNA complex. The enzyme and AMP are released. The tRNA complexed with amino acid is called charged tRNA.

$$\underset{\text{(Amino acid)}}{\text{AA}} + \text{ATP} + \underset{\substack{\text{(Amino acyl}\\ \text{tRNA synthetase)}}}{\text{Enzyme}} \xrightarrow{Mg^{2+}}$$

$$\text{AA—AMP—Enzyme} + \underset{\text{(Pyrophosphate)}}{\text{PPi}}$$

$$\text{AA} - \text{AMP} - \text{Enzyme} + t\text{RNA} \rightarrow \underset{\substack{\text{(Amino acyl tRNA}\\ \text{complex)}}}{\text{AA} - \text{tRNA}} + \text{AMP} + \text{Enzyme.}$$

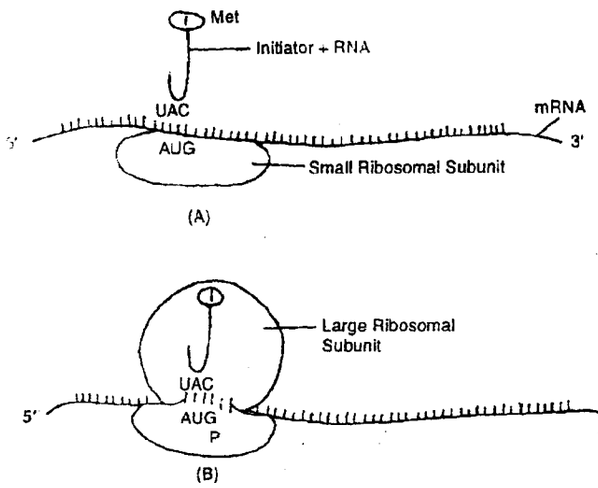
2. Attachment of mRNA. mRNA attaches itself to smaller subunit of ribosome in the region of tunnel. Its cap has nucleotides complementary to the nucleotides present on rRNA. The combination is such that initiation codon (AUG) on mRNA comes to lie at P site (peptidyl transfer side on the ribosome, which is jointly contributed by the two ribosomal subunits). A protein factor called **initiation factor-3** is required in this process.

3. Initiation of Polypeptide Chain (Fig. 2.17). The synthesis of protein or polypeptide chain starts with amino acid methionine and, therefore, methionyl-tRNA ($tRNA_f^{met}$) charged with methionine, binds to the initiator codon on mRNA at the P site. Since the codon for methionine is AUG, the $tRNA_f^{met}$ would have UAC at the anticodon site. The anticodon of ($tRNA_f^{met}$) establishes temporary hydrogen bonds with the initiator codon on mRNA. This process occurs in presence of **initiation factor-2.** It also requires energy which is provided by GTP. The initiation factors involved in attachment and initiation processes are designated as **IFs** in prokaryotes and **eIFs** in eukaryotes.

prokaryotes, elongation factors are called **EF-Tu, EF-Ts** and **EF-G.** Eukaryotes require a more complex set of accessory factors. The GTP is hydrolysed to GDP and inorganic phosphate to release energy for the process. As a result of translocation the third codon comes into the A site and an appropriate tRNA charged with a third amino acid would bind at the A site. The process of peptide bond formation and translocation would be repeated. Thus, as the mRNA moves with respect to the ribosome,

NOTES

NOTES



Steps in protein synthesis (A) Initiation : The small ribosomal sub-unit binds to the presence of initiation factors. The interaction is facilitated by a ribosomal RNA recognition sequence on the messenger RNA. At the same time, the initiator methionyl-tRNA interacts with the messenger RNA through codon-anticodon interaction. (B) The large ribosomal subunit binds and an initiation complex is thus formed. The initiator methonyl-tRNA is located at the P-site on the ribosome.

The amino acid is linked to the tRNA through its carboxyl group and the $—NH_2$ group of the first amino acid (methionine) is formylated in prokaryotic systems. This would block the $–NH_2$ group of the first amino acid from getting into peptide bond formation. The tRNA used to introduce methionine at the initiation site ($tRNA_f^{met}$) is different from the one that inserts methionine elsewhere in the protein chain. With the initiator tRNA at the P site, the large ribosomal subunit now attaches to the complex and the intact ribosome (active ribosome) is formed.

4. Elongation (Polypeptide chain formation ; Fig. 2.18). A second tRNA charged with an appropriate amino acid enters the ribosome at the A site. Its anticodon binds to the complementary codon of mRNA chain by hydrogen bonds. A peptide bond is formed between the first amino acid (methionine) and the second amino acid. The reaction is catalysed by the enzyme **peptidyl transferase.** The 23S rRNA of large subunit has been assigned this catalytic function. In this process, the linkage between methionine and its tRNA is broken and the —COOH group now forms a peptide with the free $—NH_2$ group of the second amino acid. Thus, the second tRNA bound at the A site (amino acyl or acceptor site, situated on the larger subunit of the ribosome and faces the tunnel between the two subunit.) carries a dipeptide (a peptide formed with two amino acids) and the first methionyl tRNA is removed from the P site. Now, the tRNA at the A site is pulled to the P site along with the mRNA. This process is called **translocation.** All these steps require GTP and a number of proteins called **Elongation Factors (EFs).** In

NOTES

Protein Translocation

When protein synthesis is terminated, the ribosomal subunits, fall off the mRNA. In case of free cytoplasmic polyribosomes, the polypeptides or proteins are released into the cytoplasm (cytosol). These proteins remain in the cytoplasm and are employed in the synthesis of more cytoplasm and other cellular components. Some specific proteins are translocated into mitochondria and nucleus through special mechanisms. In the case of membrane bound polyribosomes, the polypeptide chain, as it grows on the messenger RNA, is inserted into the lumen to the endoplasmic reticulum. Some of the proteins get integrated into the membrane and thus become integral membrane proteins. Some others are released into the

STUDENT ACTIVITY

1. Give a schematic representation of Kreb's cycle.

2. Give a brief account of glycogenesis.

NOTES

2.12 SUMMARY

- Proteins are among the most important macromolecules of organisms and are considered to be the basis of cellular organisation and functions that impart biological specificity to the organism. They are highly organised linear polymers of amino acids.
- Each one of the amino acids undergoes its own metabolism and performs specific functions. Thus, protein metabolism is more appropriately learnt as metabolism of amino acids.
- Amino acid metabolism includes synthesis and degradation of amino acids. Amino acids are synthesised from keto acids produced during the respiratory breakdown of sugars by the process of animation at α-carbon of keto group. The catabolism of amino acids involves the removal of $—NH_2$ group and formation of corresponding keto acid. The released ammonia is converted to urea in the liver, while keto acids are oxidised through acetoacetate or acetyl CoA.
- Nucleic acids are polymeres of nucleotides. Each nucleotide is made of a nitrogen base, pentose sugar and a phosphate. The sugar molecule in a nucleotide may be ribose or deoxyribose. The nitrogen bases are of two types—purines and pyrimidines. Purines are of two types—adenine and guanine, while pyrimidines are of three types—thymine, cytosine and uracil. A nitrogen base joins to the sugar molecule to form a nucleoside. The nucleotides are phosphoric acid esters of nucleosides.
- Replication of DNA takes place semiconservately, in which the two strands act as templates to allow the synthesis of new strands, which have a complementary base sequence. Replication is achieved with the help of several enzymes, and starts at a specific point, known as origin of replication.
- Protein synthesis consists of two steps : transcription and translation. Transcription leads to the synthesis of three species of single stranded RNA under the guidance of the enzyme RNA polymerase from a DNA template. The three types of RNA have different functions in protein synthesis. The mRNA carries the message from the gene or the DNA. rRNA along with some proteins constitutes the ribosomes, the site at which translation takes place and tRNA transfers the required amino acid to the ribosome during translation. Both, transcription and translation involve initiation, elongation and termination, and like replication rely on complementary base pairing.
- The genetic information is stored in the DNA in the form of a code, which is triplet, degenerate unambiguous, non-overlapping

and commaless in nature. The complete code dictionary consists of 64 possible codes, of which 61 codons code for 20 essential amino acids including the initiation codon, and 3 are meant to terminate translation. Any mutational change may result into a new codon coding for different amino acids.

2.13 GLOSSARY

- **Proteins:** Proteins are nitrogen containing macromolecules consisting of highly organised linear polymers of amino acids.
- **Nucleic Acids:** Nucleic acids are polymeres of nucleotides, and hence, may be called polynucleotide sequences.
- **Nucleotide:** It is made up of three small chemicals—a pentose sugar, a cyclic nitrogenous base and one to three phosphate groups.
- **Nitrogenous:** A nitrogenous base is linked to the pentose sugar through a N-glycosidic linkage to form a nucleoside.
- **Trancription:** The process of copying genetic information from on strand of the DNA into RNA is called transcription.
- **Genetic Code:** The relationship between the sequence of amino acids in a polypeptide and nucleotide sequence of DNA or mRNA is called genetic code.
- **Silent Mutation:** The mutation that does not cause any change in the proteins.

2.14 REVIEW QUESTIONS

I. Very Short Answer Type Questions:

1. What are proteins?
2. Why is protein metabolism more appropriately learnt as metabolism of amino acids?
3. What is a codon?
4. What is an anticodon?
5. What is genetic code?
6. Of the 64 code triplets, how many code for amino acids and how many for stop signals?
7. Name the enzymes that catalyses.
   (*i*) Replication of DNA (*ii*) Formation of RNA
   (*iii*) Joining of okazaki segments.
8. If one strand of double stranded DNA has the following sequence.
   5′ ..... ACCATTCG ..... 3′
   What would be the sequence of the opposite strand in its 5′ – 3′ direction?

NOTES

II. Short Answer Type Questions:

1. Explain the pathway of urea formation in liver.
2. Differentiate between
   (*i*) Codon and anticodon
   (*ii*) Leading strand and lagging strand.
   (*iii*) Transcription and translation.
3. Enlist the role of the following in protein synthesis.
   (*i*) mRNA (*ii*) rRNA (*iii*) tRNA
   (*iv*) Ribosomes (*v*) Amino acids (*vi*) ATP.
4. Given below a transcribing strand of the DNA duplex.
   3′ ..... TAC CGA TCC GAG CTG ..... 5′
   (*i*) Draw the complementary DNA polynucleotide chain.
   (*ii*) Construct the RNA molecule, which will be transcribed.
5. Given below in sequences of the processed mRNA ready for translation
   5′—AUG CUA UAC CUC CUU UAU CUG UGA ..... 3′
   (*i*) How many amino acid residues will make up the polypeptide corresponding to this mRNA?
   (*ii*) How many different tRNA molecules would be necessary to translate this mRNA?

III. Long Answer Type Questions:

1. Give an account of synthesis of amino acids.
2. Describe the process of DNA replication.
3. Describe the process of transcription.
4. Give the account of biosynthesis of pyrimidine monophosphates.
5. Describe the process of synthesis of purine monophosphate.
6. Describe the process of translation with reference to initiation, elongation and termination.

2.15 FURTHER READINGS

- **Nutrition and Dietetics;** Joshi, Shubangini A; Tata McGraw Hill, New Delhi; 1992.
- **Fundamentals of Food and Nutrition;** Mudumbi; S.R. and Rajagopal, M.U; New Age Int. Pub. New Delhi; 2008.
- **Outlines of Biochemistry;** Conn E.E., Stumpf P.K., Bruening G., Doi R.H; Wiley India (P) Ltd. New Delhi; 2007.

CHAPTER 3 LIPID METABOLISM

NOTES

OBJECTIVES

After going through this chapter, you should be able to:

- describe hydrolysis of fats
- explain glyoxylate cycle
- know about synthesis of fats.

STRUCTURE

3.1 INTRODUCTION

Lipids are stored in large amounts as neutral, highly insoluble triacylglycerols in both plants and animals. They can be metabolized and degraded to meet the energy need of the cell. In fact, much of the carbohydrates present in the diet are first converted into triglyceride (triacylglycerol), before being utilized for energy production. Triacylglycerol is the most concentrated form in which potential energy is stored. It has definite advantages over carbohydrates or proteins particularly in its caloric value which is more than double in lipids as compared to carbohydrates or proteins. Moreover, fatty acids upon oxidation yield more metabolic water than other metabolic fuels thereby solving much of the water problem of mammals living in the terrestrial environment. The metabolic interrelationships among lipids and their relationship to carbohydrate metabolism is depicted in Fig. 3.1.

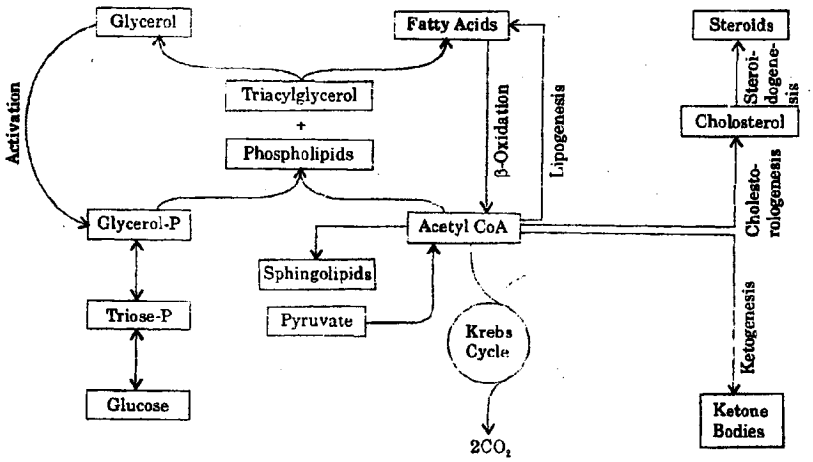


Fig. 3.1. *Principal pathway of lipid metabolism.*

3.2 HYDROLYSIS OF FATS

Fat deposits are not stationary *i.e.,* lipids are constantly being metabolized and deposited. Normally, the quantity of body lipids is kept constant over long periods of time by regulation of appetite by an unknown mechanism. When stress conditions develop in the animal such as starvation, prolonged exercise, or rapid fear responses in terms of violent exercise, adrenalin from the blood stream binds to a specific receptor in the fat cell surface and triggers a response as shown in Fig. 3.2.

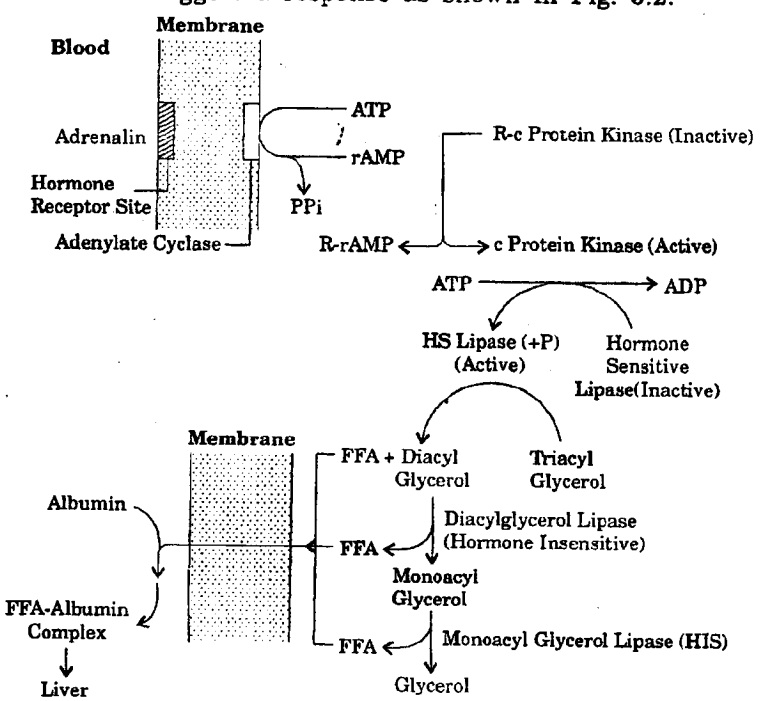
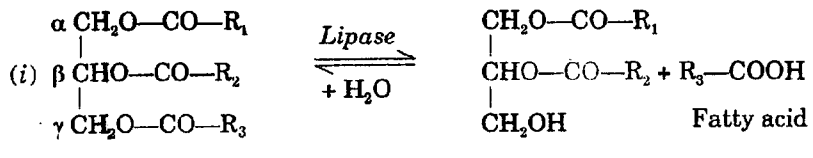
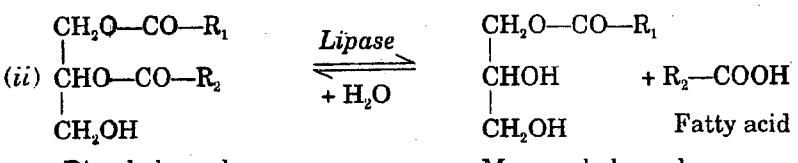


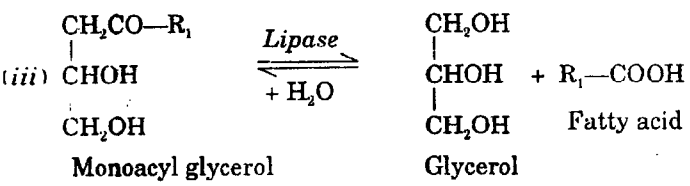
Fig. 3.2. *Events of fat catabolism in the cells of adipose tissue.*

A hormone sensitive *lipase* is activated, rapidly converting triacylglycerols to diacylglycerols and Free Fatty Acids (FFA).

Triglycerides which form the major lipid sources are essentially fatty acid esters of glycerol. The enzyme hydrolyze them by splitting the ester bonds to free the fatty acids from glycerol moiety.

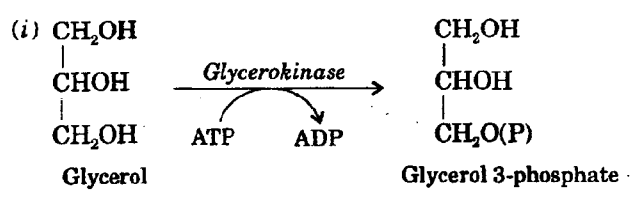


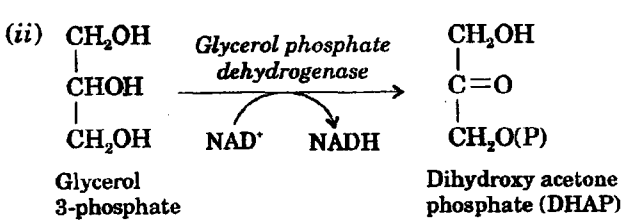




Breakdown of Glycerol

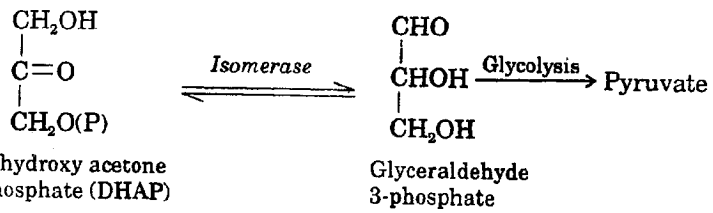
Glycerol formed by the action of *lipase* on fats is phosphorylated and oxidised to dihydroacetone phosphate, which in turn is isomerized to glyceraldehyde 3-phosphate. The glyceraldehyde 3-phosphate is converted to pyruvate through glycolytic pathway.





NOTES

(*iii*)



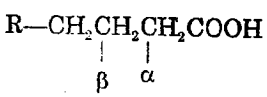
Dihydroxy acetone Phosphate (DHAP)

Glyceraldehyde 3-phosphate

Oxidation of Fatty Acids

Oxidation of fatty acids take place in the inner mitochondrial compartment of the cells of liver, kidney, heart and various other tissues. The fatty acids are oxidised to CO_2 and H_2O, accompanied with the production of large amount of ATP, NADH and $FADH_2$.

There are two mechanism of oxidation of fatty acids : β-oxidation and α-oxidation. The names β and α, indicate the position of carbon atom which is oxidised first. The position of β and α carbon is given as:



1. β-oxidation (Beta Oxidation)

β-oxidation of fatty acids is the primary pathway of fatty acid oxidation in plants and animals. It was first reported by F. knoop (1904). The oxidation produces two carbon acetate unit as acetyl CoA and releases $FADH_2$ and NADH. A summary reaction of oxidation of a fatty acid, palmitate is given as under.

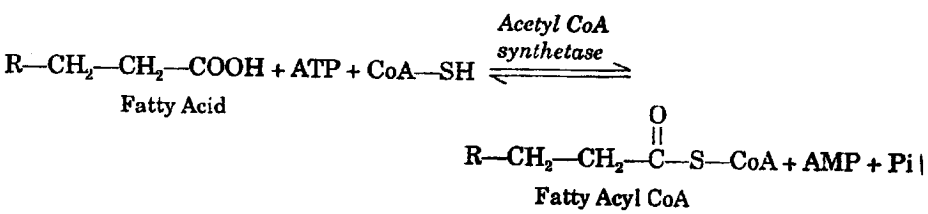
Palmitate + ATP + $7NAD^+$ + 7FAD + $7H_2O$ + 8CoASH

$\longrightarrow$ 8 Acetyl CoA + AMP + Pyrophosphate + 7 NADH + $7H^+$ + 7 $FADH_2$

In the oxidation process, the removal of 2 carbon fragments, acetyl CoA, takes place in a sequence. At the end of each series of reactions, one acetyl CoA is removed.

The reactions occur as follows :

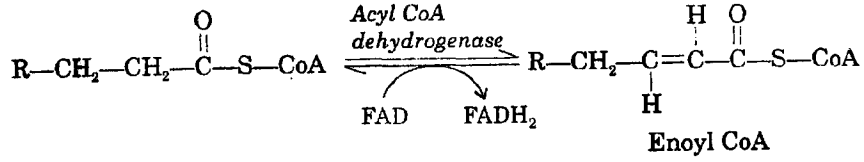
(*i*) First the fatty acid is activated with ATP to form its CoA derivative in presence of enzyme *acetyl CoA synthetase.*



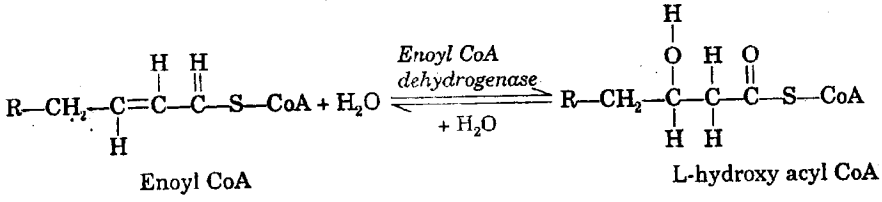
This activation reaction occurs on the outer mitochondrial membrane. The activated fatty acid diffuses into the mitochondria for its further oxidation.

NOTES

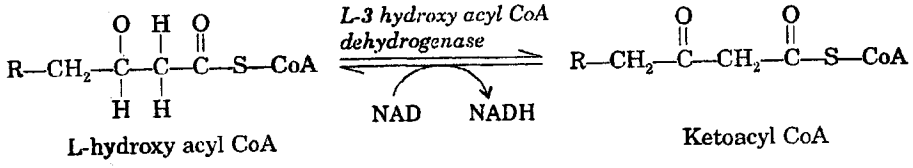
(*ii*) The acyl CoA undergoes dehydrogenation to give rise enoyl CoA. The reaction is catalysed by the enzyme *acyl CoA dehydrogenase* and FAD is reduced to $FADH_2$.



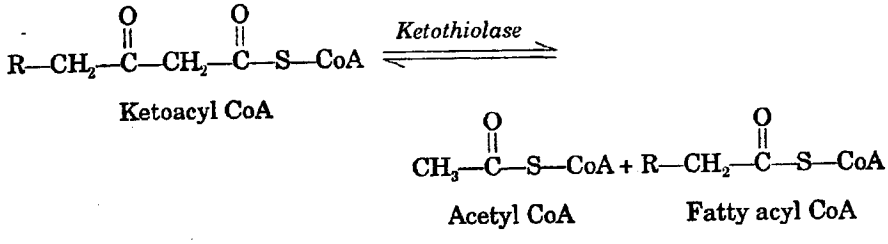
(*iii*) In the next step, the enoyl CoA is hydrolysed to hydroxy acyl CoA, with the help of enzyme *enoyl CoA hydratase.*



(*iv*) In the next step, the hydroxyl group is converted to ketogroup to produce ketoacyl CoA in presence of enzyme *L-3 hydroxy acyl CoA dehydrogenase.* NAD is reduced to NADH in the reaction.



(*v*) In the last step of removal of 2 carbon fragment of the fatty acid, the ketoacyl CoA is cleaved by the thiol group of a second molecule of CoA, to yield acetyl CoA and a fatty acid CoA, which is shortened by two carbon atom. The reaction is catalysed by the enzyme *ketothiolase.*



The shortened fatty acyl CoA then undergoes another cycle of oxidation involving all the steps (*ii*) to (*v*). In each cycle, an acyl CoA is produced which is short by two carbon atoms and one molecule of each of $FADH_2$, NADH and acetyl-CoA are also produced. The general reaction of shortening of carbon chain can be represented as follows.

$$C_n \text{ acyl CoA} + FAD + NAD^+ + H_2O + CoA \longrightarrow C(n-2) \text{ acyl CoA} + FADH_2 + NADH + H^+ + \text{acetyl CoA.}$$

NOTES

Various steps of β-oxidation are shown diagrammatically in Fig. 3.3.

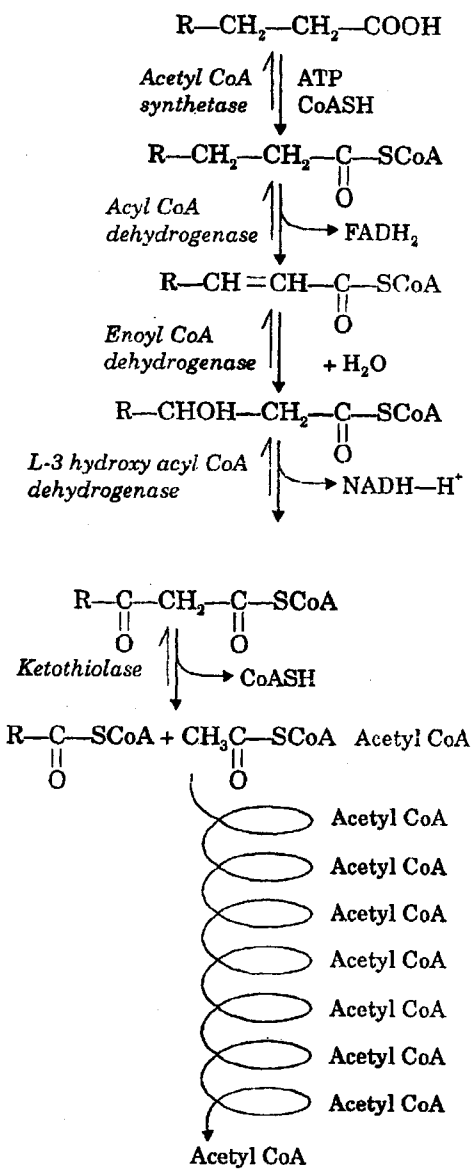


Fig. 3.3. *Scheme of β-oxidation of fatty acid.*

The degradation of a 16 carbon acyl CoA (*e.g.*, palmitoyl CoA) requires seven reaction cycles. In the seventh reaction, the 4 carbon keto acyl CoA is cleaved to two molecules of acetyl CoA. The energy yield in terms of ATP in complete oxidation of palmitic acid molecule is given in Table 3.1.

Table 3.1. ATP Yield During Oxidation of Palmitic Acid

S. No.	Molecules Produced during Oxidation	Quantity	ATP Production
1.	$FADH_2$ in seven turns of reaction cycle (oxidation of each mole of $FADH_2$ produces 2 ATP)	7	7 × 2 = 14
2.	NADH in seven turns of reaction cycle (oxidation of each mole of NADH produces 3 ATP)	7	7 × 3 = 21
3.	Acetyl CoA in seven turns of reaction cycle (oxidation of each mole of acetyl CoA through TCA Cycle produces 12 ATP)	8 —	8 × 12 = 96
			= 131
4.	Total ATP consumed in activation of fatty acid (ATP split into AMP + 2 Pi)		− 2
Net gain in complete oxidation of one molecule of palmitate = 129 ATP			

2. α-oxidation

α-oxidation is an alternate mechanism of oxidation of fatty acids, in which the acid is oxidised to produce another acid with one carbon atom less. In the reaction one molecule of CO_2 is eliminated. The reaction results in the production of fatty acids with odd number of carbon atoms. The reaction takes place as follows :

(*i*) In the first step, the fatty acid is oxidatively decarboxylated, utilizing H_2O_2 by the enzyme *fatty acid peroxidase.* The product is an aldehyde with one carbon less than the fatty acid and CO_2.

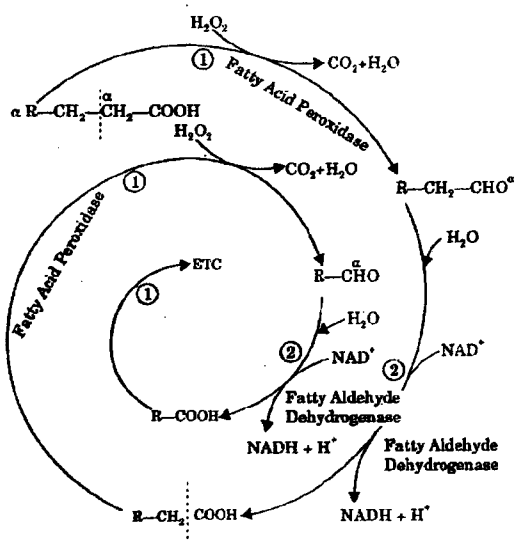


Fig. 3.4. *α-Oxidation of fatty acids.*

NOTES

$$R(CH_2)_nCH_2—COOH + H_2O_2 \xrightarrow{\text{Fatty acid peroxidase}} R(CH_2)_n\,CHO + CO_2 + H_2O$$

(*ii*) Now the aldehyde is oxidised with NAD^+ in the presence of enzyme *fatty aldehyde dehydrogenase.* NAD^+ is reduced to NADH + H^+ and a fatty acid with one carbon atom less is formed.

$$R(CH_2)_2CHO + NAD^+ + H_2O \xrightarrow{\text{Fatty aldehyde dehydrogenase}} R(CH_2)_nCOOH$$

The new acid becomes the substrate for the enzyme *fatty acid peroxidase* and takes another turn in α-oxidation spiral (Fig. 3.4). This type of oxidation is found in plants especially in oily seeds.

3.3 GLYOXYLATE CYCLE

Fats are degraded to form fatty acids and glycerol. Glycerol undergoes glycolysis. Fatty acids form acetyl CoA by the process of β-oxidation. The function of glyoxylate cycle is to convert two molecules of acetyl CoA to succinic acid (succinate). β-oxidation and glyoxylate cycle occur in glyoxysomes. The glyoxylate produced in glyoxylate cycle keeps the cycle operating but succinate is exported to mitochondria for further processing (Fig. 3.5 and 3.6). These all changes are taking place in glyoxysomes.

Further changes occur in mitochondria where succinic acid finally forms oxaloacetic acid (Fig. 3.5). Glyoxylic acid in glyoxysome can combine with another molecule of Acetyl CoA to form malic acid. Oxaloacetic acid is decarboxylated to form Phosphoenol Pyruvic Acid (PEP) in presence of ATP. PEP can form glucose and fructose by reversal of glycolysis.

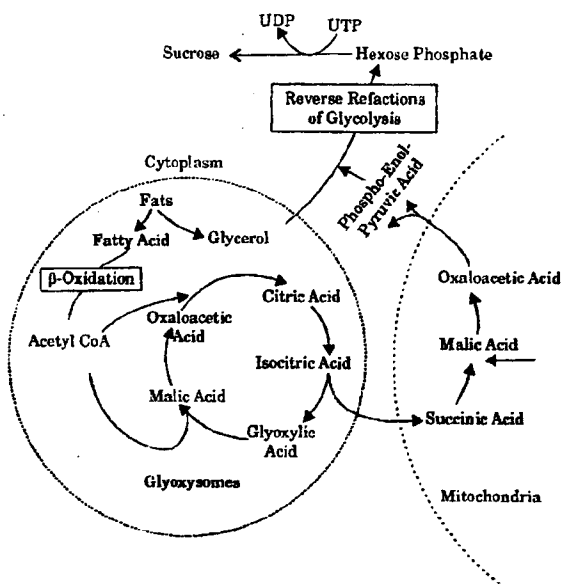


Fig. 3.5. *Glyoxylate cycle.*

Reactions can be given as under:

$$\text{Isocitric acid} \xrightarrow{\text{Isocitratase}} \text{Succinic acid} + \text{Glyoxylic acid}$$

Succinic acid follows the normal Krebs cycle. Glyoxylic acid combines with acetyl CoA to form malic acid.

$$\text{Glyoxylic acid} + \text{Acetyl CoA} + H_2O \xrightarrow{\text{Malate synthetase}} \text{Malic acid} + \text{CoA}$$

Malic acid forms pyruvic acid by two methods.

(*a*) **Ochoa reaction.** By oxidative decarboxylation

$$\text{Malic acid} + \text{NADP} \longrightarrow \text{Pyruvic acid} + NADPH_2 + CO_2$$

(*b*) **Wood Workman reaction.** By oxidation to oxaloactic acid followed by reductive decarboxylation.

$$\text{Malic acid} + \text{NAD} \longrightarrow \text{Oxaloacetic acid} + NADH_2$$

$$\text{Oxaloacetic acid} \longrightarrow \text{Pyruvic acid} + CO_2$$

β-oxidation spiral and glyoxylate cycle take place within glyoxysomes. In glyoxysomes enzymes like isocitratase and malate synthetase are present. Presence of these two enzymes enables the Acetyl CoA to by pass many steps of Krebs cycle and being oxidized to CO_2 and H_2O.

3.4 STORAGE AND MOBILIZATION OF FATTY ACIDS

Fats and oils are mainly found in the form of triacylglycerols (acyl represents the fatty acid portion) or triglycerides in which fatty acid molecules are linked by ester bonds to three hydroxyl groups of glycerol.

Triacylglycerols in most seeds are stored in cytoplasm or endosperm cells in organelles known as oleosomes (spherosomes or oil bodies).

After germination, oil containing seeds metabolize stored triglycerols by **converting lipids to sucrose.** Plants are not able to transport fats from endosperm to various parts, as a result stored lipids are converted into mobile form *i.e.,* sucrose.

The process requires several steps located in different sites like oleosomes, glyoxysomes, mitochondria and cytosol.

Lipids to Carbohydrates in Germinating Seeds

In some fat storing seeds *e.g.,* castor bean, bacteria and fungi, lipids are readily converted into sucrose and other carbohydrates. Some of the carbon atoms of fatty acid are also diverted towards amino acids during this conversion. The lipids are converted into sugars and then translocated to growing axis like root and shoot apices where it is used for growth. The conversion of lipids to sucrose starts with the hydrolysis of triacylglycerols

NOTES

stored in oil bodies to free fatty acids, followed by oxidation of fatty acids to produce acetyl CoA.

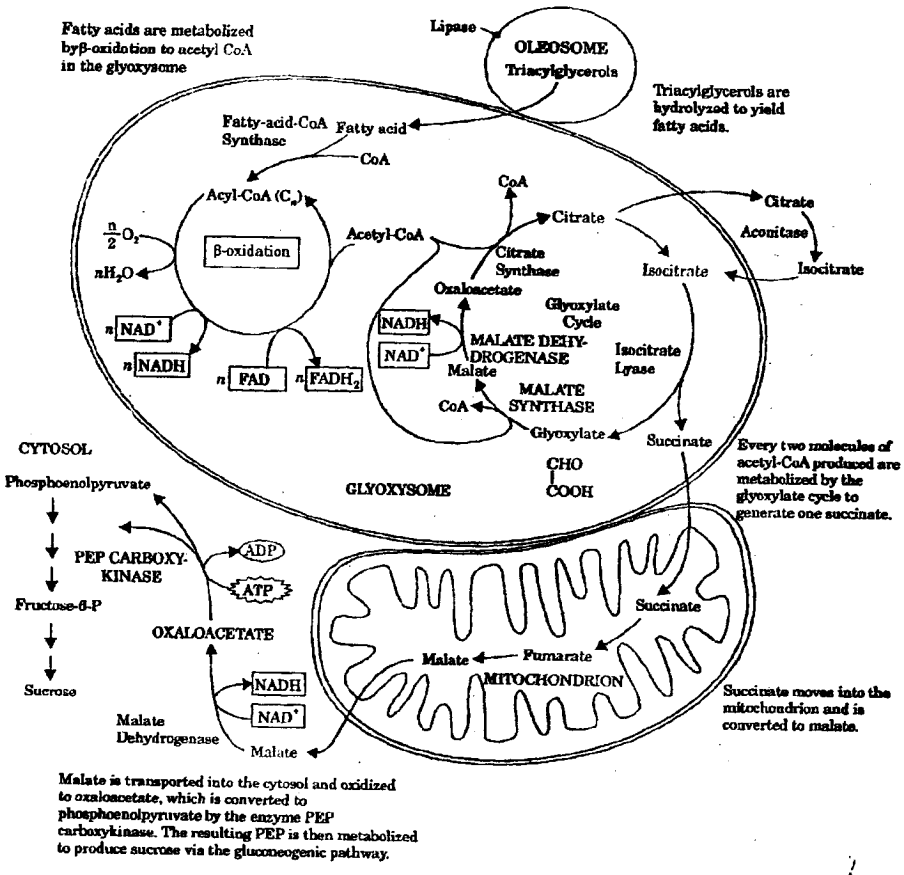


Fig. 3.6. *The conversion of fats to sugars during germination in oil-storing seeds.*

The fatty acids are oxidised in glyoxysome. The acetyl CoA in glyoxysome forms succinate. Succinate moves from glyoxysome to mitochondria to form **malate.**

In cytosol malate is converted into glucose via glucogenesis and then sucrose.

1. First step is breakdown of triglycerides to three molecules of fatty acids and glycerol with enzyme lipase.
2. Now fatty acids enter glyoxysomes. Here they are activated by fatty acyl CoA and undergo the process of β-oxidation. Due to this C_n fatty acids are converted into $n/2$ molecules of **acetyl CoA.** In potato tuber and mung bean hypocotyl, β oxidation however occurs in peroxisomes.
3. Now glyoxylate cycle occurs which converts two molecules of acetyl CoA to succinate.

4. **Succinate migrates to mitochondria.** Here succinate is converted into malate. Malate is oxidized to form oxaloacetate in cytosol. Oxaloacetate forms the carbohydrate. Sucrose is the final product of process.

3.5 SYNTHESIS OF FATS

Fats are synthesized from fatty acids and glycerol by the reversal of hydrolytic process. The synthesis occurs in three phases : synthesis of fatty acid, synthesis of glycerol and synthesis of triacyl glycerols (fats).

1. Synthesis of Fatty Acids

The fatty acids are synthesized from 2 carbon compound acetyl coenzyme A (Acetyl CoA). The acetyl CoA is produced as an intermediate in the respiratory pathway. Two enzyme complexes and five cofactors (ATP, NADPH, CO_2, biotin and Mn^{2+}) are required for the synthesis of fatty acids.

The synthesis of fatty acid occurs in the cytosol at the endoplasmic reticulum. It takes place as under.

(*i*) The synthesis starts with the carboxylation (CO_2 from bicarbonate) of acetyl CoA (2*c*) to malonyl CoA (3*c*). The reaction is catalysed by the enzyme *acetyl CoA carboxylase,* which contains biotin as prosthetic group.

$$CH_3Co \sim SCoA + CoA + CO_2 + ATP \xrightarrow[Mn^{2+}]{\text{Acetyl CoA carboxylase}}$$

Acetyl CoA (2C)

$$H\text{—}C(H)(C(=O)\sim SCoA)\text{—}C(=O)\text{—}OH + ADP + Pi \text{ (Inorganic Phosphate)}$$

Malonyl coenzyme A (3C)

(*ii*) In the next step, malonyl CoA (3*c*) is acylated and undergoes reduction several times to produce saturated fatty acids. These reactions are catalysed by a multienzyme complex, *fatty acid synthetase* complex, and NADPH provide hydrogen for the reduction.

The malonyl CoA (3*c*) formed in the first step reacts with another molecule of acetyl CoA (2*c*) to form acetomalonyl CoA (5*c*), one molecule of coenzyme A is also released.

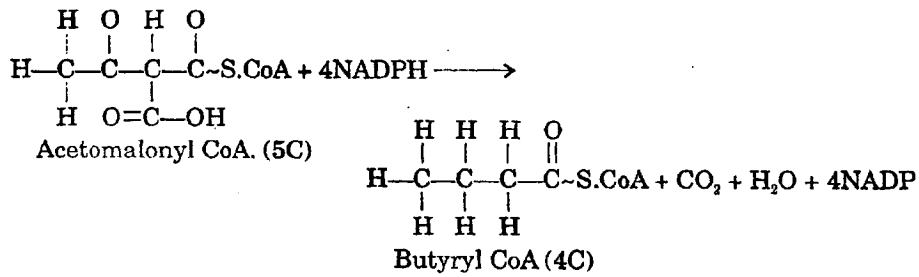
$$H\text{—}C(H)(C(=O)\sim SCoA)\text{—}C(=O)\text{—}OH + CH_3CO\sim S.CoA \longrightarrow H\text{—}C(H)(C(=O)\sim SCoA)\text{—}C(H)\text{—}C(=O)\text{—}OH + HS.CoA$$

Acetyl CoA (2C)

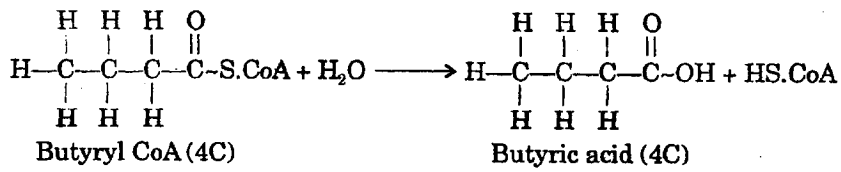
Malonyl coenzyme A(3C) — Acetomalonyl CoA (5C)

The acetomalonyl CoA (5*c*) is decarboxylated and reduced by NADPH to produce butyryl CoA (4*c*), CO_2, water and NADP.

NOTES



The butyryl CoA (4c) is hydrated to produced butyric acid (4c) and coenzyme A.



The butyryl CoA (4c) may further condense with malonyl CoA (3c) t form 7c chain and the process is repeated to cause elongation of chair Through the process of chain elongation different types of fatty acid are synthesised (Fig. 3.7).

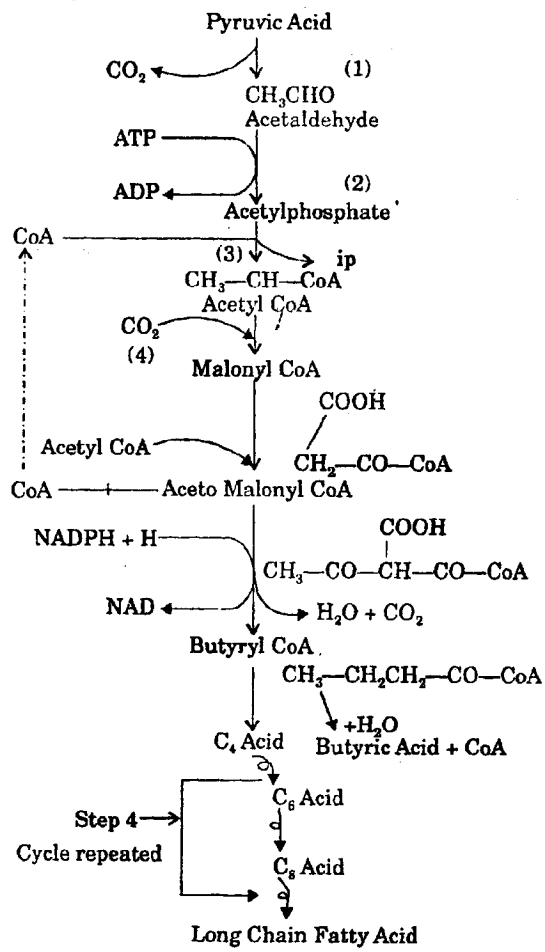
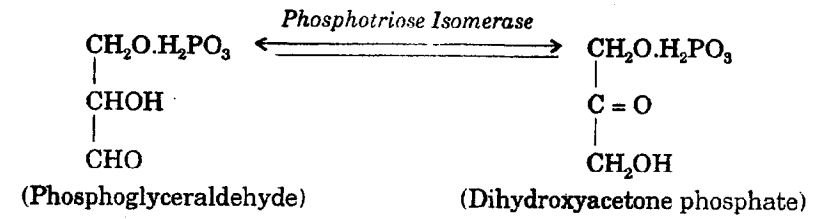


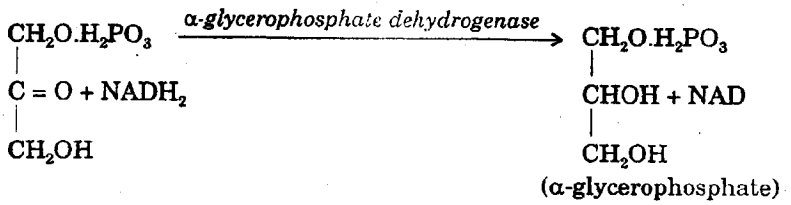
Fig. 3.7. *Schematic representation of steps in the synthesis of fatty acid.*

2. Synthesis of Glycerol

Glycerol is synthesized from dihydroxy acetone phosphate as glycerol phosphate through glyceraldehyde phosphate. The reaction is catalysed by NADH dependent *glycerol 3-phosphate dehydrogenase.*

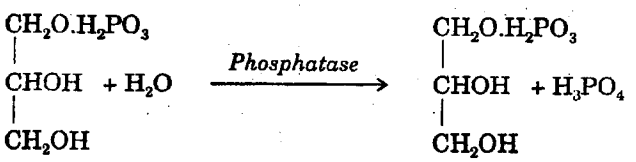


(Phosphoglyceraldehyde) (Dihydroxyacetone phosphate)



(α-glycerophosphate)

Dephosphorylation of α-glycerophosphate results in its conversion into glycerol

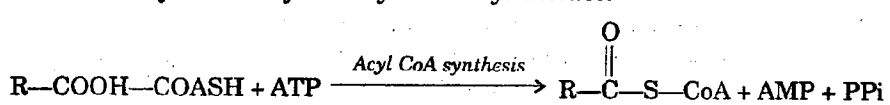


(Glycerol)

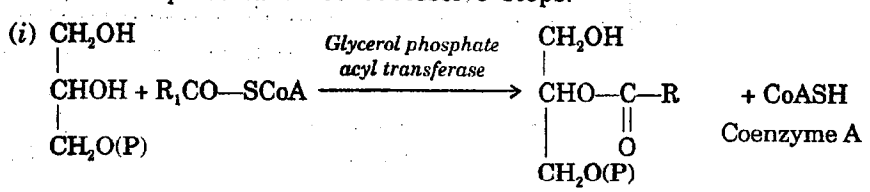
3. Synthesis of Triacyl Glycerol (Fats)

The biosynthesis of triacyl glycerols occurs primarily on the surface of endoplasmic reticulum. Three fatty molecules join sequentially to a glycerol phosphate molecule to form a triacylglycerol.

The fatty acids are first activated by their conversion to respective acyl derivatives by the enzyme *Acyl CoA synthetase.*

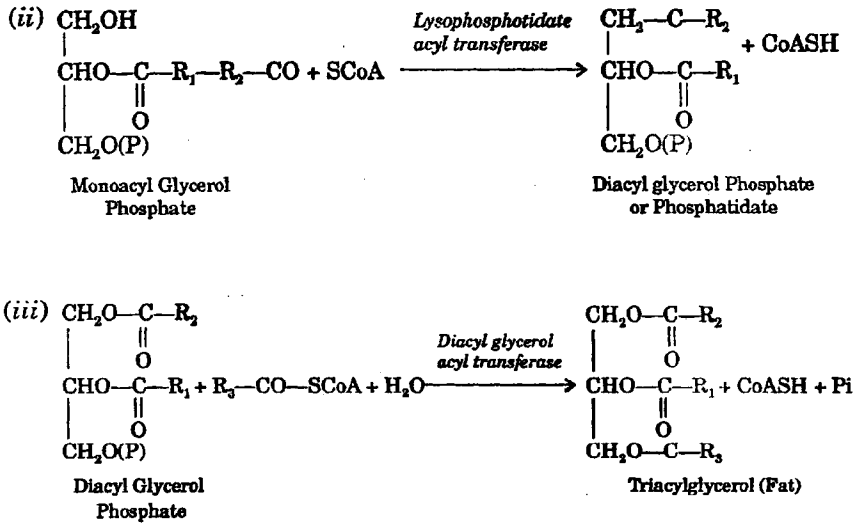


The esterification of glycerol phosphate with this fatty acyl CoA (RCO—SCoA) takes place in three successive steps.



Mono Glycerol Phosphate
or
Lysophosphatidate

NOTES



The enzymes carrying out acylations of glycerol phosphate are part of membrane bound *triacyl glycerol synthetase* complex.

Ketone Bodies and their Metabolism

During periods of excess formation of acetyl CoA from fatty acid oxidation or pyruvate oxidation acetyl CoA is diverted enzymatically into free **acetoacetate** and **β-hydroxy butyrate** in many vertebrates. These pass into the blood by diffusion. Acetoacetate spontaneous decarboxylation to form **acetone**. The acetoacetate, β-hydroxybutyrate and acetone are collectively called as **ketone bodies** (Fig. 3.8).

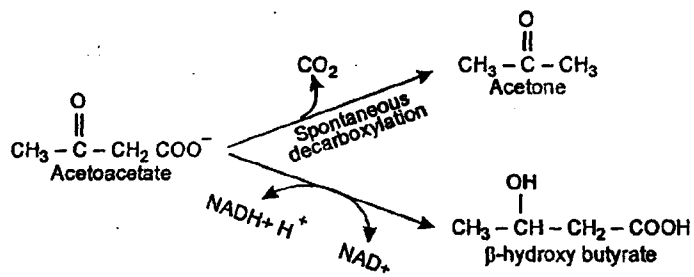


Fig. 3.8. *Interrelationships of the ketone bodies.*

From the blood, ketone bodies are transported to the peripheral tissues (such as skeletal muscles, cardiac muscles, renal cortex *etc.,*) where they may be oxidised *via* kreb's cycle. The formation utilization and excretion of ketone bodies is depicted in Fig. 3.9.

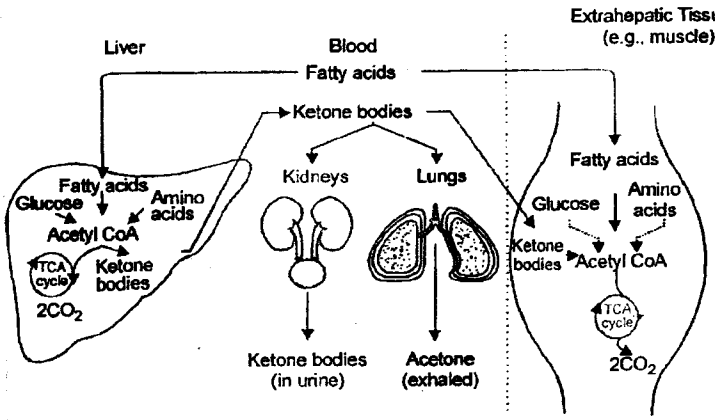


Fig. 3.9. *Summary of ketone body synthesis, utilization and excretion.*

Ketogenesis (Synthesis of Ketone Bodies)

The synthesis of ketone bodies occurs in the **liver**. The enzymes for ketone body synthesis are located in the **mitoch-ondrial matrix.** Acetyl CoA, formed by oxidation of fatty acids, pyruvate or some amino acids acts as precursor for ketone bodies. The synthesis of ketone bodies involves following reactions (Fig. 3.10).

- Two molecules of acetyl CoA condense to form acetoacetyl CoA, with the help of enzyme *thiolase.*
- Acetoacetyl CoA combines with another molecule of acetyl CoA to produce β-hydroxy β-methyl glutaryl CoA (HMG CoA). The enzyme *HMG CoA Synthase* catalyses this reaction.
- HMG CoA is splitted into acetoacetate and acetyl CoA, with the help of enzyme *HMG CoA lyase.*
- Acetoacetate can undergo spontaneous decarboxylation to form acetone.
- Acetoacetate can be reduced by enzyme *dehydrogenase* to form β-hydroxy butyrate.

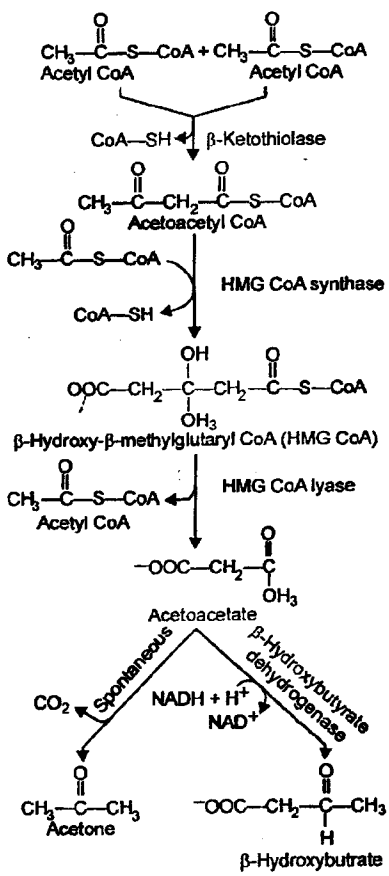


Fig. 3.10. *Synthesis of ketone bodies (ketogenesis).*

NOTES

Utilization of Ketone Bodies

The two ketone bodies—acetoacetate and β-hydroxybutyrate serve as important source of energy for the peripheral tissues like skeletal muscles, cardiac muscles, renal cortex etc. Liver lacks the enzyme *thiophorase*, hence, ketone bodies are not utilized in the liver. Being water soluble, ketone bodies are easily transported from liver to other tissues for their utilization.

The production of ketone bodies and their utilization become more important during the conditions like starvation and diabetes mellitus, when glucose is in short supply to the tissues. During prolonged starvation. Ketone bodies are the major source of energy for the brain and other parts of central nervous system. They can meet 50–70 per cent of the brain's energy needs. It is an adaptation for the survival of the organism during the period of starvation.

The steps (Fig. 3.11) of utilization of ketone bodies are as follows.

- β-hydroxy butyrate is first converted to acetoacetate
- Acetoacetate is activated to acetoacetyl CoA by a mitochondrial enzyme *thiophorase (succinyl CoA acetoacetate CoA transferase)*. The CoA is donated by succinyl CoA (an intermediate in citric acid cycle)
- The enzyme *thiolase* leaves acetoacetyl CoA into two molecules of acetyl CoA.

Ketosis

In well fed mammals, the concentration of total ketone bodies in the blood does not normally exceed 1 mg/day. In human beings, loss of ketone bodies via urine is normally less than 1 mg/day. In blood, if ketone bodies are present in higher quantities than the normal, the condition is termed as **ketonaemia**. Similarly, higher concentration of ketone bodies in the urine than the normal, the condition is described as **ketonuria** and the overall condition is termed as **ketosis**. The simplest form of ketosis occurs during starvation. Here, it involves depletion of carbohydrates and also there is mobilization

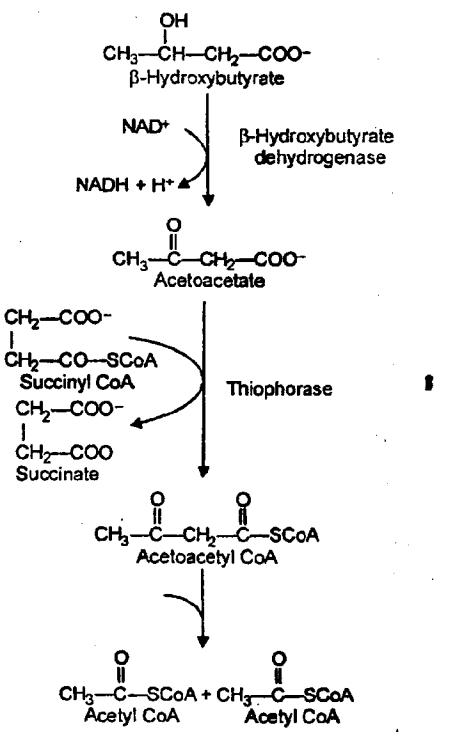


Fig. 3.11. *Metabolism (utilization) of ketone bodies to acetyl CoA.*

of Free Fatty Acids (FFA). Other forms of ketosis occur under conditions of high fat feeding and after severe exercise in the post absorptive state.

Metabolism of Bile Pigments

NOTES

Bile is secreted by the liver and is stored in gall bladder. It plays a significant role in the emulsification of fats during digestion of the later. It is golden or brownish in colour which is due to the presence of two pigments - **bilirubin** and **biliverdin.** These two pigments are termed as **bile pigments**. These two pigments are inter convertible involving oxidation-reduction process. Biliverdin is green in colour and is a tetrapyrrole compound, which does not have a metallic atom (Fig. 3.12).

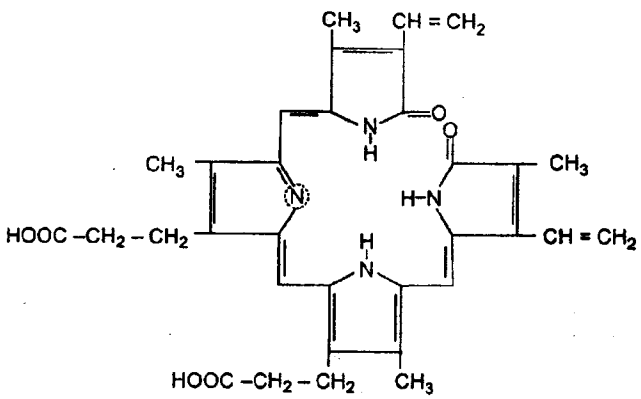
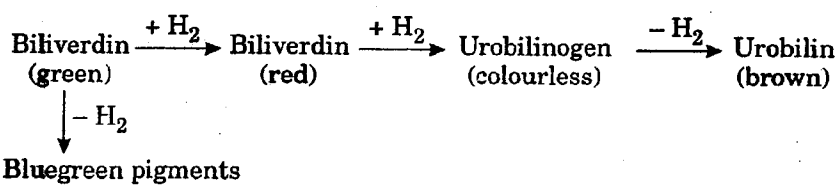


Fig. 3.12. *Structure of Biliverdin.*

The bile pigments are produced form the haeme part of haemoglobin of aged erythrocytes (RBCS) and other haeme proteins like myoglobin, cytochromes, peroxidase *etc.*

The oxidation of biliverdin yields blue green pigment whereas reduction produces biliverdin having red colour.



The bile pigment on further degradation by the microorganisms in the intestine yield brown pigment, called **stercobilin.** It is the chief pigment of faeces.

Haeme ⟶ Protoporphyrin IX ⟶ bilirubin and biliverdin ⟶ stercobilin.

STUDENT ACTIVITY

1. Name the bile pigments present in the bile.

2. Give a brief account of breakdown of glycerol.

3.6 SUMMARY

- Lipids can be metabolised and degraded to meet the energy needs of the cell. Triacylglycerols are the major lipids which on oxidation yield more energy metabolic water than any other fuels. Triacylglycerols are hydrolysed by enzyme lipase into fatty acids and glycerol moiety.
- Glycerol is catabolised through glycolytic pathway. There are two mechanism of oxidation of fatty acids—β-oxidation and α-oxidation. β-oxidation is the primary pathway of oxidation of fatty acids and occur in a cyclic manner. In each cycle, an acyl CoA is produced which is short by two carbon atoms and one molecule of each $FADH_2$, NADH and acetyl CoA are also produced. α-oxidation is an alternate mechanism of oxidation of fatty acids, in which the acid is oxidised to produce another acid, with one carbon atom less. Glyoxylate cycle is also operative to oxidise acetyl CoA along with Kreb's cycle.
- During the germination of many fat storing seeds, lipids are converted into sugars to be transported to the growing root and shoot apices where it is used for growth.
- Fats are synthesized from fatty acids and glycerol by the reversal of hydrolytic process. Synthesis of fatty acids, synthesis of glycerol and synthesis of triacyl glycerol are the main phases of fat synthesis.
- Acetoacetate, β-hydroxy butyrate and acetone are collectively called ketone bodies. These are produced during the period of excess formation of acetyl CoA. The synthesis of ketone bodies occurs in the liver. The ketone bodies serves as a source of energy in the peripheral tissues during the conditions like starvation and diabetes mellitus. The presence of excess of ketone bodies in the blood and the urine is called ketosis. It occurs during starvation and under condition of high fat feeding.

3.7 GLOSSARY

- **β-oxidation:** The oxidation produces two carbon acetate unit as acetyl CoA and releases $FADH_2$ and NADH.
- **α-oxidation:** It is an alternate mechanism of oxidation of fatty acids, in which the acid is oxidised to produce another acid with one carbon atom less.
- **Glyoxylate Cycle:** The function of glyoxylate cycle is to convert two molecules of acetyl CoA to succinic acid (Succinate).
- **Ketone Bodies:** The acetoacetate, β-hydroxybutyrate and acetone are collectively called as ketone bodies.

- **Ketonaemia:** In blood, if ketone bodies are present in higher quantities then the normal, the condition is termed as ketonaemia.
- **Ketonuria:** Higher concentration of ketone bodies in the urine than the normal, the condition is described as ketonuria.
- **Stercobilin:** The bile pigment on further degradation by the microorganisms in the intestine yield brown pigment, called stercobilin.

3.8 REVIEW QUESTIONS

I. Very Short Answer Type Questions:

1. Name the common type of lipid stored in plants and animals.
2. How many molecules of water are used up in complete hydrolysis of one molecule of a neutral fat into fatty acid and glycerol?
3. Name the enzyme complex involved in the biosynthesis of fatty acids.
4. Name the types of oxidation of fatty acids.
5. What are the products of hydrolysis of triacyl glycerol?
6. Name the ketone bodies present in the blood/urine.
7. What is the principal role of ketone bodies in the human body?

II. Short Answer Type Questions:

1. In what way lipid fuels are advantageous in the terrestrial mammals?
2. Explain, the regulation of hydrolysis of fats in animal tissues.
3. Explain the following:

   (*i*) Glyoxylate cycle (*ii*) Mobilisation of lipids.
4. Write short notes on:

   (*i*) α-oxidation of fatty acids (*ii*) Synthesis of glycerol.
5. Give an account of formation of triacyl glycerol from fatty acids and glycerol.
6. What are bile pigments and how are they formed in the body?

III. Long Answer Type Questions:

1. Give a concise account of b-oxidation of fatty acids.
2. Write a brief account of the synthesis of fatty acids.
3. Give a brief account of ketone bodies and their metabolism.

3.9 FURTHER READINGS

- **Biochemistry;** Satyanarayan U., Chakrapani U.; Books and Allied (P) Ltd. Kolkata, 2008.
- **Essentials of Food and Nutrition;** Swaminathan M.; Ganesh, Madras, India; 1985.

CHAPTER 4 METABOLIC INTER-RELATIONSHIP

NOTES

OBJECTIVES

After going through this chapter, you should be able to:

- define integration of major pathways
- understand citric acid cycle
- explain the organs involved in matabolic integration
- know about metabolism in starvation.

STRUCTURE

4.1 INTRODUCTION

Metabolism is a continuous process, with thousands of reactions, simultaneously occurring in the living cell. Carbohydrate, fat and protein metabolisms integrate to meet the body's energy requirements. The organisms possess variable energy demands, hence the supply (input) is also equally variable. The consumed metabolic fuel may be burnt (oxidised to CO_2 and H_2O) or stored to meet the energy requirements as per the body's requirements. ATP serves as the energy currency of the cell in this process.

NOTES

4.2 INTEGRATION OF MAJOR PATHWAYS

In the living organisms Acetyl CoA is the key and common metabolic produced from different fuel sources (carbohydrates, lipids, amino acids). Acetyl CoA enters citric acid (Krebs) cycle and get oxidised to release energy which is trapped in the form of NADH and $FADH_2$. The inter-relationship between the important metabolic pathways concerned with fuel metabolism are described below.

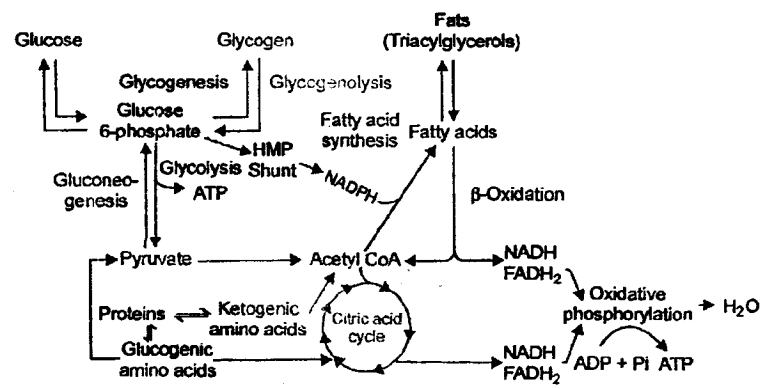


Fig. 4.1. *An overview of integration of metabolic pathways of energy metabolism (HMP shunt–Hexose monophosphate shunt).*

1. **Citric Acid Cycle (Krebs cycle):** Citric acid cycle (Krebs cycle) is the major pathway for the synthesis of reduced coenzymes (NADH and $FADH_2$) and controlled release of energy during respiration. It is a common pathway of oxidative breakdown of carbohydrates, fatty acids and amino acids (Fig. 4.1).
2. **Glycolysis:** During respiration glucose is degraded to pyruvate (lactate under an aerobic condition) and generates NADH and ATP. The pyruvate thus formed is converted into acetyl CoA.
3. **Degradation of Amino Acids:** Amino acids enter the Krebs cycle directly as glutamate (for α-ketoglutarate) and aspartate (for oxaloacetate) after their deamination.
4. **Oxidation of Fats:** Fats produce fatty acids and glycerol. Glycerol is phosphorylated and oxidised to form glyceraldehyde 3-phosphate. Fatty acids undergo β-oxidation to produce acetyl CoA. Acetyl CoA enters Krebs cycle and most of the energy is trapped in the form of NADH and $FADH_2$.
5. **Oxidative Phosphorylation:** The NADH and $FADH_2$ produced in different metabolic pathways, are finally oxidised in the Electron Transport Chain (ETC). The ETC is coupled with oxidative phosphorylation to generate ATP.
6. **Hexose Monophosphate Shunt (HMP shunt):** This pathway is primarily concerned with the liberation of NADPH and

5-carbon sugar ribose. NADPH is utilized for the biosynthesis of several compounds, including fatty acids. Ribose is an essential constituent of RNA (Ribose Nucleic Acid).

7. **Gluconeogenesis:** It refers to synthesis of glucose from non-carbohydrate sources a number of compounds (such as pyruvate, glycerol, amino acids) can serve as raw materials for the synthesis of glucose.
8. **Glycogen Metabolism:** Glycogen is the storage form of glucose, mostly found in liver and muscles. It is synthesized (glycogenesis) and degraded (glycogenolysis) by different, independent pathways, glycogen serves as a fuel reserve to meet the body's need between the meals.

4.3 CITRIC ACID CYCLE—AN AMPHIBOLIC PATHWAY

An amphibolic pathway in the one which is used for both breakdown (catabolism) and build up (anabolism) reactions. Respiratory pathway is mainly a catabolic process which serves run the living system by providing energy. The pathway produces a number of intermediates. Many of them are raw materials for building up both primary and secondary metabolites. For example, acetyl CoA is helpful not only is using fatty acids in Krebs cycle, but is also raw material for synthesis of fatty acids, steroids and aromatic compounds.

4.4 ORGANS INVOLVED IN METABOLIC INTEGRATION

In human body, various tissues and organs work in a well co-ordinated manner to meet the metabolic demands The major organs involved in the metabolic integration (Fig. 4.2) in a well fed absorptive state are discussed below.

Liver

The liver is the central metabolic clearing house, which processes and distributes the nutrients to different tissues for utilization. After a meal , the liver takes up the carbohydrates, lipids and most of the amino acids, processes them and sends to other tissues. In an absorptive state, there is an increase in glycolysis, glycogenesis, synthesis of fatty acids and triglycerides and also synthesis of proteins. However, the synthesis of glucose from non-carbohydrate sources (gluconeogenesis) is decreased.

NOTES

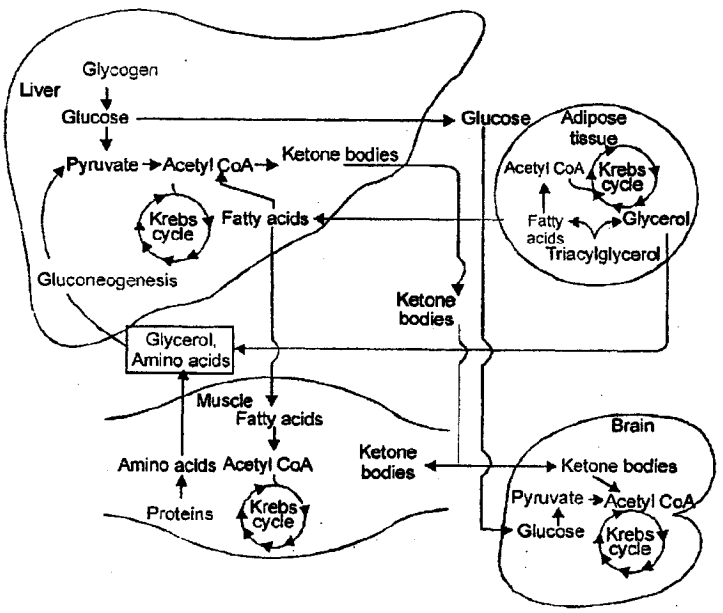


Fig. 4.2. *Metabolic interrelationship among the major tissues in a well fed state (HMP shunt-Hexose monophosphate shunt)*

Skeletal Muscles

The glucose uptake and synthesis of glycogen is increased. The incorporation of amino acids into proteins is also higher. Fatty acids serve as important fuel source for the muscles.

Adipose Tissues

There is an increase in glucose uptake and synthesis of fatty acids and triglycerides.

Brain

In an absorptive state, glucose is the only fuel source to the brain. The free fatty acids unable to cross the blood-brain barrier, hence their contribution for the supply of energy to the brain is insignificant.

4.5 METABOLISM IN STARVATION

Starvation is the condition which is caused due to food scarcity or certain clinical conditions. It is a type of metabolic stress that imposes certain metabolic compulsions on the organism. The metabolism is recognised to meet the new demands of starvation.

Under starvation, the carbohydrate reserve of the body is too low to meet the energy requirements even for a day. Under such a condition, fats (triglycerides) stored in the adipose tissue acts as the predominant energy reserve of the body, thus, obese individuals can survive longer than the lean individuals during starvation.

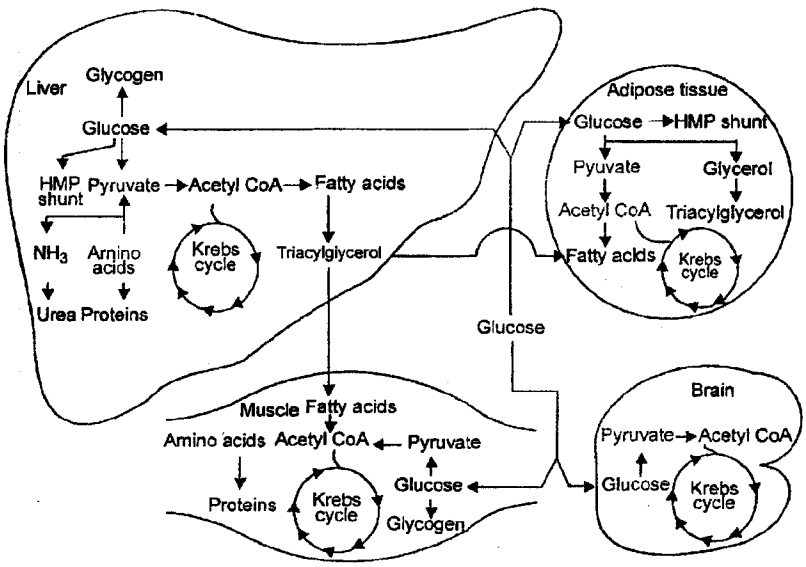


Fig. 4.3. *Metabolic interrelationship among major tissues during starvation.*

Though proteins are basically a structural constituents, mostly present in the muscles. However, during starvation, proteins can also meet the fuel demands of the body. It has been found that about 1/3 rd of the body's protein can be utilized to meet the energy without compromising the vital functions. Starvation in associated with a decrease in insulin level and an increase in glucagon level in the blood, resulting in the conversion of stored glycogen into glucose. The important metabolic changes that occur in major organs (Fig. 4.3) are described below.

1. **In Liver:** Liver acts as a blood glucose buffering organ. During starvation, increased gluconeogenesis and enhanced degradation of glycogen, provide glucose to the needy tissues, especially to brain. Fatty acid oxidation is increased with an enhanced synthesis of ketone bodies. It is because, citric acid cycle (Krebs cycle) cannot cope with the excess production of acetyl COA, and the latter is diverted to the synthesis of ketone bodies. The ketone bodies (mainly β-hydroxybutyrate) effectively serve as fuel source for the peripheral tissues.
2. **In Adipose Tissue:** In adipose tissue, the uptake of glucose and its metabolism are slowed down. The degradation of triglycerides is enhanced leading to an increased release of fatty acids from the adipose tissue, which serve as fuel source for various tissues

NOTES

(brain in an exception). The glycerol released by the breakdown of fats serves as precursor for glucose synthesis by liver.

3. **In Skeletal Muscles:** In skeletal muscles, the uptakes of glucose and its metabolism are very much lowered. Both fatty acids and ketone bodies are utilized by the muscle as fuel source. During the early period of starvation, muscle proteins are degraded to liberate the amino acids, which are effectively utilized by the liver for glucose synthesis (gluconeogenesis). On prolonged starvation, however, protein breakdown is reduced.
4. **In Brain:** Glucose is the principal fuel source for the brain. During the first two weeks of starvation, brain depends on the glucose supplied by the liver's gluconeogenesis. Starvation beyond three weeks, generally marked by an increase in plasma ketone bodies. Now the brain depends on ketone bodies for the energy needs.

4.6 REGULATION OF METABOLIC PATHWAYS

In general, metabolic pathways are regulated by the availability of the fuel substrate, physical condition of the body and hormones. The details of these regulatory processes have been discussed under the individual pathways in respective chapters.

STUDENT ACTIVITY

1. What are the substances that meet the body's energy requirements?

2. Explain the role of liver in metabolic integration.

NOTES

4.7 SUMMARY

- Carbohydrate, fat and protein metabolisms integrate to meet the body's energy requirements. Acetyl CoA is the key and common metabolite produced from different sources. It enters citric acid cycle and get oxidised to release energy.
- Various tissues and organs work in a well co-ordinated manner to meet the metabolic demands. The major organs involved in the metabolic integration are liver, skeletal muscles, adipose tissue and brain. Under starvation, the carbohydrate reserve of the body is too low to meet the energy requirements even for a day. Hence, fats stored in adipose tissue serve as the predominant energy reserve of the body. Structural proteins can also meet the fuel demands of the body under starvation. In general, metabolic pathways are regulated by the availability of fuel substrate, physical condition of the body and hormones.

4.8 GLOSSARY

- **Metabolism:** It is a continuous process, with thousands of reactions, simultaneously occurring in the living cell.
- **Glycolysis:** During respiration glucose is degraded to pyruvate and generates NADH and ATP.
- **HMP Shunt:** This pathway is primarily concerned with the liberation of NADPH and 5-Carbon sugar ribose.
- **Gluconeogenesis:** It refers to synthesis of glucose from non-carbohydrate sources.
- **Liver:** The liver is the central metabolic clearing house, which processes and distributes the nutrients to different tissues for utilization.

4.9 REVIEW QUESTIONS

I. Very Short Answer Type Questions:

1. Name the key and common metabolite produced from different fuel sources.
2. Through which pathway, Acetyl CoA is oxidised to release energy.

II. Short Answer Type Questions:

1. Why is citric acid cycle called an amphibolic pathway?
2. Why do obese individuals survive longer than the lean individuals under starvation?

3. How does liver works with respect to metabolic pathways under starvation?

III. Long Answer Type Questions:

1. Explain, the integration of metabolic pathways in the human body.
2. Explain, the changes give metabolisms in different tissues and organs during starvation.

4.10 FURTHER READINGS

- **Biochemistry;** Stryer L.; W.H. Freeman and Company; New York, 1981.
- **Essentials of Food and Nutrition;** Swaminathan M; Ganesh, Madras, India; 1985.
- **Nutrition and Dietetics;** Joshi, Shubangini A; Tata McGraw Hill, New Delhi, 1992.

UNIT-II

NOTES

CHAPTER 5 MINERAL METABOLISM

OBJECTIVES

After going through this chapter, you should be able to:

- explain macroelements
- describe phosphorus
- know about microelements.

STRUCTURE

5.1 INTRODUCTION

Minerals occur in living organisms as components of organic and inorganic molecules and ions. They perform several vital functions which are absolutely essential for the very existence of the organism. Minerals are involved in clacification of bone, coagulation of blood, neuromuscular irritability, acid-base equilibrium, fluid balance and osmotic regulation. Certain minerals are integral components of biologically active substances such as iron (Fe) in haemoglobin, iodine (I) in thyroxine, zinc (Zn) in insulin and cobalt in vitamin B_{12}. Sulphur is present in thiamine, biotin, lipoic acid and coenzyme A. Several minerals *e.g.*, Mg, Mn, Cu, Zn, K participate as cofactors for enzymes in metabolism.

5.2 CLASSIFICATION

Minerals are classified as macrominerals or macroelements (principal elements and micro-minerals or macroelements (trace elements).

The macroelements are required in amounts greater than 100 mg/day. These include calcium, phosphorus, magnesium, sodium, potassium, chlorine, sulphur.

The microminerals are required in amounts less than 100 mg/day. These include iron, copper, iodine, manganese, zinc, molybdenum, cobalt, fluorine, selenium, chromium.

The important characteristics of macrominerals and microminerals are respectively given in table 5.1 and 5.2.

Table 5.1. A Summary of Important Characteristics of Macrominerals

Element	*Major functions*	*Deficiency disease / symptoms*	*Recommended dietary allowance*	*Major sources*
Calcium	Constituent of bones and teeth; muscle contraction, nerve transmission.	Rickets; osteomalacia, osteoporosis	0.8–1.0 g/d	Milk and milk products, leafy vegetables, beans
Phosphorus	Constituent of bones and teeth; in the formation of high energy phosphates, nucleic acids, nucleotide coenzymes.	Rickets, osteomalacia	0.8–1.0 g/d	Milk, cereals, leafy vegetables
Magnesium	Constituent of bones and teeth; cofactor for enzymes *e.g.*, kinases.	Neuromuscular weakness, irritation	300–350 mg/d	Cereals, vegetables, fruits, milk
Sodium	Chief cation of extracellular fluids; acid-base balance, osmotic pressure, nerve and muscle function.	Almost unknown on normal diet	5–10 g/d	Table salt, salt added foods
Potassium	Chief cation of intracellular fluids; acid-base balance; osmotic pressure; muscle function.	Muscular weakness, mental confusion	3–4 g/d	Fruits, nuts, vegetables
Chlorine	Regulation of acid-base balance; formation of HCl	Almost unknown on normal diet	5–10 g/d	Table salt
Sulfur	Constituent of sulfur containing amino acids, certain vitamins (thiamine, biotin) and other compounds (heparin, chondrotin sulfate).	Almost unknown	—	Sulfur containing amino acids

NOTES

Table 5.2. A Summary of Important Characteristics of Microminerals

Element	*Major functions*	*Deficiency disease / symptoms*	*Recommended dietary allowance*	*Major sources*
Iron	Constituent of heme *e.g.*, hemoglobin, myoglobin, cytochromes; involved in O_2 transport and biological oxidation.	Hypochromic, microcytic anemia	10–15 mg/d	Organ meats (liver, heart), leafy vegetables, iron cookware
Copper	Constituent of enzymes *e.g.*, cytochrome C oxidase, catalase, tyrosinase; in iron transport.	Anemia, Menke's disease	2–3 mg/d	Organ meats cereals, leafy vegetables
Iodine	Constituent of thyroxine and triiodothyronine	Cretinism, goitre, myxedema	150–200 µg/d	Iodized salt, sea foods
Manganese	Cofactor for enzymes *e.g.*, arginase, pyruvate carboxylase, glycoprotein synthesis.	Almost unknown	2–9 mg/d	Cereals, leafy vegetables
Zinc	Cofactor for enzymes *e.g.*, alcohol dehydrogenase, carbonic anhydrase, lactate dehydrogenase.	Growth retardation, poor wound healing, hypogonadism	10–15 mg/d	Meat, fish, milk
Molybdenum	Constituent of enzymes *e.g.*, xanthine oxidase	Almost unknown	75–250 µg/d	Vegetables
Cobalt	Constituent of vitamin B_{12}, required for the formation of erythrocytes	Pernicious anemia (as in vitamin B_{12} deficiency)	5–8 µg/d	Foods of animal origin
Fluorine	Helps in the proper formation of bones and teeth	Dental caries, osteoporosis	2–4 mg/d	Drinking water
Selenium	Involved in antioxidant function along with vitamin E; constituent of glutathione peroxidase and selenocysteine	Muscular degeneration, cardiomyopathy	50–200 µg/d	Organ meats, sea foods
Chromium	Promotes insulin function (as glucose tolerance factor)	Impaired glucose tolerance	10–100 µg/d	Brewer's yeast, meat, whole grains

NOTES

5.3 MACROELEMENTS

Calcium

Calcium is the most abundant among the minerals in the body. It is required for the development of bones and teeth, muscle contraction, blood coagulation, nerve transmission, activation of certain enzymes and many more activities.

Milk and milk products are best sources of calcium. Beans, leafy vegetables, fish, cabbage, egg yolk are the good sources of calcium.

Absorption of Calcium

The absorption of calcium mostly occurs in duodenum by an energy dependent process called active absorption. However, the absorption is affected by a number of factors.

Factors Promoting Ca Absorption

- The active form of vitamin D, calcitriol induces the synthesis of calcium binding protein in the intestinal epithelial cells and promote Ca absorption.
- Parathyroid hormone (PTH) promote Ca absorption by increasing synthesis of calcitriol.
- Low pH (acidity) favours absorption of calcium.
- Lactose promote calcium uptake by intestinal cells.

Factors Inhibiting Ca Absorption

- Oxalates and phytates interfere with calcium absorption by forming insoluble salts.
- High dietary phosphate contents form insoluble calcium phosphate and, thus, prevent calcium uptake.
- High pH (alkalinity) is unfavourable for calcium absorption.
- When fat absorption is impaired, the free fatty acids react with Ca and form insoluble calcium soap, thereby preventing Ca absorption.
- High content of dietary fibre interferes with calcium uptake.

Excretion of Calcium

Excretion of calcium occurs partly through the kidneys and mostly through the gut. Excess intake of proteins causes increased calcium excretion in urine. This is mainly due to an increase in the acidity of urine as a result of high protein diet. Deficiency of vitamin D, results an increased elimination of calcium through faeces.

Factors Regulating Plasma Ca Level

The hormones—*calcitriol, parathyroid hormone* (PTH) and *calcitonin* are the major factors involved in the regulation of plasma calcium, within a narrow range *i.e.*, 9–11 mg/dl. (Fig. 5.1)

NOTES

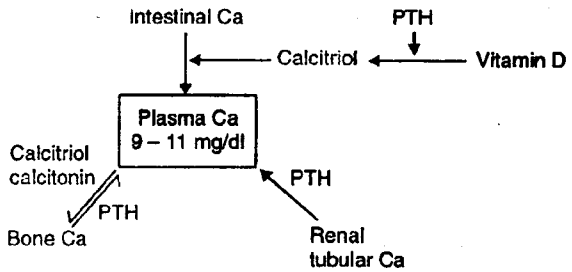


Fig. 5.1. *Calcium homeostasis.*

1. **Calcitriol.** Calcitriol or 1, 25-dihydroxy cholecalciferol (1, 25 DHCC) is the physiologically active form of vitamin D. It induces the synthesis of a specific calcium binding protein in the intestinal cells. This protein increases the intestinal absorption of calcium as well as phosphate. Thus blood Ca level is increased by calcitriol. Calcitriol also stimulate Ca uptake by osteoblasts of bone and promotes calcification or mineralization and remodelling of bones.
2. **Parathyroid Hormone (PTH).** Parathyroid hormone is secreted by parathyroid glands. The secretion of PTH is under the negative feedback regulation of serum Ca^{2+}. Therefore, low serum Ca^{2+} concentration promotes PTH secretion.

   PTH elevates serum calcium level in three ways.

   - It causes decalcification or demineralization of bones, leading to an increase in the blood Ca^{2+} level.
   - PTH increases the calcium reabsorption by kidney tubules. It also promotes production of calcitriol in the kidney by stimulating 1 hydroxylation of 25-hydroxycholecalciferol.
   - PTH also increases the intestinal absorption of calcium by promoting the synthesis calcitriol.
3. **Calcitonin.** Calcitonin (CT) is secreted by parafollicular cells of thyroid gland. The action of CT on calcium metabolism is antagonistic to that of PTH. Thus, calcitonin promotes calcification by increasing the activity of osteoblasts. It decreases bone resorption and increases the excretion of Ca into urine. Therefore, CT has a decreasing influence on blood calcium.

Disorders

The homeostatic control of blood Ca level is mainly under the control of PTH. Therefore, abnormalities in Ca metabolism are mainly associated with alteration of PTH.

- **Hypocalcemia.** It is characterised by a fall in the serum Ca to below 7 mg/dl, which causes tetany. Neuromuscular irritability, spasms and convulsions are the main symptoms of tetany. Hypocalcemia is a serious disorder and may be life threatening.

Hypocalcemia is mostly caused by **hypoparathyroidism** (under activity of parathyroid glands). It may be caused due to surgical removal of parathyroid gland or due to autoimmune disease.

- **Hypercalcemia.** It is characterised by an increase in serum Ca level (*i.e.* above 9–11 mg/dl). Hypercalcemia caused by increased activity of parathyroid glands (hyperparathyroidism).

  Hypercalcemia causes lethargy, muscle weakness, loss of appetite, constipation, nausea, increased myocardial contractility and susceptibility to fractures.

- **Osteoporosis.** It is characterised by demineralization of bone resulting in the progressive loss of bone mass. It results from excessive reabsorption of calcium and phosphorus from the bones. Osteoporosis occurs in postmenopausal women and elderly men. It is more common in women than in men. It results in frequent bone fractures which are a major cause of disability among the elderly.

  Decreased level of estrogen is the common cause of osteoporosis. The other causative factors include hormonal imbalance like calcitonin, parathormone and deficiency of calcium and vitamin D. Administration of estrogen along with calcium (in combination with vitamin D) to postmenopausal women reduce the risk of fractures.

Phosphorus

An adult body contains about 1 kg of phosphate. About 80 percent of it occurs in combination with calcium in the bones and teeth. About 10 percent of body's phosphorus is found in muscles and blood with proteins, carbohydrates and lipids. The remaining 10 percent is widely distributed in various chemical compounds.

Phosphorus is essential for the development of bones and teeth, and is required for the formation of phospholipids, phospho-proteins and nucleic acids (DNA and RNA). It is an essential component of nucleotide coenzymes such as NAD^+, $NADP^+$, ADP, AMP and pyridoxal phosphate. It helps in maintenance of pH in the blood as well as in the cells. It plays key role in the formation and utilization of high energy phosphate compounds like ATP, GTP, creatine phosphate etc.

Milk, cereals, leafy vegetables, meat, eggs are the best sources of phosphorus.

Absorption of Phosphorus

- Absorption of phosphate occurs mainly in jejunum.
- Calcitriol promotes phosphate uptake along with calcium.
- Acidity also favours phosphate uptake by intestinal cells.
- Phytate decreases phosphate uptake by intestinal cells.

NOTES

Excretion

About 500 mg phosphate is excreted in urine per day. The reabsorption of phosphate by renal tubules is inhibited by PTH.

Serum Phosphate

The whole blood contains about 40 mg of phosphate per decilitre (40 mg/dl), while the phosphate level of serum is about 3–4 mg/dl. It is because the RBC and WBC have very high content of phosphate. The fasting serum phosphate level is higher than the post-prandial level. It is because, that after ingestion of food, serum phosphate is drawn for metabolism by the cells.

Disorders

- In diabetes mellitus, serum content of organic phosphate is lower, but that of inorganic phosphate is higher.
- Serum phosphate level is elevated in hypoparathyroidism and decreased in hyperparathyroidism.
- In vitamin D deficient rickets, the serum phosphate is low. Renal rickets is also associated with low serum phosphate content, but increased alkaline phosphatase activity.
- In severe renal disease, acidosis is caused due to high serum phosphate content.

Magnesium

About 20 g. of magnesium is present in an adult human body. About 70% of it is found in bones in combination with calcium and phosphorus. The remaining 30% occurs in the soft tissues and body fluids.

Magnesium is required for the formation of bones and teeth. Mg^{2+} ions are necessary for proper neuromuscular function and serves as cofactor for several enzymes requiring ATP.

Cereals, nuts, beans, vegetables, meat, milk and fruits are good sources of magnesium.

Absorption of Magnesium

About half of the dietary magnesium is absorbed by the intestinal mucosa. Magnesium absorption is decreased by alcohol and calcium phosphate consumption in large quantity, whereas PTH increases Mg absorption.

Serum Magnesium

In serum, about 60 percent of the magnesium occurs in ionized form, 10 percent in combination with other ions and 30 percent is bound to proteins.

Disorders

- Low level of serum magnesium may occur due to urenia, rickets and abnormal pregnancy.

- Deficiency of magnesium causes neuromuscular irritation, weakness and convulsions. The symptoms are more or less similar to that of tetany caused by Ca deficiency. Magnesium deficiency may be caused by malnutrition and alcoholism that lead to liver cirrhosis.

Sodium

Sodium is an important cation in extracellular body fluid. About 50% of it is present in the bones, 40% in the extracellular fluid and the remaining 10% in the soft tissues.

Sodium ions help in the conduction of nerve impulse, maintain electric potential across the cell membranes for selective permeability and retaining water in the body. Sodium is necessary for initiating and maintaining heartbeat, and is involved in the absorption of glucose and amino acids. Along with chloride and bicarbonate ions, sodium helps in maintaining acid-base balance in the body.

About 5–10 g of sodium is required per day for a normal individual. It is important to note that 10 g of common salt (NaCl) contains 4 g of sodium. The common salt used in the cooking medium is the major source of sodium. Food items such as grains, leafy vegetables, nuts, eggs and milk are also good sources of sodium.

Absorption of Sodium

Sodium is readily absorbed in the gastrointestinal tract hence very little of it is eliminated through faeces. However, in diarrhoea large quantities of sodium is lost in faeces.

Excretion of Sodium

Sodium is mainly excreted from the body through kidney. About 800 g of sodium is filtered by the glomeruli per day, 99% of this is reabsorbed by the renal tubules actively. A considerable amount of sodium is also lost through sweating.

Plasma Sodium

Sodium is an extracellular cation hence blood cells contain much less *i.e.*, 35 mEq/l of sodium. It is mainly present in the plasma. The normal concentration of sodium in plasma is 135–145 mEq/l.

Disorders

- **Hyponatremia.** It refers to the decreased concentration of sodium in the blood. Hyponatremia is characterised by low blood pressure and circulatory failure. It may occur due to diarrhoea, vomiting, chronic renal diseases and Addison's disease (adrenocortical insufficiency). Decreased plasma sodium concentration may also be caused in edema caused due to congestive heart failure or cirrhosis.

NOTES

- **Hypernatremia.** It refers to the increased concentration of sodium in the blood. Hypernatremia is characterised by increase in blood volume and blood pressure.

  Hypernatremia is caused due to prolonged administration of cortisone, ACTH and/or sex hormones. Hyperactivity of adrenal cortex (cushing's syndrome) and diabetes insipidus resulting in excess loss of water may also cause hypernatremia. During pregnancy, steroid and placental hormones retain sodium and water in the body, leading to hypernatremia.

Potassium

Potassium occur as principal intracellular cation but is also equally important in the extracellular fluid for specific functions. It maintain intracellular osmotic pressure and required for the transmission of nerve impulse and regulation of acid and base balance in the cells. An adequate intracellular concentration of potassium is essential for the synthesis of proteins. The activity of cardiac muscles is influenced by extracellular potassium.

About 3–4 g of potassium is the daily dietary requirement of a normal human body. Banana, orange, pineapple, potato, beans, coconut water, chicken and liver are good sources of potassium.

Excretion

Excretion of potassium occurs mainly through urine. Adrenal cortical hormone-aldosterone increases excretion of potassium.

Plasma Potassium

Since potassium is an important intracellular cation, the plasma contains potassium concentration at the level of 3.4–5.0 m Eq/l. However, the whole blood contains a much higher level of potassium *i.e.*, 50 mEq/l.

Disorders

- **Hypokalemia.** It refers to a decreased concentration of plasma potassium. It may be caused due to overactivity of adrenal cortex (cushing's syndrome), prolonged cortisone therapy, intravenous administration of K^+ free fluids, administration of insulin to treat diabetic coma or prolonged diarrhoea and vomiting.

  Hypokalemia is characterised by muscular weakness, irritability, tachycardia (abnormal rapidity of heart action), cardiomegaly (hypertrophy of the heart) and cardiac arrest. Flattened waves with inverted T wave are observed in ECG of a affected person.
- **Hyperkalemia.** It refers to an increased concentration of serum potassium. It may be caused due to renal failure, decreased activity of adrenal cortex (Addison's disease), diabetic coma, severe dehydration or intra-venous administration of fluids with excessive potassium salts.

Hyperkalemia is characterised by mental confusion, numbness, bradycardia (slow heartbeat) with reduced heart sound and finally cardiac arrest. Elevated T-waves are observed in ECG of the affected person.

NOTES

Chlorine

Chlorine and sodium are intimately related, because chlorine is a constituent of common salt (sodium chloride).

Chloride ions are important components of blood plasma and help in the maintenance of its pH, and are involved in the regulation of acid-base equilibrium, fluid balance and osmotic pressure. Chloride ions are necessary for the formation of HCl in stomach. Acidic medium is essential for digestion of food in the stomach.

About 5–10 g of chloride as NaCl is the daily dietary requirement of normal human body. It is obtained as common salt and from grains, leafy vegetables, eggs and milk.

Absorption

Absorption of chloride occurs in the gastrointestinal tract. Under normal conditions, almost all the chloride is absorbed in the intestine.

Excretion

Chloride is excreted through urine. The renal threshold for Cl^- is about 110 mEq/l. A small amount of chloride is also lost through sweat.

Plasma Chloride

The normal concentration of plasma chloride is 95–105 mEq/l. However, cerebrospinal fluid (CSF) contains higher level of chloride (*i.e.*, 125 mEq/l) so as to maintain membrane donnan equilibrium as CSF contains a low concentration of proteins.

Disorders

- **Hypochloremia.** It refers to decreased chloride plasma concentration. Hypochloremia may be caused due to vomiting, diarrhoea, respiratory alkalosis, excessive sweating and Addison's disease.
- **Hyperchloremia.** It refers to an increased concentration of plasma chloride. Hyperchloremia may be caused due to dehydration, respiratory acidosis and cushing's syndrome.

Sulphur

Most of the body's sulphur is present in proteins that contain sulphur containing amino acids—methionine, cysteine and cystine.

A number of vitamins *e.g.* thiamine, biotin, lipoic acid and coenzyme A of pantothenic acid contain sulphur. The disulphide linkage (S–S) and sulfhydryl groups (–SH). Provide structural conformation and functional

NOTES

activity to a number of proteins. Heparin (an anticoagulant present in the blood), chondroitin sulphate, glutathione, taurocholic acid are some other important sulphur containing compound present in the body.

Food items containing proteins with sulphur containing amino acids methionine and cysteine are the sources of sulphur. Adequate intake of sulphur containing amino acids meets the body's requirement for sulphur.

Sulphur is excreted from the body through urine in the form of sulphate and sulphur containing amino acids.

5.4 MICROELEMENTS

Iron

Iron occurs in the body as a constituent of several proteins/enzymes such as haemoglobin, myoglobin, cytochromes, xanthine oxidase, catalase, peroxidase, ferritin, transferrin etc.

Haemoglobin is involved in the transport of O_2 and CO_2, myoglobin store O_2 in the muscles, cytochromes and certain non-heme proteins are involved in electron transport chain and oxidative phosphorylation. Iron is associated with effective immuno competence of the body.

An adult man requires 10 mg of iron per day, mensturating woman 18 mg of iron per day and pregnant and lactating woman 40 mg of iron per day.

Organ meats (such as liver, heart, kidney) are rich source of iron. Pulses; cereals, leafy vegetables; apple, fish and molasses are good source of iron.

Absorption

In foods, iron is mostly found in ferric (Fe^{3+}) form, bound to proteins or organic acids. In stomach, HCl of gastric juice releases iron from the food. Reducing compounds like ascorbic acid (vitamin C) and cysteine convert ferric ion (Fe^{3+}) to ferrous ion (Fe^{2+}). Ferrous form of iron is soluble and is readily absorbed in the stomach and duodenum.

Factors Promoting Fe Absorption

- Absorption of iron increases in low pH (acidity) and in presence of ascorbic acid and cysteine.
- Fe absorption is increased to 2–10 times that of normal in case of iron deficiency anemia.
- Amino acids and small peptides favour iron absorption.

Factors Inhibiting Fe Absorption

- Fe absorption is decreased when the diet is rich in phosphate.
- Oxalate (present in leafy vegetables) and phylate (present in cereals) interfere with Fe uptake.

- Fe absorption is severely impaired in patients undergone stomach or intestinal surgeries.

Transport and Metabolism

Iron is absorbed in ferrous form (Fe^{2+}) by the mucosal cells of GIT (Gastro Intestinal Tract). The absorbed iron is oxidised to ferric form (Fe^{3+}) by the enzyme *ferroxidase*. It then combines with apoferritin to form ferritin and is stored temporarily. From the mucosal cells, iron may enter the blood stream.

From mucosal cells iron enters plasma in ferrous form (Fe^{2+}) and is oxidised by a copper containing protein, ceruloplasmin having *ferroxidase* activity. There is another copper containing protein ceruloplasmin (*ferroxidase II*) to convert Fe^{2+} to Fe^{3+}. Here, Fe^{3+} binds with a specific iron binding protein called transferrin (siderophilin). The plasma contains 250 mg of transferrin/dl which can bind with 400 mg of iron. Thus, the Total Iron Binding Capacity (TIBC) of plasma is 250 mg/dl.

Storage of Iron

Iron is stored in iron storing proteins—**ferritin** and **hemosiderin.** Ferritin can hold about 25% of iron by weight, whereas, iron content of hemosiderin is about 35%. Iron is stored in liver and spleen in the form of ferritin and hemosiderin, in the bone marrow as haemoglobin (Hb) in muscles in the form of myoglobin (Mb) and some other tissues as cytochromes (cyt) and non-heme iron (NH1). In mucosal cells, ferritin is the temporary storage form of iron. In case of the supply of iron is in excess of the body's demand, it is stored in the form of hemosiderin in spleen and liver (Fig. 5.2).

Iron is very efficiently utilized and reutilized by the body. The loss from the body is minimum (*i.e.*, < 1mg/day) which may occur through bile, sweat and menstrual flow, and due to hair loss. Unlike other inorganic and organic substances, iron neither inactivated or excreted through urine.

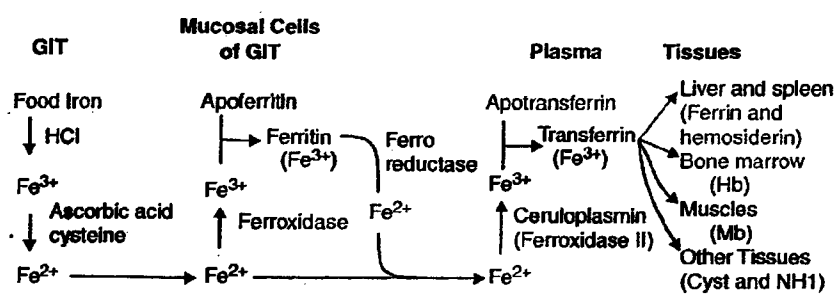


Fig. 5.2. *Absorption, transport and storage of iron.*

NOTES

Generally, a constant level of Fe is maintained in the body tissues. About 1–2 mg of iron is absorbed per day to replace the loss. A generalised metabolism of iron is shown in Fig. 5.3.

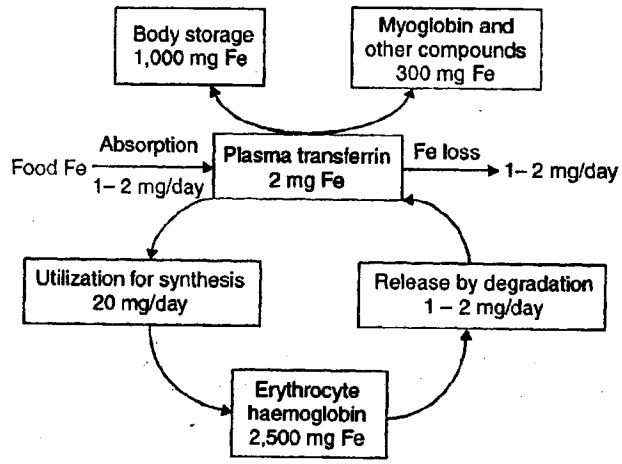


Fig. 5.3. *A general view of iron metabolism.*

Disorders

- **Iron Deficiency Anemia.** It is a nutritional disorder prevalent worldover. It is caused due to inadequate intake or defective absorption of iron, chronic blood loss, repeated pregnancies and hookworm infections.

  Iron deficiency anemia is characterised by reduced blood haemoglobin level (*i.e.*, 12 g/dl of blood), which is manifested with sluggish metabolic activities, retarded growth, loss of appetite and apathy (dull and inactiveness). This condition is often described as **microcytic hypochromic anemia.**

  Iron deficiency anemia is more common in growing children, adolescent girls, pregnant and lactating women. Vegetarians are more prone to iron deficiency anemia because of the presence of iron absorption inhibitors in the vegetarian foods, besides the relatively low content of iron.

- **Hemosiderosis.** It is a condition characterised by the deposition of excessive iron in the body, especially in liver and spleen in the form of hemosiderin. It occurs in case of diseases in which there is marked Red Blood Corpuscle (RBC) destruction such as hemolytic anemia, pernicious anemia and chronic infection.

  Hemosiderosis is commonly observed among the Bantu tribe in South Africa. It is due to high iron content in their staple food and cooking of the foods in iron vessels.

- **Hemochromatosis.** It is a disorder related to iron metabolism in which iron accumulates in body tissues. The liver becomes

enlarged, the skin is bronze pigmented, and there is diabetes and frequent cardiac failure. Hemochromatosis is also described as bronze diabetes.

NOTES

Copper

Copper occurs as constituent of several enzymes and other biomolecules. The human body contains about 100 mg copper distributed in different organs, and is involved in several functions.

Copper is a constituent of a number of enzymes including cytochrome oxidase, catalase, tyrosinase, ascorbic acid oxidase, superoxide dismutase, monoamine oxidase, ALA synthase, phenol oxidase and uricase, hence involved in a number of metabolic activities. Copper is required for the synthesis of haemoglobin (ALA synthase required heme synthesis), melanin and phospholipids. It is needed for the formation of myelin sheet and development of bone. Hemocyanin, a copper containing protein complex present in some invertebrates helps in oxygen transport. Copper containing ceruloplasmin is involved in conversion of iron from Fe^{2+} to Fe^{3+} to be stored in body tissues. Certain copper containing proteins viz. hepatocuprein (in liver), cerebrocuprein (in brain) and hemocuprein (in RBC) with unknown functions are known to occur in the body.

The requirement of copper for an adult is 2–3 mg/day while for infants and children is 0.5–2 mg/day. Liver, kidney, meat, egg yolk, cereals, nuts and green leafy vegetables are the good source of copper.

Absorption

Copper is absorbed mainly in duodenum. About 10% of the food Cu is absorbed in the duodenum and is facilitated by a transport protein, metallothionein. Copper uptake is decreased by phylate, zinc and molybdenum.

Plasma Copper

Plasma contains 100–200 mg/dl of copper. About 95% of it is bound to ceruloplasmin and the remaining is loosely held to albumin.

Disorders

- **Menke's disease.** It is caused due to defecting intestinal absorption of copper, and is characterized by the decreased copper in plasma and urine, anemia and greying (depigmentation) of hair.
- **Wilson's disease.** It is caused due to abnormal copper metabolism. It is characterized by deposition of copper in liver and lenticular nucleus of brain, leading to hepatic cirrhosis and brain necrosis. Deposition of copper in kidney leads to excretion of amino acids, glucose, peptides and haemoglobin in urine resulting in renal failure. Low levels of copper and ceruloplasmin in plasma with increased excretion of copper in urine. Copper absorption increases by 4–6 times than the normal in the intestine.

NOTES

Failure of ceruloplasmin or/and impairment of binding capacity of copper to this protein seem to be propable cause of Wilson's disease. As a result, free plasma copper enters the tissues *i.e.*, liver, brain, kidney, etc., and get deposited there. Reduced intestinal excretion of copper may be responsible for Wilson's disease.

- **Deficiency of copper.** It causes demineralization of bones, anemia, demyelination of neural tissue, fragility of arteries, mycocardial fibrosis, hypopigmentation of skin and greying of hair.

Iodine

Human body contains about 20 mg of iodine. Most of it is present in thyroid gland. Small amount of iodine is also present in muscle, salivary glands and ovaries.

Iodine is required, for the synthesis of thyroxine hormones—thyroxine (T_4) and tri-iodothyronine (T_3).

The requirement of iodine for adults is 100–150 μg/day and that of pregnant woman is 200 μg/day. Sea foods, drinking water, vegetables and fruits grown in coastal areas are rich source of iodine. In hilly regions, underground water and soil are deficient of iodine. Therefore, plant and animal foods of these regions are deficient of iodine. Iodised salt (common salt supplemented with iodine) is recommended for consumption for these regions.

Absorption and Excretion

Small intestine is the main site of iodine uptake. About 30% of dietary iodine is absorbed by the intestinal cells. Iodine is mainly stored in the form of glycoprotein, iodothyroglobulin in the thyroid gland. The protein contains thyroxine, di-iodotyrosine and tri-iodothyronine. Iodine is mostly excreted through urine by the kidney. Loss of iodine also occur through saliva, bile and lactation.

Plasma Iodine

Plasma iodine present mainly in the circulating thyroid hormones. The normal concentration of plasma iodine is 4–10 mg/dl. Its concentration decreases in hypothyroidism and increases in hyperthyroidism.

Disorders

- **Hyperthyroidism (Exophthalmic Goitre).** It is caused due to over secretion of thyroid hormone, due to cancer of thyroid gland or due to development of nodules of thyroid gland. The excess of thyroid hormones increase metabolic rate and accelerates oxidation.

  Hyperthyroidism is characterised by a peculiar oedema (fluid accumulation) behind the eyes called exophthalmia (protrusion

of eye balls). Weight loss, slight rise in body temperature, excitability, tachycardia (rapid heartbeat), nervousness and restlessness are some other symptoms of hyperthyroidism.

Hypothyroidism. It is a disorder of infants and children caused due to low secretion of thyroid hormone. It can result from primary failure of thyroid gland, inadequate supply of iodine in the diet.

Hypothyroidism leads to cretinism (dry skin, dwarfism and mental retardation), myxoedema (accumulation of interstitial fluid and fats) and simple goitre (enlargement of thyroid gland).

Manganese

Human body contains about 15 mg of manganese Mn. It is found mainly in association with nucleic acids in the cells. Liver and kidney are rich in Mn content.

Manganese acts as cofactor for a number of enzymes such as pyruvate carboxylase, isocitrate dehydrogenase, arginase, dismutase and peptidase. It is required for the synthesis of mucopolysaccharides and glycoproteins, and is involved in synthesis of hemoglobin and cholesterol.

About 2–9 mg/day of Mn is recommended for an adult person. Cereals, nuts, leafy vegetables, fruits and tea are rich sources of manganese.

Absorption

Manganese is absorbed in the small intestine. About 3–4% of dietary Mn is absorbed by the intestinal epithelium. Iron inhibits Mn absorption.

Plasma Manganese

The normal plasma manganese concentration is 5–20 mg/dl. It is mainly present in a carrier protein transmagnanin (α β - globulin).

Disorders

Manganese deficiency results in retarded growth, bone deformities, decreased insulin secretion (low activity of β-cells of pancreas) and increased activity of serum alkaline phosphatase and sterility.

Zinc

About 2 g of zinc is present in an adult human being mainly as intracellular element. The highest concentration of zinc (*i.e.*, about 100 mg/g) in prostate gland.

Zinc is an essential component of several enzymes such as carbonic anhydrase, alcohol dehydrogenase, alkaline phosphatase, carboxy peptidase, dismutase etc. Zinc being a component of enzyme dismutase protects the body against free radical damage hence acts as an **antioxidant.** It is required for the secretion and storage of insulin, and is ncessary to

NOTES

maintain the normal level of vitamin A in serum. Zinc promotes cell growth and division, thus, helps in wound healing. A zinc containing protein, gusten present in saliva helps in taste sensation. Zinc is also essential for proper reproduction.

The dietary requirement of zinc for an adult is 10–15 mg/day. Its requirement is increased during pregnancy and lactation. Fish, egg, meat, milk, beans and nuts are the good source of zinc.

Absorption

Duodenum is the main site of Zn absorption. Zinc is better absorbed from the animal sources. Amino acids and small peptides promote Zn absorption, while calcium, copper, iron phytate interfere with Zn absorption.

Plasma Zinc

The concentration of zinc in plasma is about 100 mg/dl. A fairly high content of zinc (*i.e.*, 1.5 mg/dl) is present in the RBCs in association with the enzyme carbonic anhydrase.

Disorders

- Deficiency of zinc causes poor wound healing, loss of appetite and taste sensation, impaired spermatogenesis and growth retardation. It may result in depression, dementia and other psychiatic disorders.
- Zinc toxicity is caused due to inhalation of zinc oxide fumes and often observed in workers engaged in welding and metal fabrication works. The symptoms of Zn toxicity include nausea, gastric ulcer, pancreatitis, anemia and excessive salivation.

Molybdenum

Molybdenum is a constituent of some enzymes like xanthine oxidase, aldehyde oxidase and sulfite oxidase. Molybdenum is sufficiently present in natural foods, and is absorbed by the small intestine.

Excessive consumption of molybdenum causes a rare disorder, **molybdenosis.** It results in impairment of growth, diarrhoea and anemia.

Cobalt

Cobalt is a constituent of cobalamin (vitamin B_{12}). Cobalt as a constituent of vitamin B_{12} is required for the process of erythropoiesis (formation of erythrocytes). Cobalt toxicity causes polycythemia (increased count of RBCs in the blood).

Fluorine

Fluorine occurs in the body mainly as fluoride in bones and teeth. It has beneficial effect only in trace amount, excess consumption is hazardous to health.

Fluoride forms a acid resistant layer of fluoroapatite with hydroxyapatite of the enamel and prevent dental caries (tooth decay) caused by bacterial acid. It is necessary for the proper development of bones.

Drinking water is the main source of fluoride. An intake of less than 2 ppm of fluoride in water is sufficient to meet the daily requirements.

Disorders

- **Dental caries (Tooth decay).** It is associated with consumption of water less than 0.5 ppm of fluoride. Fluoridation of water and the use of toothpastes prevent dental caries in children.
- **Fluorosis.** It is caused due to excessive intake of fluoride. An intake of fluoride above 2 ppm in children causes discolouration of teeth and mottling of enamel. The teeth become weak and rough with brown or yellow patches on their surface. This condition is referred as **dental fluorosis.**

  The consumption of fluoride above 20 ppm is toxic, which causes hyper calcification of bones, stiff joints and neurological disturbances. This condition is referred as skeletal fluorosis.

Selenium

Selenium is biologically important only in small amounts with vitamin E, selenium prevents the development of muscular dystrophy and hepatic necrosis. It protects the body against carcinogenic chemicals. Se, prevent lipid peroxidation and protects the cells against free radicals.

Disorders

- Selenium deficiency leads to muscular dystrophy, pancreatic fibrosis and reproductive disorders.
- **Selenosis** refers to selenium toxicity caused due to excessive intake of Se. It results in weight loss, diarrhoea, hair loss and bad breath.

Chromium

About 6 mg of chromium is present in human body. Chromium is a constituent of protein **chromodulin** which helps in binding of insulin to cell receptor sites and thus promotes glucose utilization. It participate in the transport of amino acids and lowers serum cholesterol level.

About 10–100 mg/day is the daily requirement, chromium, which can be obtained from cereals, yeast, cheese and meat.

Deficiency of Cr disturbs carbohydrate, lipid and protein metabolism. Excess of Cr may damage liver and kidneys.

STUDENT ACTIVITY

1. What is the importance of calcium in human body?

2. How do calcium affect PTH secretion?

NOTES

5.5 SUMMARY

- Minerals are required for normal growth and maintenance of the body. They are classified as macrominerals like Ca, P, Mg, Na, K, Cl and S and microminerals such as Fe, Cu, I, Zn, Mn, Mo, Co, Fe, Se and Cr.
- Calcium is required for the development of bones and teeth, muscle contraction, nerve impulse conduction, blood coagulation and many more activities. Absorption of calcium is promoted by acidity, vitamin D and PTH, while oxalate, phytate, free fatty acid and fibres inhibit its absorption. Plasma calcium is regulated by PTH, calcitriol and calcitonin.
- Phosphorous is essential for the development of bones and teeth, sodium potassium and chlorine play a role in the regulation of acid-base equilibrium, fluid balance and osmotic pressure in the body. Iron is required for the formation of haemoglobin, myoglobin and several enzyme proteins. Iron deficiency results in anemia, while excess of it causes hemosiderosis. Copper is an essential constituent of several enzymes. Iodine is an important constituent of thyroid hormones, while cobalt is a constituent of vitamin B_{12}. Zinc is essential for secretion and storage of insulin and maintenance of normal level of vitamin A in the plasma. Fluorine in trace amount prevent dental caries, while its higher intake results in fluorosis. Selenium protects the cells from free radicals. Chromium promotes the utilization of glucose and reduces serum cholesterol.

5.6 GLOSSARY

- **Anaemia:** Reduction in the number of red blood cells, packed cell volume or circulating haemoglobin, resulting in paler appearance.
- **Bone Matrix:** The protein ground substance of bones in which minerals are deposited.
- **Calcification:** Process by which an organic tissue becomes hardened by a deposit of calcium suits.
- **Cretinism:** Dwarfism usually caused in the infant due to severe depletion of iodine of the mother in her pregnancy.
- **Dementia:** Mental disorder resulting in impairment of transfer of nerve impulses.
- **Element:** Anyone of the fundamental atoms of which all matter is composed.
- **Enamel:** Calcified tissue covering of a teeth.
- **Gastrointestinal:** Part of the digestive system made up of stomach and intestines.

NOTES

- **Hypertension:** Refers to high blood pressure or higher than normal blood pressure.
- **Hypothyroidism:** Condition in which thyroid gland secretes too little thyroxine.
- **Iodised Salt:** Table salt to which potassium and sodium iodine and a very small amount of magnesium carbonate is added.
- **Osmotic Pressure:** The pressure that causes water/any other solvent to move from a solution with low concentration of solute to one having high concentration of solute.
- **Phytate:** Salt of phytic acid a phosphorus containing compound formed in the outer layer of cereals.
- **Thyroxine:** Hormone containing iodine produced by thyroid gland.

5.7 REVIEW QUESTIONS

I. Very Short Answer Type Questions:

1. What is the disorder caused due to hypocalcemia?
2. Define the following:
   (*i*) Hyponatremia, (*ii*) Hyperkalemia
   (*iii*) Hypochloremia
3. Name the elements that act as antioxidant.

II. Short Answer Type Questions:

1. What factors affect absorption of calcium?
2. Explain in the regulation of calcium level in the plasma.
3. Describe the disorders related with the calcium.
4. Write notes on:
   (*i*) Osteoporosis (*ii*) Wilson's disease
   (*iii*) Factors affecting Fe absorption
5. Write the role of sodium and potassium in the human body.
6. Write a note on the disorders related to iodine.
7. Write the roles of the following in the body.
   (*i*) Zinc (*ii*) Molybdenum
   (*iii*) Selenium (*iv*) Chromium

III. Long Answer Type Questions:

1. Give an account of biochemical functions, dietary requirement and absorption of calcium.
2. Write an essay on iron metabolism in the body.
3. Give an account of importance and disorders of fluorine.
4. Describe the metabolism zinc and manganese.

5.8 FURTHER READINGS

- **Biochemistry:** Stryer L.; W.H. Freeman and company; New York, 1981.
- **Biochemistry;** Satyanaryan V., Chakrapani V.; Books and Allied (P) Ltd. Kolkata, 2008.
- **Outlines of Biochemistry;** Conn E.E., Stumpf P.K., Bruening G., Doi R.H.; Wiley India (P) Ltd, New Delhi, 2007.
- **Essentials of Food and Nutrition;** Swaminathan M.; Ganesh Madras, India; 1985.

UNIT–III

NOTES

CHAPTER 6 VITAMIN METABOLISM

OBJECTIVES

After going through this chapter, you should be able to:

- describe fat soluble vitamins
- explain vitamin A, D, E, K
- describe water soluble vitamins.

STRUCTURE

6.1 INTRODUCTION

Vitamins are organic compounds of diverse structure, which are not used for energy or tissue framework, but are required in minute amounts for the normal functioning of living organisms. **Dr. Casimir Funk** (1911) for the first time found that food, apart from ordinary nutrients contains substances that are essential for normal functioning of the animal body. He named these substances as **Vitamins.**

Vitamins have been defined as *"a group of organic substances which function catalytically in cellular metabolism or act as accessory food factors."* Plants can make vitamins from simple substances, but animals are unable to synthesize them. Animals must obtain vitamins from their food. Fresh fruits and raw vegetables are rich source of vitamins.

Vitamins are broadly classified into two groups: **fat soluble vitamins,** which include vitamins A, D, E and K; and **water soluble vitamins** that include vitamin B complex and C.

In the following pages individual vitamins with respect to biochemical functions and metabolisms are discussed.

NOTES

6.2 FAT SOLUBLE VITAMINS

Vitamin A

The term vitamin A is collectively used to represent many structurally related and biologically active molecules. The term **retinoids** is often used to include the natural and synthetic forms of vitamin A. Retinol, retinal and retinoic acid are considered as vitamers of vitamin A. β-carotene also called provitamin A is a precursor of retinal and is found in plant foods.

Absorption and Mobilization

The retinyl esters present in food are hydrolysed by the pancreatic or intestinal hydralases to release retinol and free fatty acids. Carotenes of the food are hydrolysed by the enzyme β-*carotene 15-15-dioxygenase* to release two molecules of retinal which is reduced to retinol. In the intestinal mucosal cells, retinol is re-esterified to long chain fatty acids, incorporated into chylomicrons and transferred to the lymph. The retinal ester of chylomicrons are transported to the liver and stored as retinyl palmitate.

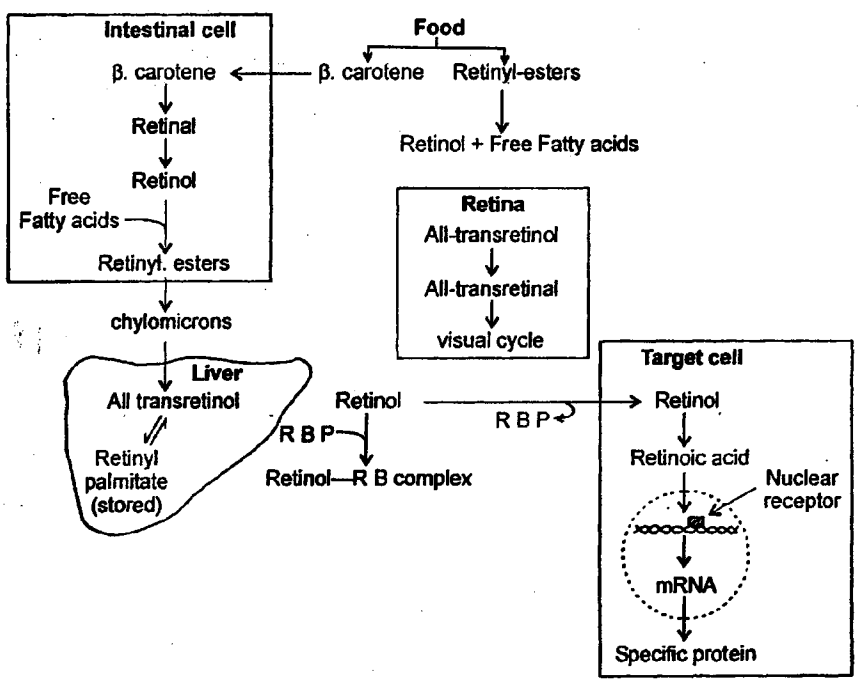


Fig. 6.1. *Absorption and mobilization of vitamin A.*

Vitamin A is released from the liver as free retinol, when required. The transportation of retinol in the circulation occurs by the **plasma retinol**

NOTES

binding protein (RBP) in association with pre-albumin. The retinol-RBP complex binds to specific receptors on the cell membrane of the target tissues that carry retinol to the nucleus and binds to the chromatin and exert its function analogous to a steroid hormone (Fig. 6.1)

Biochemical Functions

Vitamin A is required for a variety of functions, including vision, growth, reproduction and differentiation, reproduction and maintenance of epithelial cells.

- ***Vision***

The retina of eye possesses two type of visual receptors—**rods and cones.** Human eye has about **110–125** million rods and 6–6.5 million cones. The rods contain **rhodopsin** (= visual purple) pigment, and enable a person to see in darkness. The cones contain **iodopsin** (= visual violet) pigment and are chiefly concerned with the distinction of colour and light vision during daytime.

Biochemistry of Vision. Light splits rhodopsin (visual purple) into a pigment **retinol** (= retinene). An aldehyde derivative of vitamin A, and a protein **scotopsin** (opsin). The process of splitting is called **bleaching.** This depolarizes the rod cells to release a neurotransmitter, transmitting the nerve impulse to the bipolar cells, ganglion cells and then to the optic nerve fibres. In night, light is received from the moon and stars. In the dark, rhodopsin is resynthesized from retinol and scotopsin making rods functional.

Rhodopsin $\xrightarrow{\text{Bleaching}}$ Retinol + Scotopsin + Energy $\longrightarrow$ Nerve impulse

In Darkness. Retinol + Scotopsin + Energy from ATP $\longrightarrow$ Rhodopsin.

Resynthesis of rhodopsin takes some time so when we go suddenly from bright light into darkness or semidarkness, we can see things only after a few minutes. Similarly, when we go from darkness into bright light we remain blinded for a few minutes till rhodopsin depleted cones to become active visual cells.

As vitamin A is an important constituent of retinol, hence its deficiency causes **night blindness** (nyctolopia).

Colour Vision. The pigment iodopsin (visual violet) work in day light and artificial light. This pigment is sensitive to bright light and colours. It is considered that there are three different kinds of cones, each of which contains a different light sensitive pigment. (*i*) cones containing **erythrolable,** are most sensitive to red light, (*ii*) cones containing **chlorable,** are most sensitive to green light, and (*iii*) cones containing **cyanolable** are

most sensitive to blue light. Combinations of these three colours of light produce all the colours human can see.

- Retinol and retinoic acid function almost like steroid hormones. They regulate the protein synthesis and thus are involved in cell growth and differentiation.
- Retinol and retinoic acid prevent keratin synthesis and thus help in maintaining healthy epithelial tissue.
- Vitamin A is required to maintain proper immune system to fight against various infections.
- Vitamin A is required for the synthesis of cholesterol. In absence of vitamin A, mevalonate (an intermediate in cholesterol synthesis) is diverted to co-enzyme Q synthesis.
- Retinol and retinoic acid are involved in the synthesis of a iron transport protein, **transferrin.**
- Carotenoids, the precursor of vitamin A act as antioxidants and reduce the risk of cancers and heart attack.

Vitamin D

Vitamin D is a fat soluble vitamin. It resembles sterols in structure, and functions like a hormone. Ergocalciferol (also called vitamin D_2) and cholecalciferol (also called vitamin D_3) are the sources of vitamin D, and are often referred to as provitamins. Ergocalciferol is synthesized from ergosterol and is present in plants. Cholecalciferol is found in animals. Cholecalciferol is formed from 7. dehydrochole-sterol (an intermediate of cholesterol biosynthesis) on exposure to sunlight. Vitamin D is regarded as **sun-shine vitamin.** The synthesis of vitamin D in the skin is proportional to the exposure to sunlight. Dark skin (melamine pigment) adversely influences the synthesis of cholecalciferol.

Absorption and Storage

Small intestine is the site of vitamin D absorption. The absorbed vitamin D reaches to lymph and circulates in the body bound to plasma α_2. globulin. Small amounts of vitamin D is stored in liver and other tissues.

Metabolism

Calcitriol is the active form of vitamin D. It is formed from cholecalciferol. In liver, the cholecalciferol is first hydroxylated to 25-hydroxy cholecalciferol (25 OH D_3) with the help of enzyme **hydroxylase.** The 25 OH D_3 is the major storage and circulatory form of vitamin D. In kidney, 25 hydroxycholecalciferol is further hydroxylates at position 1 to form 1, 25-hydroxycholecalciferol (1, 25-DHCC), with the help of enzyme *25-hydroxycholecalciferol 1-hydroxylate.* The 1, 25-DHCC is commonly called

NOTES

calcitriol due to the presence of 3 hydroxyl groups at 1, 3 and 25 carbons. The both hydroxylase enzymes *i.e.*, of liver and kidney require cytochrome P_{450}, NADPH and molecular oxygen for the hydroxylation process (Fig. 6.2).

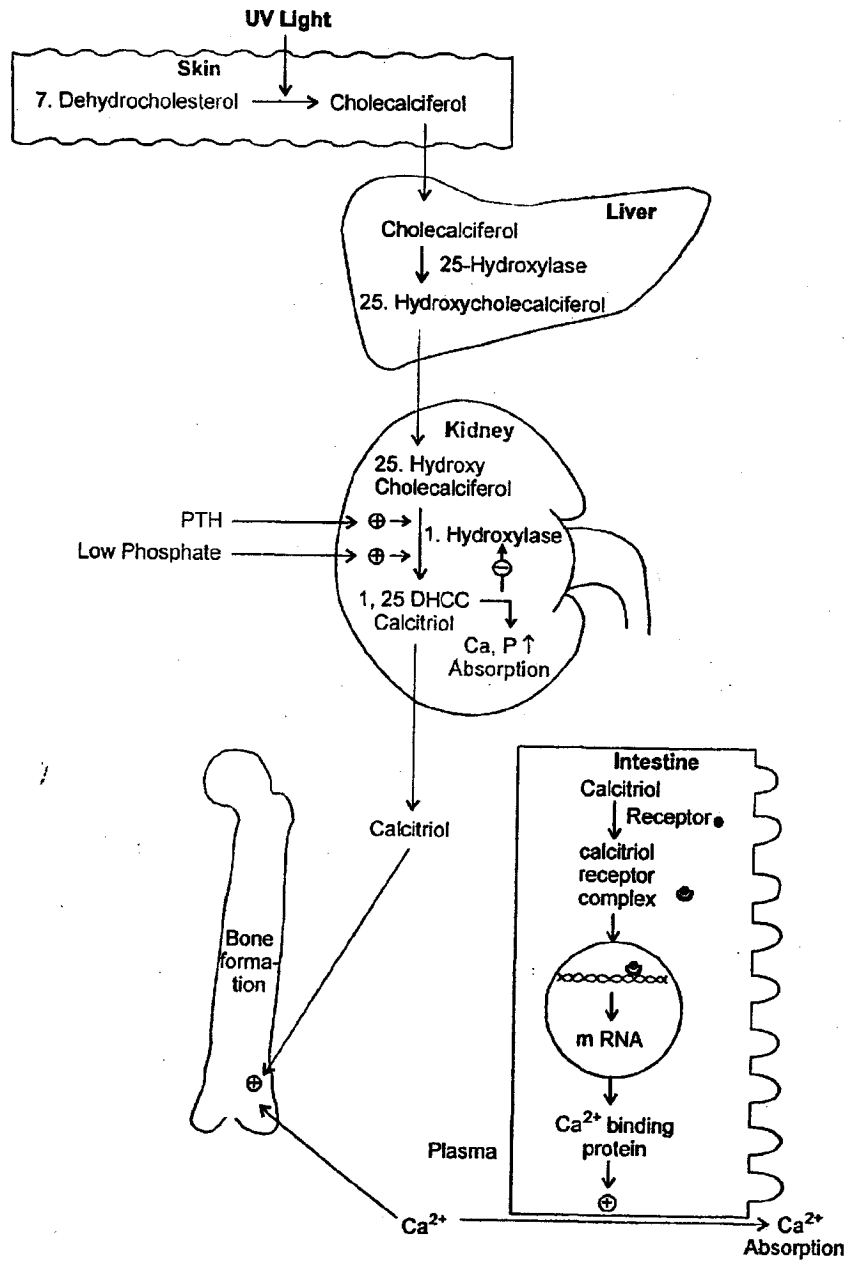


Fig. 6.2. *Metabolism of vitamin-D.*

NOTES

Regulation of Calcitriol Synthesis

The plasma levels of calcium and phosphate regulate the concentration of 1, 25 DHCC by controlling hydroxylation reaction at position 1. The low plasma phosphate increases the activity of *25-dihydroxychole-calciferol 1-hydroxylase.* Low plasma calcium enhances the production of parathyroid hormone (PTH), which in turn activates *1-hydroxylase.* Thus, the action of phosphate on the enzyme is direct, while that of calcium is indirect.

Biochemical Functions

Plasma levels of calcium and phosphate the 1, 25-DHCC (Calcitriol) is the biologically active form of vitamin D. It regulates the plasma levels of calcium and phosphate. It maintains plasma calcium level normal *i.e.*, 9–11 mg/dl. To regulate plasma calcium and phosphate levels, calcitriol acts at three levels *i.e.*, intestine, kidney and bone.

- It the intestinal cells, calcitriol binds with a cytosolic receptor to form a calcitriol-receptor complex. This complex enters the nucleus and interacts with a specific DNA to synthesize **calcium binding protein.** This protein **increases the calcium** uptake by the intestine. The action of calcitriol on intestinal cells in analogous to steroid hormone.
- In kidney, calcitriol is involved in minimizing the excretion of calcium and phosphate by decreasing their elimination and enhancing reabsorption.
- The calcitriol is essential for bone formation. It stimulates calcium uptake by osteoblasts for deposition as calcium phosphate. Bones are important reservoir of calcium and phosphate. Along with parathyroid hormone (PTH), calcitriol increases the mobilization of calcium and phosphate from the bones. This causes elevation of calcium and phosphate levels in plasma.

Calcium homoeostasis. In kidney, a metabolite of vitamin D called 24, 25-Dihydroxycholecalciferol (24, 25-DHCC) by the enzyme **24-hydroxylate.** It is believed that under adequate concentration of calcitriol, **24-hydroxylase** produces a less important compound 24, 25-DHCC so as to maintain calcium homoeostasis.

Vitamin E

Vitamin E represents to a group of **tocopherols and tocotrienols.** There are about eight tocopherols (vitamin E vitamers), identified as α, β, γ, δ etc. Tocopherols are the derivatives of *6-hydroxychromane* (tocol) ring with isoprenoid side chain. The chromane ring provides antioxidant property to vitamin E.

NOTES

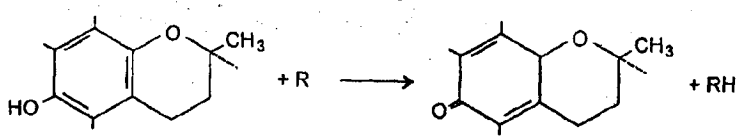
Absorption and Storage

Small intestine is the site of vitamin E absorption. Vitamin E is absorbed along with fats, hence bile salts are required for its absorption. The normal plasma level of tocopherol is less than 1 mg/dl. It is incorporated into lipoproteins (VLDL and LDL) in liver for transportation. It is stored in adipose tissues, liver and muscles.

Biochemical Functions

Most of the biochemical functions of vitamin E are related to its antioxidant property. It prevents the non-enzymatic oxidations of various cell components by molecular oxygen and free radicals such as super oxide (O_2^-) and hydrogen peroxide (H_2O_2). The element **selenium** helps in the antioxidant functions of vitamin E. It is a component of the enzyme ***glutathione peroxidase*** that destroys free radicals. Thus, selenium and vitamin E act synergistically in preventing non-enzymatic oxidation. The important antioxidant properties of vitamin E are given, below.

- Vitamin E is lipophilic and is found in association with lipoproteins, fat deposits and cellular membranes. It protects polyunsaturated fatty acids (PUFA) from per oxidation reaction by free radicals by getting it self-oxidised into quinone form.



Vitamine E — Vitamine E (oxidised)

- It is essential for maintaining the structure and integrity of cells membrane, hence is regarded as a membrane anti-oxidant.
- Vitamin E maintains germinal epithelium of gonads for proper reproductive function and prevents sterility.
- It protects erythrocytes (RBCs) from hemolysis caused by oxidising agents like H_2O_2.
- Vitamin E increases the synthesis of heme by enhancing the activity of enzymes *δ-aminolevulinic acid (ALA) synthase* and ALA *dehydratase*.
- It is believed to stabilize co-enzyme Q of electron transport chain, hence required for cellular respiration.
- It is required for optimal absorption of amino acids from the intestine, and proper storage of creatine in skeletal muscles.
- Vitamin E prevents the oxidation of vitamin A and carotenes.
- It protects liver from harmful effects of toxic compounds like carbon tetrachloride.

- In association with vitamins A, C and β carotene, vitamin E delay the onset of cataract.

NOTES

Vitamin K

Vitamin K is needed for the synthesis of blood clotting factors essential for coagulation (= roagulation) of blood, hence the name K for the vitamin. It is the only fat soluble vitamin with specific co-enzyme function.

Vitamin K exists in different forms. All the forms of vitamin K are naphthoquinone derivatives. Phylloquinone (referred as K1) occurs in plants, menaquinone (referred as K2) is produced by the intestinal bacteria and is also found in animals, and menadione (called K3) is a synthetic form.

Absorption and Storage

Vitamin K is obtained from the food or synthesize by some bacteria present in the intestine. The absorption of vitamin A takes place along with fat (chylomicrons) and requires bile salts. The absorbed vitamin K is transported with LDL and is stored mainly in liver. Small amounts of vitamin K is also stored in other tissues.

Biochemical Functions

Vitamin K is involved mainly in blood clotting process. It brings about modification of certain blood clotting factors (proteins) after their synthesis called post translational modification. The clotting factors II (prothrombin), VII, IX and X are synthesized in the form of inactive precursors or zymogens in the liver. Vitamin K acts as co-enzyme for the enzyme **carboxylase,** which causes carboxylation of glutamic acid (Glu) present in clotting factors (proteins) to γ-carboxy-glutamate (Gla). The reaction requires O_2 and CO_2 in addition to vitamin K. Dicumarol (an anticoagulant found in spoilt sweet clover) and warfarin (a synthetic analogue of dicumarol) can inhibit the action of vitamin K in post translational modification (Fig. 6.3).

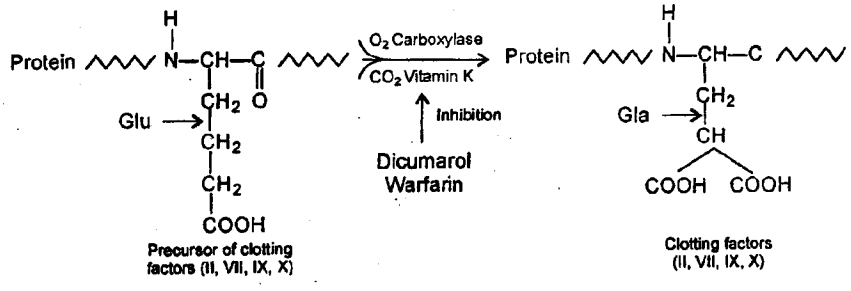


Fig. 6.3. *Role of vitamin K in post translation modification of clotting factors.*

The γ-carboxy-glutamic acid (Gla) residues present in clotting factors are negatively charged (COO^-), which bind with positively charged calcium

NOTES

ions (Ca^{2+}) to form a complex. For example, clotting factor, prothrombin combine with Ca^{2+} to form prothrombin-calcium complex. This complex binds to the phospho-lipids on the membrane surface of the platelets (Fig. 6.4). This increases the conversion of prothrombin to thrombin.

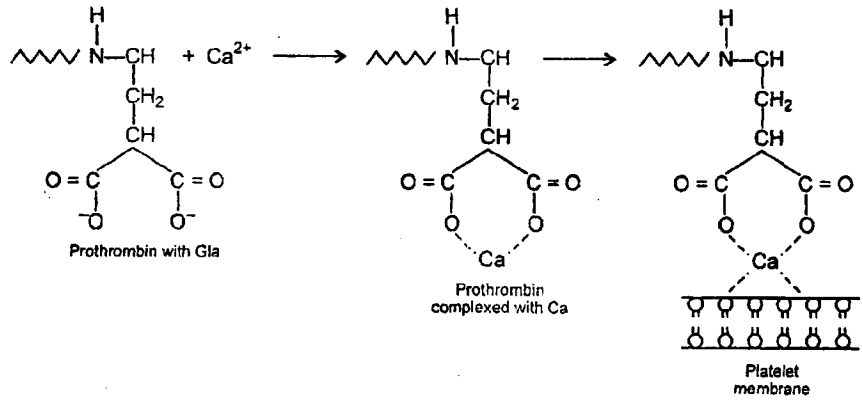


Fig. 6.4. *Action of γ-carboxyglutamate of prothrombin in blood clotting.*

Vitamin K is also involved in the carboxylation of glutamic acid residue of a calcium binding protein, osteocalcin present in the bones.

6.3 WATER SOLUBLE VITAMINS

Vitamin C (Ascorbic Acid)

Vitamin C is a water soluble vitamin, which is commonly found in sour fruits. Deficiency of vitamin C, cause scurvy, which was the first disease found to be associated with diet.

Vitamin C, also called ascorbic acid is a hexose derivative and closely resembles monosacharides in structure. The acidic property of vitamin C is due to the enolic hydroxyl groups. L-ascorbic acid produces dehydroascorbic acid on oxidation. Both ascorbic acid and dehydroascorbic acid are biologically active.

Absorption and Metabolism

Vitamin C is rapidly absorbed in the intestine. It is not stored in the body in the significant amount. Vitamin C is excreted in the urine either as such, or its metabolites diketoglulonic acid and oxalic acid (Fig. 6.5).

Biochemical Functions

Vitamin C or ascorbic acid is involved in several biochemical functions in the body. Most of its functions are related to its property of reversible conversion into dehydroascorbic acid. The important functions of ascorbic acid are discussed below.

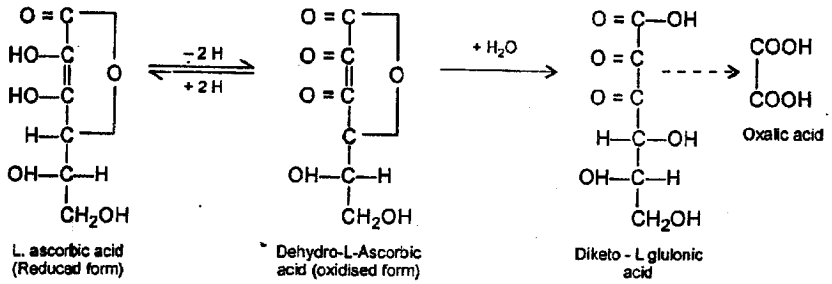


Fig. 6.5. *Vitamin C (ascorbic acid) and its related compounds.*

- **Collagen Formation.** The amino acid residues—proline and lysine present in protocollagen (precursor of collagen) undergo hydroxylation *i.e.*, post translational modification) to form collagen. The hydroxylation reaction is catalysed by *lysyl hydroxylase* (for lysine) and *prolyl-hydroxylase* (for proline). Vitamin C plays the role of co-enzyme for both the enzymes. The formation of collagen requires molecular oxygen and α-ketoglutarate, besides vitamin C (Fig. 6.6). Hydroxyproline (hydroxylated proline) and hydroxylysine (hydroxylated lysine) provide cross-linking and the strength to collagen fibres. Thus, vitamin C is required for maintaining normal connective tissue and healing of wounds.

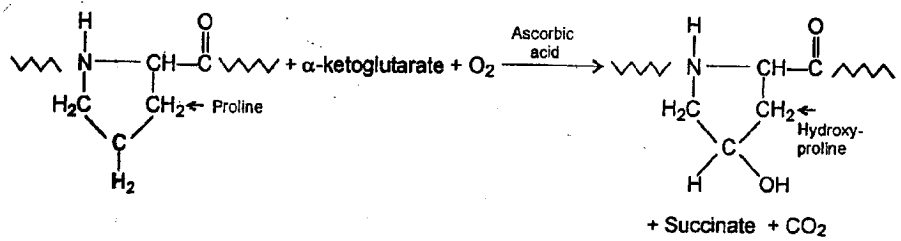


Fig. 6.6. *Hydroxylation of proline of protocollagen by ascorbic acid.*

- **Hemoglobin Metabolism.** Ascorbic acid is a strong reducing agent, hence, enhances uptake of dietary iron in stomach and duodenum by converting it into Ferrous (Fe^{2+}) form. It helps in the formation of ferritin (storage form of iron) and mobilization of iron from ferritin).

  Vitamin C is required for the reconversion of methemoglobin to hemoglobin. It is also needed in the degradation of hemoglobin to bile pigments in the liver.

- **Bone Formation.** Vitamin C is involved in the formation of collagen in bone matrix. Thus, vitamin C is required for the formation and normal growth of bones.

- **Folic Acid Metabolism.** Vitamin C is required for the formation of active form of folic acid, called tetrahydrofolate (FH_4).

NOTES

The reaction is catalysed by ascorbic acid dependent enzyme *folic acid-reductase.* In association with tetrahydrofolate, ascorbic acid is involved in the maturation of erythrocytes.

- **Synthesis of Hormones.** Many peptide hormones contain carbonyl terminal amide, which is derived from terminal glycine after hydroxylation. The hydroxylation of glycine is carried out by vitamin-C dependent enzyme *peptidylglycine hydroxylase.*
- **Protection of Vitamins.** Vitamin C acts as a strong antioxidant and protects some vitamin such as vitamin A, E and some B complex vitamin from oxidation.
- **Synthesis of Serotonin.** Vitamin C is required for the activity of enzyme *tryptophan hydroxylase,* which convert tryptophan to hydroxytryptoplan in the synthesis of serotonin.
- **Tyrosine Metabolism.** Ascorbic acid is required for hydroxylation of p-hydroxyphenylpyruvate to homogentisic acid in tyrosine metabolism.
- **Immunological Function.** Vitamin C enhances the synthesis of immunoglobins (antibodies) and increases the phagocytic action of leucocytes.
- **Prevention of Cataract.** Vitamin C reduces the risk of cataract formation.
- **Prevention of Chronic Diseases.** As an antioxidant, ascorbic acid reduces the risk of some chronic diseases such as cancer, cataract and coronary heart diseases.

Vitamin B_1 (Thiamine)

Vitamin B_1 is also called anti-beri-beri or antineuritic vitamin. It forms a specific co-enzyme, *thiamine pyrophosphate (TPP)* or co-carboxylase which is involved in carbohydrate metabolism. Thiamine contains a pyrimidine ring and a thiazole ring held by a methylene bridge (Fig. 6.7). It is the only natural compound with thiazole sing. The alcohol (–OH) group of thiamine is esterified with two molecules of phosphate to form coenzyme, thiamine pyrophosphate ATP provide phosphates to thiamine and the reaction is catalysed by the enzyme *thiamine pyrophosphate transferase.*

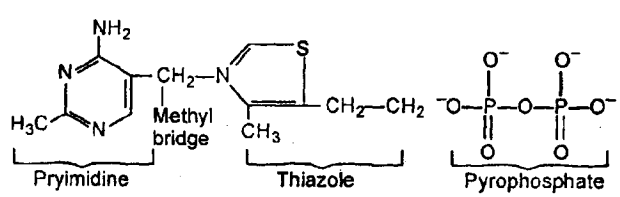


Fig. 6.7. *Structures of thiamine and pyrophosphate.*

NOTES

Biochemical Functions

Thiamine pyrophosphate or co-carboxylase is mainly involved in the energy releasing reactions of respiratory pathway (Fig. 6.8).

- The conversion of pyruvate into acetyl CoA (oxidative decarboxylation) is catalysed by the enzyme *pyruvate dehydrogenase.* The enzyme requires TPP besides the other coenzymes (*i.e.*, coenzyme A, lipoic acid and Mg^{2+} ions).
- In citric acid cycle (Krebs cycle), the conversion of α-Retoglutarate into succinyl CoA, is catalysed by the enzyme *α-Retogularate dehydrogenase,* Like pyruvate dehydrogenase, this enzyme also requires TPP besides the other coenzymes.
- The action of the enzyme *transketolase* of the hexose monophosphate shunt (HMP shunt) is dependent on TPP.
- The oxidative decarboxylation of branched chain amino acids (valine, leucine and isoleucine) to form respective ketoacids, is catalysed by a TPP dependent enzyme *α-keto acid dehydrogenase* (*decarboxylase*).
- TPP is also required for the synthesis of a neurotransmitter, *acetylcholine,* involved in the transmission of nerve impulse.

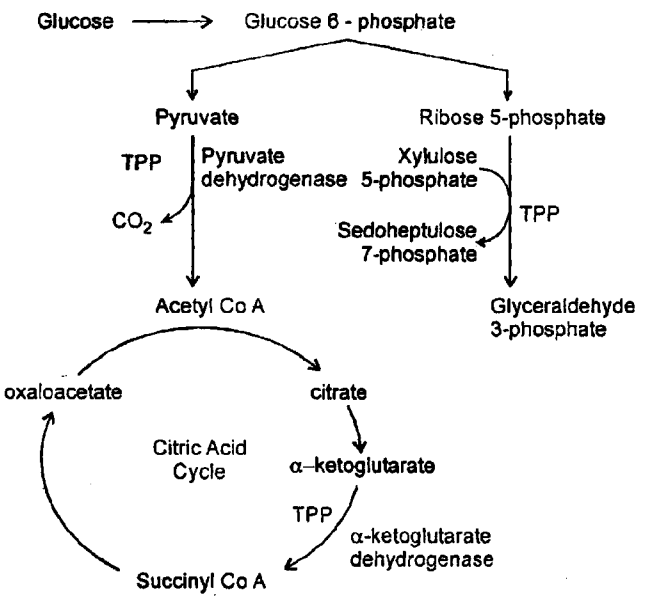


Fig. 6.8. *Respiratory pathway reactions dependent on TPP.*

Vitamin B_2 (Riboflavin)

Vitamin B_2 through its coenzymes is involved in a variety of oxidation-reduction reactions in the cells. Riboflavin is stable to heat but sensitive to light. When exposed to UV rays of sunlight, it is converted into a

NOTES

yellow florescent substance, *lumiflavin.* The substance lactoflavin (present in milk), hepatoflavin (occuring in liver cells) and ovoflavin (present in eggs) are structurally identical to riboflavin.

Riboflavin contains a heterocyclic 3 ring structure, 6, 7-dimethyl isoalloxazine attached to *D-ribitol* by a nitrogen atom. Ribitol is an open chain form of pentose sugar with the aldehyde group (–CHO) reduced to alcohol (CH_2OH).

The coenzyme forms of riboflavin are *flavin mononucleotide (FMN)* and *flavin adenine dinucleotide (FAD).* In FMN, a phosphate is attached to the ribitol of riboflavin. FAD is formed by joining AMP from ATP to FMN.

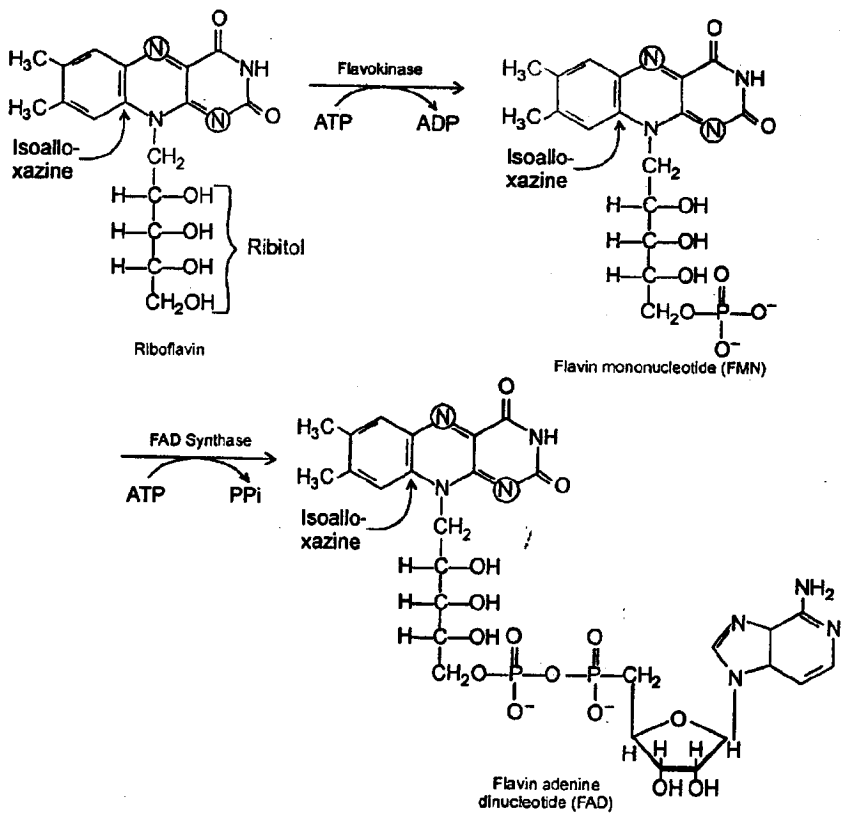


Fig. 6.9. *Synthesis of flavin mononucleotide (FMN) and flavin adenine dinucleotide (FAD).*

Biochemical Functions

Many redox-reactions responsible for energy production require coenzyme mainly FAD and to a lesser extent FMN. In both the coenzyme, the functional unit is isolloxazine sing, which serves as the acceptor of hydrogen. FMN and FAD can undergo reversible reactions accepting two hydrogen atoms forming $FMNH_2$ and $FADH_2$ (Fig. 6.10).

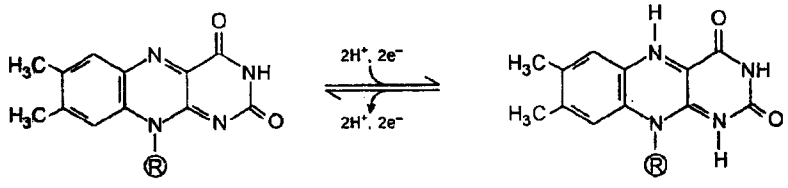


Fig. 6.10. *FMN or FAD involved in oxidation-reduction reactions (R-representing the rest of the structure of FMN or FAD).*

The coenzymes FAD and FMN are associated with certain enzymes involved in carbohydrate, lipid, protein and purine metabolisms, besides the electron transport chain. Some important examples of these reactions are given in Table 6.1

Table 6.1. Important Examples of FAD and FMN Dependent Enzymes and their Reactions.

Enzyme	*Reaction*
FAD dependent	
I. *Carbohydrate metabolism*	
Pyruvate dehydrogenase complex	Pyruvate ⟶ Acetyl CoA
α-Ketoglutarate dehydrogenase complex	α-Ketoglutarate ⟶ Succinyl CoA
Succinate dehydrogenase	Succinate ⟶ Fumarate
II. *Lipid metabolism*	
Acyl CoA dehydrogenase	Acyl CoA ⟶ α, β-Unsaturated acyl CoA
III. *Protein metabolism*	
Glycine oxidase	Glycine ⟶ Glyoxylate + NH_3
D-Amino acid oxidase	D-Amino acid ⟶ α-Keto acid + NH_3
IV. *Purine metabolism*	
Xanthine oxidase	Xanthine ⟶ Uric acid
FMN dependent	
L-Amino acid oxidase	L-Amino acid ⟶ α-Keto acid + NH_3

Vitamin B_3 (Niacin or Nicotinic Acid)

Vitamin B_3 is also called *pellagra preventive (PP) factor.* Much before it was recognised as a vitamin, nicotinic acid was well known as a chemical compound produced by the oxidation of nicotine, present in tobacco leaves. The term niacin was coined and used to emphasize the role of niacin as a vitamin and to avoid the impression that nicotinic acid is an oxidised form of nicotine.

NOTES

Structurally, niacin is pyridine 3-carboxylic acid (pyridine derivative). The amide form of niacin is called niacinamide or nicotinamide. Niacin, nicotinamide and tryptophan (an essential amino acid) are involved in the synthesis of coenzymes NAD^+ (nicotinamide adenine dinucleotide) and $NADP^+$ (nicotinamide adenine dinucleotide phosphate) (Fig. 6.11).

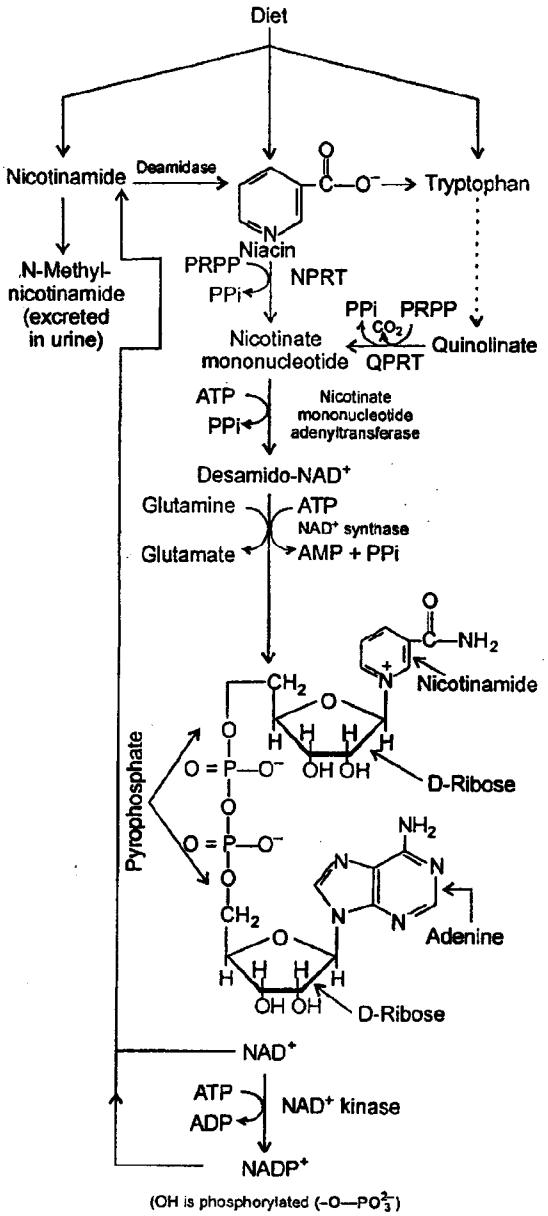


Fig. 6.11. *Biosynthesis of nicotinamide nucleotides NAD^+ and $NADP^+$.*

Nicotinamide is deaminated to niacin in the body. Niacin then undergoes a series of reactions to produce NAD^+ and $NADP^+$. Also the amino acid,

tryptophan produces quinolinate, which then forms nicotinate mononucleotide and ultimately, NAD^+ and $NADP^+$. Phosphoribosyl pyrophosphate and ATP, respectively, provide ribose phosphate and AMP moities for the synthesis of NAD^+. The amide group is contributed by glutamate. In coenzymes (NAD^+ and $NADP^+$) the nitrogen atom of nicotinamide carries a positive charge due to the formation of an extra bond, N is a quaternary state. Nicotinamide produced by the degradation of NAD^+ and $NADP^+$, is excreted in urine mostly as N-methylnicotinamide.

Biochemical Functions

The niacin coenzymes (NAD^+ and $NADP^+$) participate in a number of oxidation-reduction reactions. They undergo reduction by accepting hydride ion (hydrogen atom and one electron $:H^-$) in the pyridine ring. The nitrogen atom and the fourth carbon atom of nicotinamide ring participate in the reaction. While one atom of hydrogen (as hydride ion) from the substrate ($A H_2$) is accepted by the coenzyme, the other hydrogen ion (H^+) is released into the surrounding medium. This reaction occurs in a reversible manner.

About 40 enzymes belonging to the class oxido-reductases are dependent on NAD^+ or $NADP^+$. The oxido-reductase enzymes which are dependent on NAD^+ or $NADP^+$ participate almost in all metabolisms including carbohydrate, proteins and lipids. The important examples of NAD^+ and $NADP^+$ dependent enzymes and the reactions catalysed by them are given in Table 6.2.

Table 6.2. NAD^+ and $NADP^+$ Dependent Enzymes and their Reactions

Enzyme	*Reaction*
NAD^+ dependent	
Carbohydrate metabolism	
Glyceraldehyde 3-phosphate dehydrogenase	Glyceraldehyde 3-phosphate ⟶ 1, 3-Bisphosphoglycerate
Lactate dehydrogenase	Pyruvate ⟶ Lactate
Pyruvate dehydrogenase complex	Pyruvate ⟶ Acetyl CoA
α-Ketoglutarate dehydrogenase complex	α-Ketoglutarate ⟶ Succinyl CoA
Lipid metabolism	
β-Hydroxy acyl CoA dehydrogenase	β-Hydroxy acyl CoA ⟶ β-Keto acyl CoA
β-Hydroxybutyrate dehydrogenase	β-Hydroxybutyrate ⟶ Acetoacetate
Alcohol dehydrogenase	Alcohol ⟶ Acetaldehyde

NOTES

Protein metabolism	
Branched chain α-keto acid dehydrogenase	α-Keto acids of branched chain amino acids (Leu, Ile, Val) ⟶ Corresponding acyl CoA thioesters
Tyramine dehydrogenase	Tyramine ⟶ p-Hydroxyphenyl acetate
NAD^+ or $NADP^+$ dependent	
Glutamate dehydrogenase	Glutamate ⟶ α-Ketoglutarate + NH_3
Isocitrate dehydrogenase	Isocitrate ⟶ Oxalosuccinate
$NADP^+$ dependent	
Glucose 6-phosphate dehydrogenase	Glucose 6-phosphate ⟶ 6-phosphogluconolactone
Malic enzyme	Malic ⟶ Pyruvate
NADPH dependent	
3-Ketoacyl reductase	3-ketoacyl enzyme ⟶ 3-Hydroxy acyl enzyme
HMG CoA reductase	HMG CoA ⟶ Mevalonate
Squalene epoxidase	Squalene ⟶ Squalene oxide
Cholesterol 7α-hydroxylase	Cholesterol ⟶ 7α-Hydroxy cholesterol
Phenylalanine hydroxylase	Phenylalanine ⟶ Tyrosine
Dihydrofolate reductase	Folic acid ⟶ Tetrahydrofolic acid.

Vitamin B_5 (Pantothenic Acid)

Vitamin B_5 or pantothenic acid, formerly known as chick anti-dermatitis factor (or filtrate factor) is widely distributed in nature. Pantothenic acid consists of two components—pantoic acid and β-alanine held together by a peptide linkage. It is involved in the synthesis of coenzyme A, through a series of reaction. Pantothenate is phosphorylated with simultaneous addition of cysteine to form 4-phosphopantothenyl cysteine. The later undergoes decarboxylation followed by addition of AMP and a phosphate from ATP to form coenzyme A (Fig. 6.12).

Biochemical Functions

Pantothenic acid perform its functions through coenzyme A (CoA). Coenzyme A plays a central role in almost all important metabolisms. More than 70 enzymes which are involved in different types of metabolisms are CoA dependent.

Coenzymes A acts as a carrier of activated acetyl or acyl groups (asthiol ester). It is comparable with ATP which is a carrier of activated phosphoryl groups. Coenzyme A has a terminal thiol or sulfhydryl group (–SH), which is the active site, hence CoA-SH is also used. Acyl groups (free fatty acids are linked to coenzyme A by a thioester bond, to give acyl CoA. When bound to acetyl unit, it is called acetyl CoA.

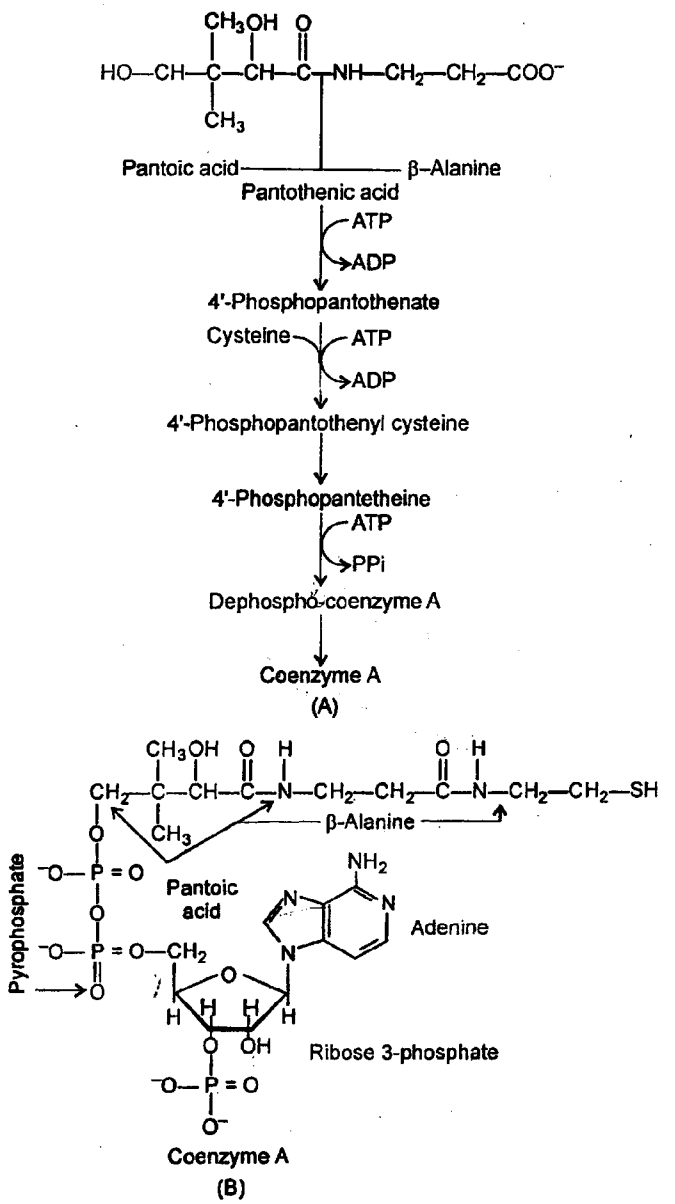


Fig. 6.12. *(A) Synthesis of coenzyme A from pantothenic acid (B) Structure of coenzyme A.*

Some important reactions involving coenzyme A are:

$$\text{Pyruvate} + \text{CoA} \xrightarrow{\textit{Pyruvate dehydrogenase}} \text{Acetyl CoA}$$

$$\alpha\text{ Ketoglutarate} + \text{CoA} \xrightarrow{\alpha\textit{ Ketoglutarate dehydrogenase}} \text{Succinyl CoA}$$

$$\text{Fatty acid} + \text{CoA} \xrightarrow{\textit{Thiokinase}} \text{Acyl CoA}$$

- Coenzyme A is responsible for metabolic integration since acetyl CoA is a central molecule for a wide variety of biochemical reaction (Fig. 6.13).

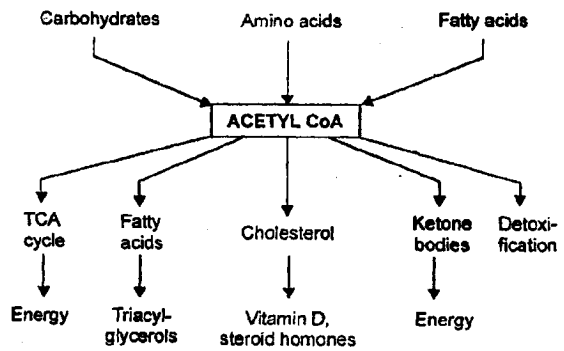


Fig. 6.13. *Coenzyme A involved in metabolic integration.*

- Succinly CoA is involved in the synthesis of porphyrins and heme.
- Pantothenic acid itself is a component of *fatty acid synthase complex* which is involved in the synthesis of fatty acids.

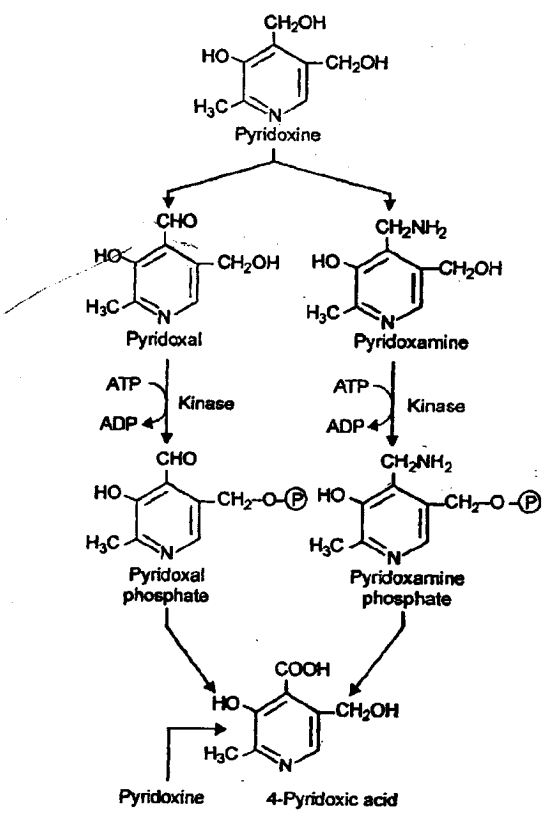


Fig. 6.14. *Phyrodoxine and its derivatives*

Vitamin B_6 (Pyridoxine)

Vitamin B_6 includes three compounds *pyridoxine, pyridoxal* and *pyridoxamine.* These three compounds are called the vitamers of B_6. The vitamers of B_6 are pyridine derivatives. They differ from each other in the structure of a functional group attached to 4rth carbon in the pyrimidine ring. Pyridoxine is a primary alcohol, pyridoxal is an aldehyde form, while pyridoxamine is an amine.

Pyridoxal and pyridoxamine are present in animal foods, whereas pyridoxamine is mostly present in plants. Pyridoxine can be converted to pyridoxal and pyridoxamine, but the latter two cannot form pyridoxine (Fig. 6.14). The active form of vitamin B_6 is the coenzyme *pyridoxal phosphate (PLP),* which can be synthesized from the three vitamers of B_6 (*i.e.*, pyridoxine, pyridoxal and pyridoxamine). Vitamin B_6 is excreted in urine as 4-pyridoxic acid.

Biochemical Functions

Pyridoxal phosphate (PLP), the coenzyme of vitamin B_6 is involved in reactions like transamination, decarboxylation, deamination, transulfuration, condensation etc. The synthesis of certain biochemicals such as serotonin, histamine, niacin coenzymes from the amino acids is dependent on pyridoxine.

1. Transamination

During amino acid catabolism, deamination of amino acids to keto acids is catalysed by the enzyme *transaminase.* The reaction also involve coenzyme pyridoxal phosphate (PLP). The keto acids, thus formed enter the citric acid cycle and get oxidised to release energy. Thus vitamin B_6 integrates carbohydrate and amino acid metabolisms (Fig. 6.15).

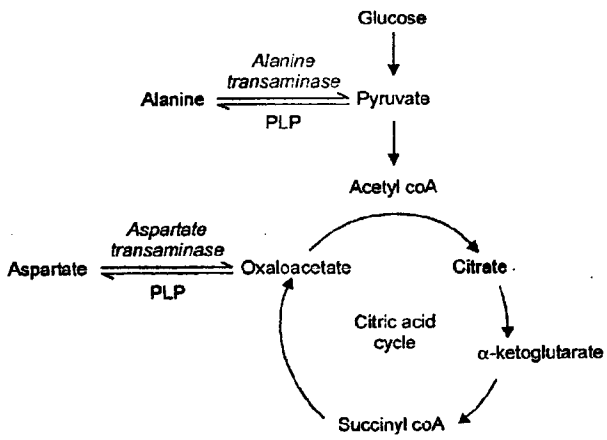


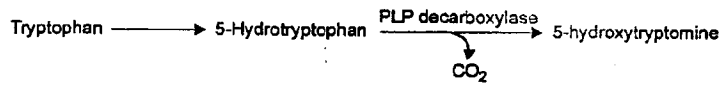
Fig. 6.15. *Integration of carbohydrate and amino acid metabolism by pyridoxal phosphate (PLP).*

NOTES

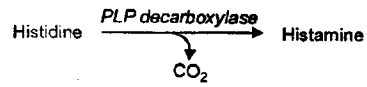
2. Decarboxylation

Decarboxylation of some α-amino acids give rise to respective amine. The reaction is catalysed by a group of enzymes called *decarboxylases*, which are dependent on coenzyme pyridoxal phosphate (PLP). Some important biogenic amines synthesized by PLP decarboxylation are described below.

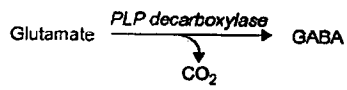
- **Serotonin.** Chemically, serotonin is 5-hydroxytryptamine (5-HT). It is an important neurotransmitter which help in nerve impulse transmission. It regulates a number of body's activities like sleep, behaviour, blood pressure etc. 5-HT is synthesized from tryptophan through PLP decarboxylation.



- **Histamine.** Histamine is another biogenic amine formed from amino acid histidine through PLP decarboxylation. It acts as a vasodilator and lowers blood pressure. Histamine is involved in inflammatory and allergic reactions. It also stimulates gastric HCl secretion.

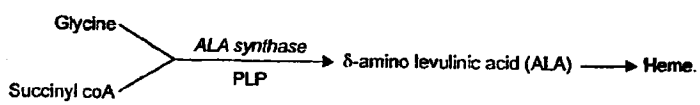


- **GABA (γ-amino butyric acid).** GABA is an inhibitory neurotransmitter, which inhibits the transmission of nerve impulses. It is synthesized from amino acid, glutamic acid through PLP decarboxylation.



- **Catecholamines.** The synthesis of catecholamines (dopamine, norepinephrine and epinephrine) takes place from amino acid tyrosine through PLP decarboxylation. Catecholamines are involved in metabolic and nervous regulation.

- **Heme precursor.** The synthesis of δ-amino-levulinic acid, the precursor for synthesis of heme is catalysed by PLP dependent enzyme *ALA synthase.*



3. Deamination

The hydroxyl group containing amino acids are deaminated with the help of a PLP dependent enzyme *dehydratase.*

$$\text{Serine} \xrightarrow[PLP]{Dehydratase} \text{Pyruvate} + NH_3.$$

$$\text{Threonine} \xrightarrow[PLP]{Dehydratase} \alpha\text{-ketoglutarate} + NH_3$$

4. Transsulfuration

PLP plays an important role in the metabolism of sulphur containing amino acids. For example, transfer of sulphur (transsulfuration) from homocysteine to serine occurs in the synthesis of cysteine. This reaction is catalysed by a PLP dependent enzyme, *cystathionine synthase.*

5. Niacin Coenzymes

The synthesis of niacin coenzymes (NAD^+ and $NADP^+$) from tryptophan is catalysed by a PLP dependent enzyme *kyrureninase.* In vitamin B_6 deficiency. 3-hydroxy anthranilic acid is diverted to xanthurenic acid. Elemination of xanthurenate in urine is an indication of deficiency of vitamin B_6. (Fig. 6.16)

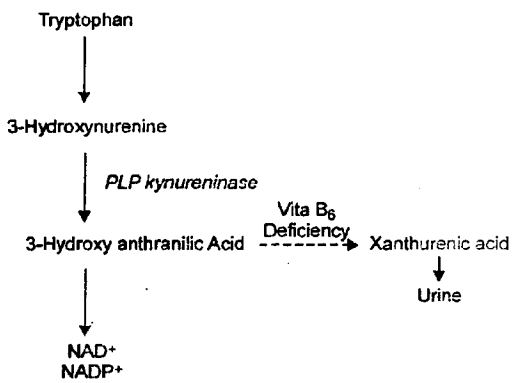


Fig. 6.16. *Formation of niacin coenzymes (NAD^+) and ($NADP^+$).*

6. Serine

Amino acid serine is synthesized from glycine by a PLP dependent enzyme *hydroxymethyl transferase.*

7. Effective Enzyme Function

PLP is covalently bound to lysine residue in enzyme *glycogen phosphorylase,* which cleaves glycogen to glucose 1-phosphate. The structure of the enzyme is stabilized by PLP for its effective functioning.

8. Absorption of Amino Acids

PLP is required for the absorption of amino acids in the intestine.

NOTES

9. Prevention of Hyperoxaluria

Vitamin B_6 prevent hyperoxaluria and urinary stone formation.

Vitamin B_7 (Biotin)

Biotin is a sulphur containing B-complex vitamin. It is also called anti-egg white injury factor or vitamin H. Vitamin B_6 directly acts as coenzyme in carboxylation reactions. Biotin is a heterorcyclic sulphur containing monocarboxylic acid. It is made up of fused imidazole and thiophene rings with a valeric acid side chain (Fig. 6.17).

Biotin is covalently linked to E-amino group of lysine to form biocytin in the enzymes. Biocytin is considered as the coenzyme of biotin.

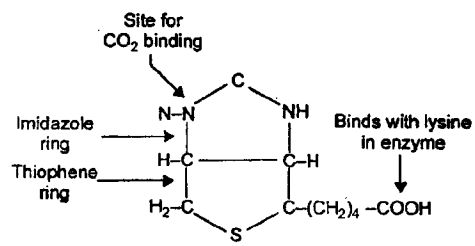


Fig. 6.17. *Structure of Biotin (Vitamin B_7)*

Biochemical Functions

Biotin acts as a carrier of CO_2 in a number of carboxylation reactions. The conversion of pyruvate to oxaloacetate is mediated by the enzyme *pyruvate carboxylase*. The enzyme has biotin which is linked to the E-amino group of lysine. The biotin enzyme reacts with CO_2 in presence of ATP to form a *carboxybiotin-enzyme complex*. This high energy complex transfer CO_2 to pyruvate to produce oxaloacetate (Fig. 6.18).

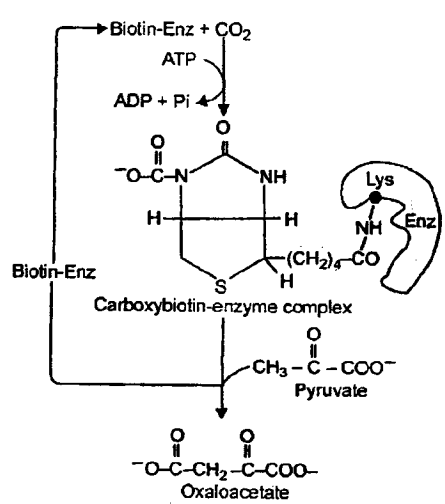


Fig. 6.18. *Carboxylation by Biotin dependent enzyme.*

- **Gluconeogenesis.** The formation of oxaloacetate from pyruvate catalysed by a biotin dependent *pyruvate carboxylase,* is required for the synthesis of glucose from many non-carbohydrate sources.
- **Synthesis of Fatty Acids.** Fatty acids are synthesized from acetyl CoA (the starting compound). The acetyl CoA undergoes carboxylation in the very first reaction by a biotin dependent enzyme *acetyl CoA carboxylase.*

  $$\text{Acetyl CoA} + CO_2 \xrightarrow[\text{Biotin}]{\text{Acetyl CoA Carboxylase}} \text{Malonyl CoA.}$$

- **Propionyl CoA.** Propionyl CoA is formed in the metabolism of certain amino acids (valine, isoleucine, threonine etc.) and degradation of odd chain fatty acids. Further metabolism of propionyl CoA is mediated by biotin dependent enzyme.

  $$\text{Proponyl CoA} \xrightarrow[\text{Biotin}]{\text{Propionyl CoA Carboxylase}} \text{Methylmalonyl CoA}$$

- **Leucine Metabolism.** Biotin dependent enzyme β-*methyl crotonyl CoA carboxylase* is involved in leucine metabolism.

  $$\beta\text{-methyl crotonyl CoA} \xrightarrow[\text{Biotin}]{\beta\text{-methyl crotonyl CoA Carboxylase}} \beta\text{-methyl glutaconyl CoA.}$$

Folic Acid (Folacin)

Folic acid occurs abundantly in green leafy vegetables. It is a part of coenzymes involved in the synthesis of certain amino acids, purines and pyrimidine (thymine). Folic acid is formed of three components—pteridine ring, p-amino benzoic acid (PABA) and glutamic acid (1 to 17 residues). Folic acid having only one glutamic acid residue is called pteroyl glutamic acid (PGA). The active form of folic acid called tetrahydrofolate (THF or FH_4) is synthesized from folic acid by the enzyme *dihydrofolate reductase* utilizing 2 molecules of NADH (Fig. 6.19)

Absorption and Storage

The dietary folic acid is mostly occurs as polyglutamates (with 3 to 7 glutamate residues held by peptide bonds) of folic acid, and is not absorbed in the intestine. The glutamate residues of folic acid is broken down by the enzyme *folate conjugase* to form the monoglutamate of folic acid. The absorption of folic acid occurs only in the form of monoglutamate of folic acid in the intestine. Inside the cells, tetrahydrofolates are found as polyglutamates (with 5 to 6 glutamate residues), which is biologically potent derivative. Liver stores folic acid to some extent (upto 10–12 mg) as polyglutamate derivative, which lasts for 2–3 months. In circulation, it is abundantly present as N^5 methyl tetrahydrofolate. (N^5 methyl THF).

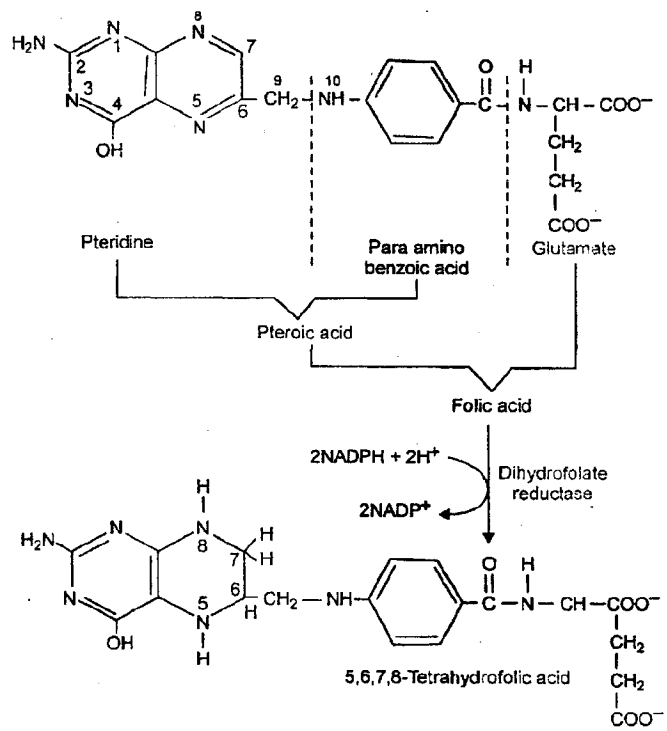


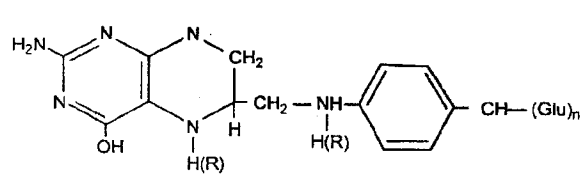
Fig. 6.19. *Folic acid and its active form tetrahydrofolate*

Biochemical Functions

The coenzyme of folic acid tetrahydrofolate (THF or FH_4) participates in the one carbon metabolism. It acts as an acceptor or donor of one carbon unit (formyl, methyl etc.) in a variety of reactions of amino acid and nucleotide metabolisms. THF binds with one carbon units at position N^5 or N^{10} or on both N^5 and N^{10} of pteroyl structure. For example, the attachment of formyl (–CHO) at position 5 of THF forms N^5 formyl tetrahydrofolate which is commonly called folinic acid (citrovorus factor).

Vitamin B_{12} is required for the conversion of N^5_ methyl THF to THF, in which homocysteine is converted to methionine. This step is essential for the liberation of free THF and for its repeated use in one carbon metabolism. In case of vitamin B_{12} deficiency conversion of N^5_ methyl THF to THF is blocked, affecting the one carbon metabolism.

Some other important derivatives of THF are given below:



R group (1 Carbon unit)	THF (1 Carbon derivatives)
– CHO	N^5_ Formyl THF
– CHO	N^{10}_ Formyl THF
– CH = NH	N^5_ Formino THF
≡ CH	N^5, N^{10} Methenyl THF
= CH_2	N^5 N^{10}_ Methylene THF
– CH_3	N^5_ Methyl THF

One carbon derivatives of THF are involved in the synthesis of a number of important compounds (Fig. 6.20). The important compounds synthesized through one carbon metabolism are listed below.

- Synthesis of purines (carbon 2,8), which are the constituents of nucleic acids (DNA and RNA).
- Synthesis of pyrimidine nucleotide-deoxythymidylic acid (dTMP) which is involved in DNA synthesis.
- Formation of glycine, serine, ethanolamine and choline.
- Synthesis of N-Formyl methionine, which is the initiator of protein biosynthesis.

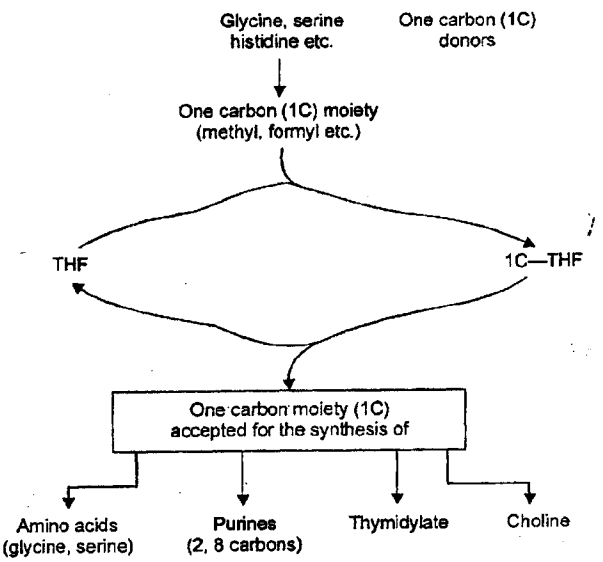


Fig. 6.20. *Synthesis of some important compounds involving one carbon metabolism.*

The involvement of one carbon THF derivatives in the synthesis of different compounds is summarised in Fig. 6.21.

NOTES

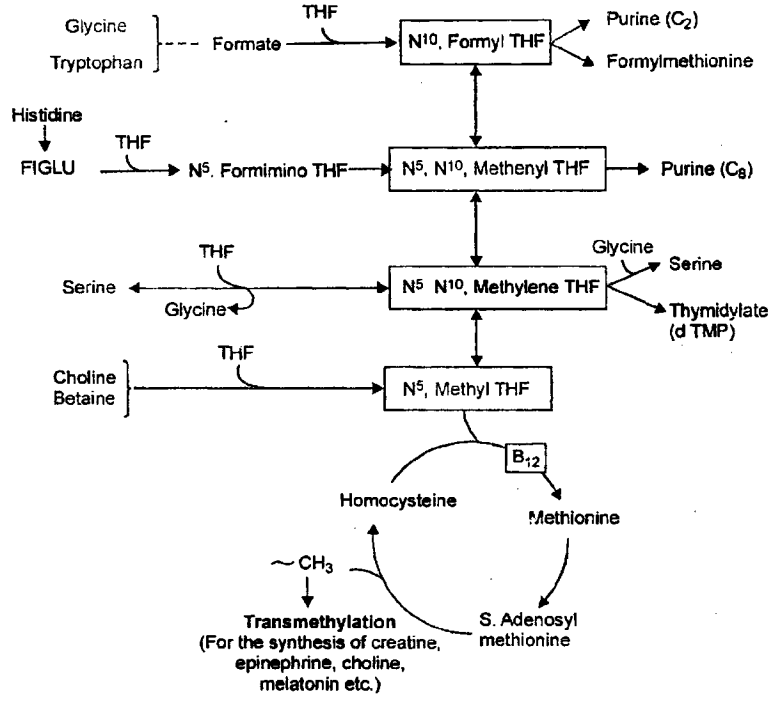


Fig. 6.21. *Summary of one carbon metabolism.*

Vitamin B_{12} (Cobalamin)

Cobalamin is the only vitamin synthesized by only microorganisms and not by animals and plants. It is also known as antipernicious anemia vitamin. Vitamin B_{12} has a complex structure with empirical formula $C_{63}H_{90}$ $N_{14}O_{14}$ PCO. Vitamin B_{12} consists of a corrin ring with a central cobalt (Co) atom. The corrin ring has a structure almost similar to tetrapyrrole ring found in porphyrin compounds like heme (with Fe) and chlorophyll (with Mg). The corrin ring has four pyrrole units A, B, C and D. The units A and D are directly bound to each other, whereas, the units B and C are held by methane bridges. The group namely methyl, acetamide and propionamide are the substituents on the pyrrole rings. The cobalt is present in the centre of the corrin ring, and is bounded to the four pyrrole nitrogens. It also holds below (*i.e.*, below the corrin plane) dimethylbenzimidazole (DMB) containing ribose 5-phosphate and aminoisopropanol. The cobalt is linked to a nitrogen atom of DMB. The amide group of aminoisopropanol is linked to D unit of the corrin. The cobalt atom also has a sixth substituent group located above the plane of corrin ring (Fig. 6.22). The substituent group may be:

- Cyanide in cyanocobalamin (also called $B_{12}a$)
- Hydroxyl in hydroxocobalamin (also called $B_{12}b$)

- Nitrate in nitrocobalamin (also called B_{12})

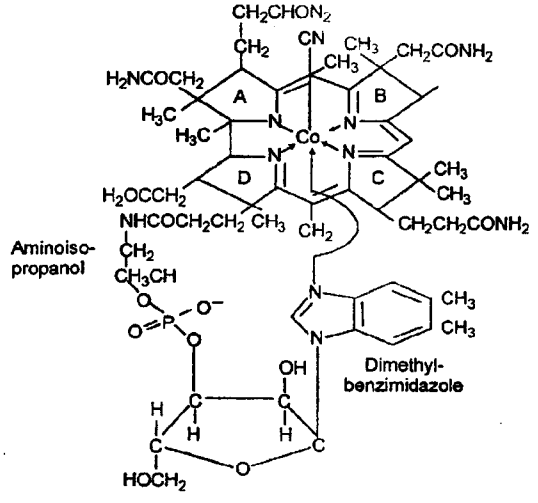


Fig. 6.22. *Structure of vitamin B_{12} (cyanocobalamin)*

Vitamin B_{12} has two coenzyme derivatives: –5-Deoxyadenosyl cobalamin and methylcobalamin (Fig. 6.23).

- In 5-Deoxyadenosyl cobalamin cyanide is replaced by 5-deoxyadenosine forming an unusual carbon cobalt bond.
- In methylcobalamin cyanide is replaced by methyl group.

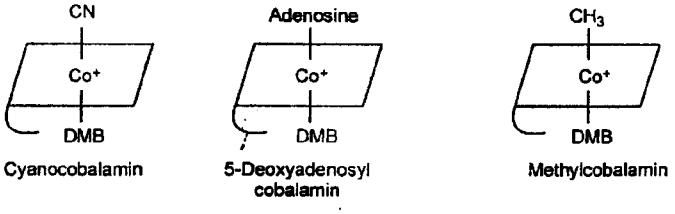


Fig. 6.23. *Coenzyme derivatives of vitamin B_{12}*

Absorption and Storage

The absorption of vitamin B_{12} is dependent on a special glycoprotein called castle's intrinsic factor (IF) secreted by oxyntic cells of gastric glands in stomach. The IF is resistant to proteiolytic digestive enzymes. Vitamin B_{12} is present in the diet in the bound form to proteins. It is released in the stomach from the food proteins by the enzymes *acid hydrolases*. The liberated vitamin B_{12} is called *extrinsic factor*.

The IF generally forms a dimer and strongly binds with 1 or 2 molecules of vitamin B_{12}. to from cobalamin IF complex. This binding protects vitamin B_{12} against it uptake and use by bacteria. The cobalamin. IF complex from stomach reaches to the intestine and binds to specific receptors present on the surface of the mucosal cells of the ileum. The

NOTES

binding of the complex and entry of B_{12} into the mucosal cells is mediated by Ca^{2+} ions. In the mucosal cells, B_{12} is converted to methylcobalamin. The methylcobalamin, then enters into circulation and binds to proteins to form *transcobalmins (TC–I, TC–II)* to be transported to various tissues. About 90% of methylcobalamin is mostly bound to TCI and about 10% to TCII. It is considered that TC-I acts as a repository (reserve form) of B_{12}. While TC-II mediates the tissue uptake of B_{12}. The excess of methylcobalamin is taken up by the liver, converted to dexyadenosyl B_{12} and stored in this form (Fig. 6.24). Liver can store about 4–5 mg which can meet the body's requirement of B_{12} for 4–6 years.

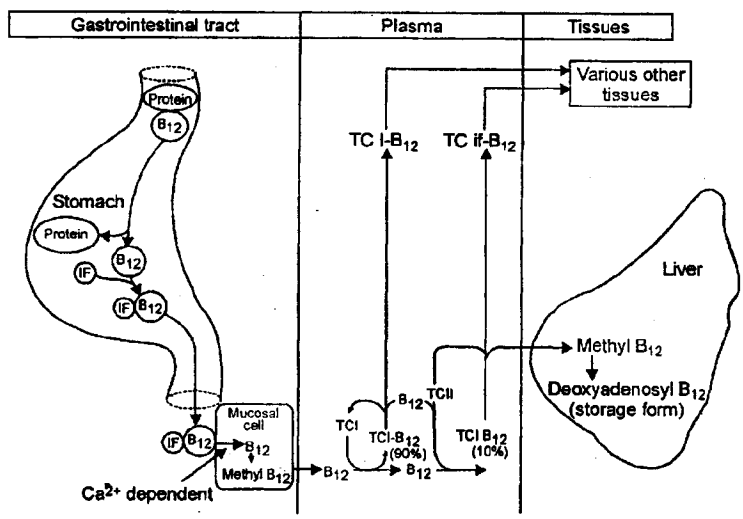
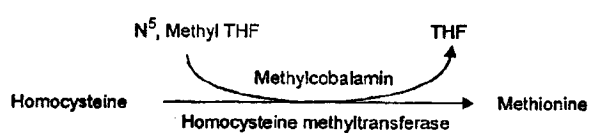


Fig. 6.24. *Absorption, transport and storage of vitamin B_{12}.*

Biochemical Functions

In humans two reactions are depends upon vitamin B_{12}.

- **Synthesis of methionine.** Vitamin B_{12} as methylcobalamin is used in the synthesis of methionine from homocysteine. It involves N^5. methyl tetrahydrofolate and the enzyme *homocysteine methyltransferase (methionine synthase).*



- **Isomerization of methylmalonyl CoA to succinyl CoA.** The breakdown of odd chain fatty acids, certain amino acids (like valine and isoleucine etc.) and pyrimidines (thymine and uracil) produces methylmalonyl CoA directly or by the mediation of

propionyl CoA. The methylmalonyl CoA is converted to succinyl CoA by the enzyme *methylmalonyl CoA mutase* in presence of B_{12} coenzyme-*deoxyadenosyl cobalamin*. In deficiency of vitamin B_{12}, methylmalonyl CoA accumulates and is excreted in urine as methyl malonic acid (Fig. 6.25).

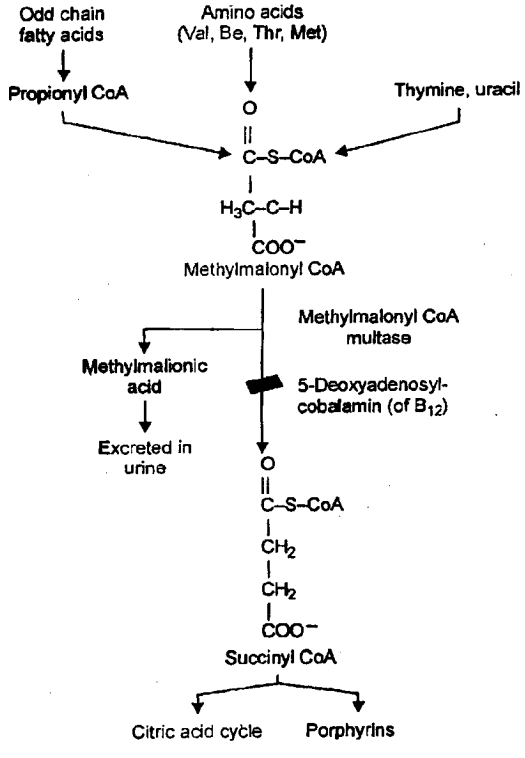


Fig. 6.25. *Isomerization of methyl malonyl CoA to succinyl CoA.*

Interrelation between folic acid and vitamin B_{12}. The deficiency of either folic acid or vitamin B_{12} causes anemia, suggesting a probable biochemical interrelation between these two vitamins. There is only one reaction known to common folate and vitamin B_{12}.

In vitamin B_{12} deficiency, the level of folate is increased in the plasma. The activity of the enzyme *homocysteine methyltransferase (methionine synthase)* is also low in B_{12} deficiency.

It blocks the conversion of N^5_methyl THF to tetrafolate, which leads in the reduction of THF pool in the body. Almost the entire body's folate becomes trapped as N^5. methyl THF. It is called *folate trap* or *methyltrap*. It results in decreased folate coenzymes leading to reduced nucleotide and DNA synthesis (Fig. 6.26).

Although, in tissues the folate level is high but, they show functional folate deficiency due to decreased THF pool. This causes *megaloblastic*

NOTES

anemia. A combined therapy of vitamin B_{12} and folic acid is generally employed to treat the patients with megaloblastic anemia.

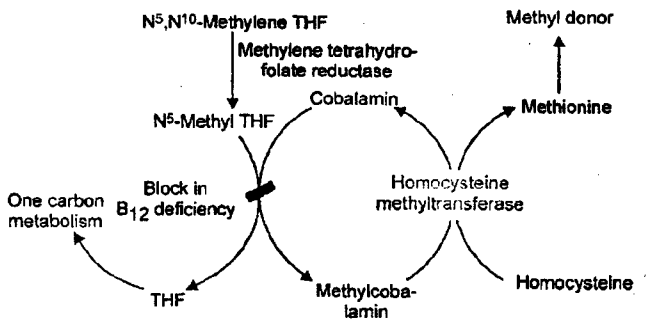


Fig. 6.26. *Interrelation between folic acid and vitamin B_{12}*

STUDENT ACTIVITY

1. Name the functionally active form of vitamin D.

2. Name the vitamers of vitamin A.

NOTES

6.4 SUMMARY

- Vitamins are organic substances which act as accessory food factors. They are classified as fat soluble (A, D, E and K) and water soluble (B complex and C). Vitamin A is involved in vision, proper growth, differentiation and maintenance of epithelial cells. The active form of vitamin D is calcitriol which functions like a steroid hormone and regulates plasma levels of calcium and phosphate. Vitamin E is a natural antioxiant necessary for normal reproduction in many animals. Vitamin K has a specific coenzyme function. It catalyses the carboxylation of glutamic acid residues in blood clotting factors (II, VII, IX and X) and convert them to active form.
- Vitamin C (ascorbic acid) is involved in the hydroxylation of proline and lysine in the formation of collagen.
- Vitamin B_1 (thiamine) as coenzyme of carboxylase (TPP) is involved in energy releasing reactions. The coenzymes of riboflavin (FAD and FMN) and niacin (NAD^+ and $NADP^+$) participates in a variety of oxidation-reduction reactions connected with energy generation. Coenzyme A (of pantothenic acid) is involved in the metabolism of carbohydrates, lipids and amino acids and their integration. Pyrodoxal phosphate (PLP), the coenzyme of vitamin B_6 is mostly associated with amino acid metabolism. PLP participates in transamination, decarboxylation, deamination and condensation reactions. Biotin participates as a coenzyme in carboxylation reactions of gluconeogenesis, fatty acid synthesis etc. Tetrahydrofolate (THF), the coenzyme of folic acid is involved in the transfer of one carbon unit (formyl, methyl) in amino acid and nucleotide metabolism. Vitamin B_{12} has two coenzymes—deoxyadenosylcobalamin and methylcobalamin.

6.5 GLOSSARY

- **Absorption:** uptake of end product of digestion through the cell membrane of the digestive fact.
- **Adolescence:** Period of years between the beginning of puberty and maturity.
- **Antivitamin:** A substance that inactivates a vitamin/inhibits its synthesis.
- **Beriberi:** A deficiency disease caused due to lack of thiamin.
- **Beta-carotene:** A fat soluble plant pigment, which is precussor of vitamin A.

- **Fortification:** Addition of one or more nutrients to a food to made it richer than the unprocessed food.
- **Hyper vitaminosis:** Undesirable effects produced by taking an excess of a concentrate or pure fat soluble vitamin.
- **Nyctolapia (Night blindness):** Inability to seen in dimlight.
- **Rickets:** A deficiency disease caused by absence of vitamin D or calcium or both which affects the skeletal system.
- **Scurvy:** A deficiency disease due to lack of vitamin C, characterised by bleeding gums, weakness and loss of weight.
- **Vitamins:** Organic compounds occurring in minute amounts in food and essential for numerous metabolic reactions.
- **Xerosis:** Abnormal dryness of skin and eye.

6.6 REVIEW QUESTIONS

I. Very Short Answer Type Questions:

1. What are retinoids?
2. Which part of vitamin E provides it antioxidant property?
3. Which vitamin is necessary for clotting of blood?
4. Name the vitamins, which are popularly known as:
   (*i*) Antineuritic vitamin.
   (*ii*) Pellagra preventive factor.
   (*iii*) Chick anti-dermatitis factor.
   (*iv*) Anti-scurvy vitamin.
5. Name the vitamin that contains cobalt.

II. Short Answer Type Questions:

1. Explain the absorption, transport and mobilization of vitamin A.
2. How is vitamin D synthesized in the skin?
3. List the main functions of vitamin E.
4. Discuss the role of vitamin K in blood clotting.
5. Explain the biochemical functions of vitamin B_1.

III. Long Answer Type Questions:

1. Describe the chemistry and biochemical functions of vitamin A.
2. Give an account of folic acid involvement in one carbon metabolism.
3. Discuss the biochemical functions of vitamin C.
4. Give a brief account of the coenzymes involved in oxidation-reduction reaction.

NOTES

5. Discuss the metabolism and biochemical functions of vitamin D.
6. Write the biochemical functions of following vitamins.
   (*i*) Thiamine (*ii*) Riboflavin
   (*iii*) Niacin (*iv*) Pantothenic acid
   (*v*) Pyridoxine (*vi*) Biotin
   (*vii*) Folic Acid (*viii*) Cobalamin.

6.7 FURTHER READINGS

- **Essentials of Food and Nutrition;** Swaminathan M; Ganesh, Madras, India; 1985.
- **Introduction to Nutrition;** Fleck Henrietta; MacMillan, New York, 1981.
- **Nutrition and Dietetics;** Joshi, Shubangini A; Tata McGraw Hill, New Delhi, 1992.
- **Fundamentals of Food and Nutrition;** Mudambi; S.R. and Rajagopal, M.U; New Age Int. Pub, New Delhi; 2008.
- **Biochemistry;** Satyanarayan U., Chakrapani U.; Books and Allied (P) Ltd. Kolkata, 2008.
- **Outlines of Biochemistry;** Conn E.E., Stumpf P.K, Bruening G., Doi R.H;. Wiley India (P) Ltd. New Delhi, 2007.

UNIT–IV

CHAPTER 7 ENZYMES

OBJECTIVES

After going through this chapter, you should be able to:

- describe historical background
- know about nomenclature
- explain IUB system of enzyme classification
- understand chemical nature of enzyme
- illustrate about enzyme kinetics.

STRUCTURE

7.1 INTRODUCTION

Enzymes are commonly proteinaceous substances which are capable of catalysing chemical reactions of biological origin without themselves undergoing any change. Therefore, they are called **biocatalysts or the catalyst of life**. The student-teacher relationship may be a good example to understand how a catalyst works. The student often find it difficult to learn from a textbook on their own. The teacher explains the subject to the students and increases their understanding capability. It is not surprising that certain difficult things which the students take days

NOTES

together to understand, and sometimes do not understand at all — are easily learnt under the guidance of the teacher. Here, the teacher acts like a catalyst in enhancing the understanding ability of students.

Thus, enzymes may be defined as *'biocatalysts synthesized by living cells. They are protein in nature (exception—RNA acting as ribozyme), colloidal and thermolabile in character, and specific in their action'.*

7.2 HISTORICAL BACKGROUND

The term 'enzyme' was coined by **Kuhne** (1878) for catalytically active substances previously called **ferments.** Isolation of enzyme system from cell free extract of yeast was achieved in 1883, by **Buchner.** He named the active principle as zymase (later found to contain a mixture of enzymes), which could convert sugar to alcohol. Buchner was awarded Nobel Prize in 1903. In 1926, James Sumner first obtained the enzyme **urease** from jack bean in crystalline form and identified it as a protein.

There are numerous enzymes as every biochemical reaction is catalysed by a separate enzyme. It is estimated that a cell contains over 5000 chemicals. The number of chemical reactions is many times more. Therefore, the number of enzymes is several thousands (Lehninger, 1993).

Enzymes are mainly functional inside the living cells. As found out by Buchner, they can be extracted from the cells and made to catalyse reactions outside the living cells. In nature some enzymes are secreted by living cells and made to perform extracellular catalysis. Digestive enzymes belong to this category. Several enzymes of medical and chemical importance are now available in the market *e.g.*, rennet tablet (from rennin of calf stomach) for coagulating milk protein casein during preparation of cheese and other milk products. Enzymes functional outside the living cells are called **exoenzymes,** *e.g.*, enzymes present in digestive juices, lysozymes of tears. Enzymes functional inside living cells are known as **endoenzymes,** *e.g.*, enzymes of krebs cycle (inside mitochondria), enzymes of glycolysis (inside cytoplasm).

The biochemical which is acted upon by an enzyme is known as **substrate.** In case two biochemicals are involved in a reaction, the same are called **'reactants'.** The substances produced in the reaction are termed as **products.** The final products are also called **end products.**

7.3 NOMENCLATURE

In the past, enzymes were given arbitrary names. For example, ptyalin, pepsin, trypsin, etc., convey no information about the function of the enzyme or the nature of substrate on which they act. Some old names indicate the source but not the action, *e.g.*, papain from papaya, bromelain from pineapple of family Bromeliaceae.

Sometimes, the suffix *'ase'* was added to the substrate for naming the enzymes. *e.g.*, lipase act on lipids; lactase on lactose; nuclease on nucleic acids and so on. These are known as **trivial names** of the enzymes which, however, fail to give complete information of enzyme reaction (*i.e.*, type of reaction, cofactor requirement etc.)

7.4 IUB CLASSIFICATION

The International Union of Biochemistry (IUB) appointed an Enzyme Commission in 1961. This committee made a thorough study of the existing enzymes and devised some basic principles for the classification and nomenclature of enzymes. The **IUB system of enzyme classification** has been in force since 1964.

According to IUB system, enzymes are grouped into six major classes. Each class represents the general type of reaction brought about by the enzymes of that class.

1. **Oxidoreductases.** They take part in oxidation and reduction reactions or transfer of electrons.
2. **Transferases.** They are involved in transfer of functional groups from one molecule to another.
3. **Hydrolases.** They bring about hydrolysis of various compounds. They catalyse hydrolysis of bonds like ester, ether, peptide, glycosidic, C–C, C–halide, P–N, etc., which are formed by dehydration condensation. Hydrolases break up large molecules into smaller ones with the help of hydrogen and hydroxyl groups of water molecules. The phenomenon is called **hydrolysis**.
4. **Lyases.** These enzymes cause cleavage, removal groups without hydrolysis, addition of groups to double bonds or removal of a group producing double bond.
5. **Isomerases. They** catalyse rearrangement of molecular structure to effect isomeric changes.
6. **Ligases (Synthetases).** They catalyse the synthetic reactions, where two molecules are joined together with the help of energy obtained from ATP.

(To remember the six classes of enzymes, the word OTHLIL the first letter of each class, may be memorised).

Each class is subdivided into a number of sub classes which are further divided. A four digit **Enzyme Commission (E.C.)** number is assigned to each enzyme in which first, second, third and fourth respectively represents class, subclass, subsub-class and the individual enzyme. Each enzyme is given a specific name indicating the substrate, coenzyme (if any) and the type of reaction catalysed by the enzyme. Although the

NOTES

IUB names for the enzymes are specific and unambiguous, they have not been accepted for general use as they are complex and cumbersome to remember. Therefore, the trivial names, along with the E.C. numbers as and when needed, are commonly used and widely accepted. The various enzyme classes with example are given in Table 7.1.

Table 7.1. Classification of Enzymes with Examples (In each class systematic names of one enzyme along with E.C. number is given in the bracket)

Enzyme Classes with Example	*Reaction Catalysed*
1. Oxidoreductases	
Alcohol dehydrogenase (alcohol : NAD+ oxidoreductase E.C. 1.1.1.1), cytochrome oxidase, L- and D-amino acid and oxidases	Oxidation ⟶ Reduction $AH_2 + B \longrightarrow A + BH_2$
2. Transferases	
Hexokinase (ATP : D-hexose 6-phosphotransferase, E.C. 2.7.1.1), transaminases, transmethylases, phosphorylase	Group transfer $A - X + B \longrightarrow A + B - X$
3. Hydrolases	
Lipase (triacylglycerol acyl hydrolase E.C. 3.1.1.3), choline esterase, acid and alkaline phosphatases, pepsin, urease	Hydrolysis $A - B + H_2O \longrightarrow AH + BOH$
4. Lyases	
Aldolase (ketose 1-phosphate aldehyde lyase, E.C. 4.1.2.7) fumarase, histidase	Addition ⟶ Elimination $A - B + X - Y \longrightarrow AX - BY$
5. Isomerases	
Triose phosphate isomerase (D-glyceraldehyde 3-phosphate ketoisomerase, E.C. 5.3.1.1) retinol isomerase, phosphohexose isomerase	Interconversion of isomers $A \longrightarrow A'$
6. Ligases	
Glutamine synthelase (L-glutamate ammonia ligase, E.C. 6.3.1.2) acetyl CoA carboxylase, succinate thiokinase	Condensation (usually dependent on ATP) $A + B \xrightarrow[ATP \quad ADP + Pi]{} A - B$

7.5 ACTIVE SITE

The whole of enzyme molecule is not active in catalysing a chemical reaction. Only a small portion of it is active. It is called **active site** or **active spot.** ***An active site or spot is an area of the enzyme which is***

capable of attracting and holding particular substrate molecules by its specific charge, 'size and shape so as to allow the chemical change'. An enzyme may have one to several active sites. Active site consists of a few amino acids and their side groups, which are brought together in a particular fashion due to secondary and tertiary folding of a protein molecule, and its association with the cofactor, if any.

7.6 CHEMICAL NATURE OF ENZYME

Enzymes are globular protein (Sumner, 1926) with the exception of recently discovered RNA enzymes. Some enzymes may additionally contain a non-protein group. Accordingly, there are two types of enzymes—simple and conjugate.

- **Simple Enzymes** are wholly made up of protein. Active site is formed by specific grouping of its own amino acids.
- **Conjugate Enzymes** are formed of two parts— a protein part called **apoenzyme** and a non-protein part named **cofactor.** The complete conjugate enzyme, consisting of an apoenzyme and a cofactor, is called **holoenzyme.** Active site is formed jointly by apoenzyme and cofactor.

Cofactor is small, heat stable and dialysable part of conjugate enzyme. It may be inorganic or organic in nature. Organic cofactors are of two types, **coenzymes** and prosthetic groups. Coenzymes are easily separable non-protein organic cofactors, *e.g.,* NAD^+, $NADP^+$, CoA etc. **Prosthetic group** are non-protein organic cofactors firmly attached to apoenzymes, *e.g.,* here, biotin, pyridoxal phosphate, etc. Hence, is iron containing prosthetic group in cytochromes, hemoglobin, myoglobin, catalase and peroxidase. Certain workers use the term **cofactor** for any loosely bound non-protein group. The organic cofactor is called coenzyme. They use the term prosthetic group similarly for both inorganic and organic group attached firmly to apoenzyme.

Proenzyme or Zymogen

Proenzyme is the inactive precursor of an enzyme. The term zymogen is often used for inactive precursor of proteolytic enzyme, *e.g.,* pepsinogen for enzyme pepsin. Many enzymes are initially produced in the proenzyme or zymogen state. They become reactive or active enzymes only at a particular pH, in the presence of substrate or some special treatment. For example, pepsinogen is changed to active enzyme pepsin in the presence of hydrochloric acid of gastric juice. Thereafter, pepsin has autocatalytic effect on further conversion of pepsinogen.

NOTES

Allosteric Enzymes

They are enzymes which have separate areas for different types of modulators that alter the conformation of the active site so as to make it effective or ineffective (Fig. 7.1). These areas are called **allosteric sites**. The substances which cause change in allosteric sites are known as **modulators, allosteric substances** or **effectors.** The latter are of two types—**activators** and **inhibitors**. Allosteric activator binds with an allosteric site in such a way as to make active site operational. Allosteric inhibitor, on the other hand, brings about such a change in the active site that it becomes unable to combine with substrate molecules. For example, the enzyme phosphofructokinase is activated by ADP and inhibited by ATP.

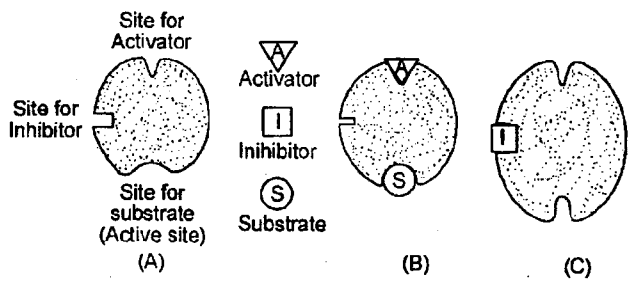


Fig. 7.1. *Allosteric enzyme. (A) an enzyme with various factors. (B) active site becomes functional in the presence of activator. (C) inhibitor distorts the enzyme molecule in such a way that the substrate cannot bind to the active site.*

7.7 ENZYME KINETICS

When chemical reactions proceed from one direction to the other, the substrate molecules have to cross an energy barrier called the **activation energy** before being converted into products. The term 'activation energy' refers to the amount of energy (in calories) required to bring all the molecules of a substrate at a given temperature to the activated state. Normally, only a part of the total substrate molecules contains enough energy to reach this barrier in order to react. Living systems employ enzymes to activate substrate molecules of the reaction. Enzymes, in fact, increase the speed of a chemical reaction by lowering the energy of activation of the said reaction. Enzymes combine with the substrate to form a complex called **enzyme–substrate complex (ES)** or the transition state having less energy of activation than the transition state of the uncatalysed reaction (Fig. 7.2). As a result more and more molecules of substrate combine with enzyme to achieve this new transition barrier and to be converted into product.

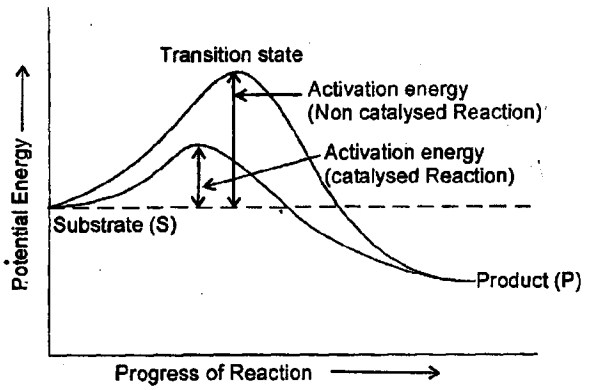


Fig. 7.2. *Energy status for uncatalysed and catalysed reaction.*

Michaelis-Menten Constant and MM Equation

In an enzymatic reaction A ⟶ P, the rate of the reaction is dependent on the substrate concentration (Fig. 7.3). At low sub-substrate concentration, the rate of reaction '*v*' is proportional to the substrate concentration. However, as the substrate concentration is increased, the velocity of reaction falls and it is no longer proportional to the substrate concentration. With the further increase in substrate concentration, the rate of reaction becomes constant and independent of substrate concentration. The enzyme at this stage shows the saturation effect *i.e.*, it has become saturated with the substrate.

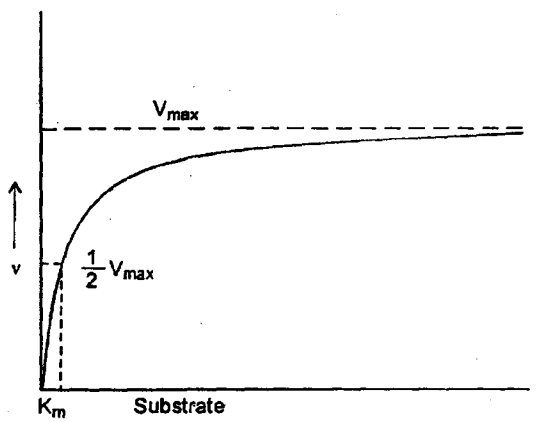


Fig. 7.3. *Effect of substate concentration on the rate of an enzyme reaction.*

This saturation effect led Michaelis and Menten to a general theory of enzyme action and kinetics in the year 1913. Accordingly, the enzyme (E) first reacts with the substrate (S) to form the enzyme substrate complex (ES) which then breaks down to form free enzyme and the product (P).

$$E + S \underset{K_2}{\overset{K_1}{\rightleftharpoons}} ES \underset{K_4}{\overset{K_3}{\rightleftharpoons}} E + P \qquad ...(7.1)$$

NOTES

Both the steps are reversible and K1, K2, K3 and K4 are specific rate constants for the said reactions. To derive Michaelis-Menten equation, rate of formation of ES and its breakdown is taken into consideration.

The rate of formation of ES from E + S is given by:

$$\frac{d[ES]}{dt} = K_1 ([E] - [ES]) [S] \quad ...(7.2)$$

(d = initial amount; dt = amount after time t)

The rate of formation of ES from E + P is negligible.

Similarly, the rate of breakdown of ES is given by —

$$\frac{-d[ES]}{dt} = K_2 [ES] + K_3 [ES] \quad ...(7.3)$$

When the reaction is in a steady state *i.e.,* when the rate of formation of ES is equal to its rate of breakdown, with the ES concentration being constant, then

$$K_1 ([E] - [ES]) [S] = K_2 [ES] + K_3 [ES] \quad ...(7.4)$$

or $$\frac{[S]([E] - [ES])}{[ES]} = \frac{K_2 + K_3}{K_1} = K_m$$

The **combined constant** K_m which is equal to $\frac{K_2 + K_3}{K_1}$ is called the **Michaelis-Menten constant.**

The steady state concentration of ES may be obtained as

$$[ES] = \frac{[E] [S]}{K_m + [S]} \quad ...(7.5)$$

Since initial rate of an enzymatic reaction (v) is proportional to the concentration of ES complex, therefore

$$v = K_3 [ES] \quad ...(7.6)$$

when substrate concentration is extremely high then whole of enzyme becomes saturated with substrate and exists as ES complex. At this state, **maximum velocity** of reaction (V_{max}) is reached

$$V_{max} = K_3 [E] \quad ...(7.7)$$

where [E] represents total concentration of enzyme. Substituting value of ES from equation 7.5 into equation 7.6, we get

$$v = K_3 \frac{[E] [S]}{K_m + [S]} \quad ...(7.8)$$

Dividing equation 7.8 by equation 7.7, we get

$$\frac{v}{V_{max}} = \frac{K_3 \frac{[E][S]}{K_m + [S]}}{K_3 [E]}$$

By solving it, we obtain

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

This is **Michaelis-Menten equation (MM Equation).**

When $v = \frac{1}{2} V_{max,}$ then

$$\frac{1}{2}V_{max} = \frac{V_{max}\ [S]}{K_m + [S]}$$

Dividing this by V_{max}, we get

$$\frac{1}{2} = \frac{[S]}{K_m + [S]}$$

or $K_m + [S] = 2\ [S]$

or $K_m = [S]$

In K_m, K stands for constant and *m* stands for Michaelis-Menten.

Thus, K_m or Michaelis-Menten constant is defined as the substrate concentration (expressed in moles/l) to produce half maximum velocity.

The value of the Michaelis-Menten constant is inversely proportional to the enzyme activity. A large value of K_m means that a high substrate concentration is needed to get half velocity of the maximum rate of reaction. In true sense it means that enzyme has lower affinity for the substrate.

When reciprocal values of enzyme activity and substrate concentration are plotted against each other, we get a straight line (Fig. 7.4). This double reciprocal plot is called the **Lineweaver-Burk plot.** From this plot, value of K_m can be obtained only by extending the line towards the abscissa.

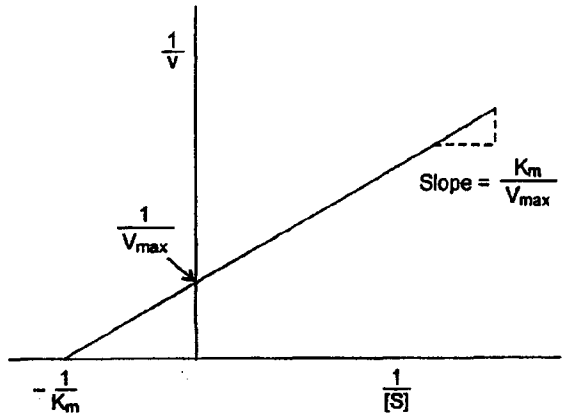


Fig. 7.4. *Lineweaver-Burk double reciprocal plot.*

Mode of Enzyme Action

There are three viewpoints by which enzymes are supposed to bring about catalytic reaction.

1. **Lock and Key Model (Fisher's Template Theory):** This theory was proposed by a German biochemist, **Emil Fischer** (1894). According to this theory, both enzyme and substrate molecules have specific geometrical shapes. The substrate fits to the active site of the enzyme just as a key fits into the proper lock or a hand into the proper glove (Fig. 7.5). The active sites also contain special groups having $-NH_2$, –COOH, –SH for establishing contact with the substrate molecule. The contact is such that the substrate molecules or reactants come together causing the chemical change. Just as a lock can be opened by its specific key, a substrate molecule can be acted upon by a particular enzyme.
2. **Induced Fit Theory (Koshland's Model):** This hypothesis was proposed by Koshland in 1958. According to this model, the active site is not rigid and pre-shaped. The essential features of the substrate binding site are present at the nascent site. The interaction of the substrate with the enzyme induces a fit or a conformation change in the enzyme, resulting in the formation of a strong substrate binding site. Further, due to induced fit, the appropriate amino acids of the enzyme are repositioned to form the active site and bring about the catalysis (Fig. 7.5 B). Induced fit model has sufficient experimental evidence from the X-ray diffraction studies. It also explains the action of allosteric modulators and competitive inhibition on enzymes.

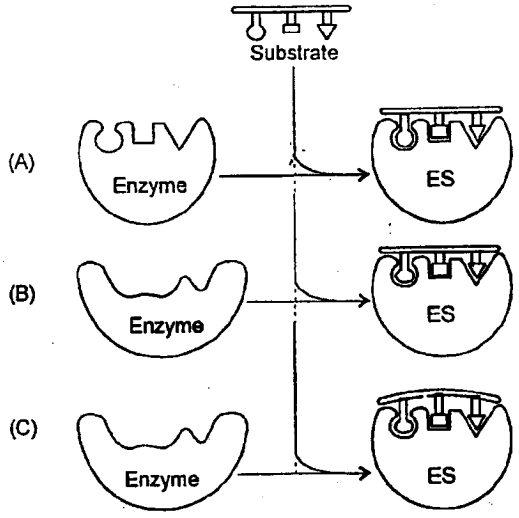


Fig. 7.5. *Mechanism of enzyme-substrate (ES) complex formation (A) Lock and key model (B) Induced fit theory (C) Substrate strain theory.*

3. **Substrate Strain Theory:** According to this model, the substrate is strained due to the induced conformation change in the enzyme. It is also possible that when a substrate binds to the pre formed active site, the enzyme induces a strain to the substrate. The strained substrate leads to the formation of product (Fig. 7.5C).

In fact, a combination of the induced fit model with the substrate strain is considered to be operative in the enzymatic action.

7.8 FACTORS AFFECTING ENZYME ACTIVITY

NOTES

The contact between the enzyme and the substrate is the most essential prerequisite for enzyme activity. The important factors that influence the enzyme activity are discussed below.

1. **Substrate Concentration:** The rate of the enzymatic reaction is influenced by the concentration of the substrate. The enzyme activity first increases with the increase in the concentration of the substrate. The enhanced rate is due to two factors: (*a*) occupation of more and more active sites by the substrate molecules; (*b*) higher number of collisions between substrate molecules. The rise in velocity is quite high in the beginning but it decreases progressively with the increase in substrate concentration. If a graph is plotted for substrate concentration *versus* reaction velocity, it appears as a hyperbolic curve. A stage is reached where velocity is maximum. It does not increase further by increasing the substrate concentration. At this stage the enzyme molecules become fully saturated and no active site is left free to bind additional substrate molecules (Fig. 7.6). This saturation effect is shown by all enzymes. Because of this, **Victor Henri** (1903), proposed the formation of enzyme substrate complex as an essential step in enzyme catalysis.

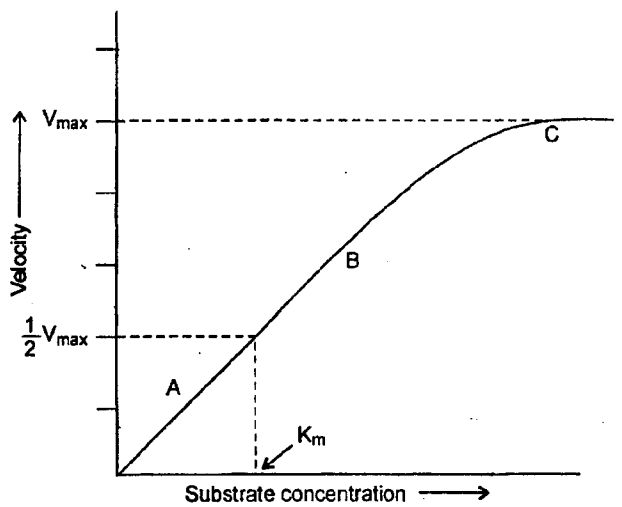


Fig. 7.6. *Effect of substrate concentration on enzyme velocity. (A. linear, B. Curve, C. Constant)*

2. **Concentration of Enzyme:** The rate of a biochemical reaction rises with the increase in enzyme concentration upto a point called limiting or saturation point (Fig 7.4). Beyond this , increase

NOTES

in enzyme concentration has little effect. This property of enzyme is made use in determining the serum enzymes for the diagnosis of diseases. By using a known volume of serum, and keeping all the other factors (substrate, pH, temperature etc.) at the optimum level, the enzyme could be assayed in the laboratory.

3. **Temperature:** Most enzymes are active within a narrow range of temperature. The temperature at which an enzyme shows its highest activity is called **optimum temperature.** Enzyme activity decreases above and below this temperature (Fig. 7.7). Enzyme becomes inactive below minimum temperature and beyond **maximum temperature.** Low temperature preserves the enzymes in the inactive state. Therefore, it is used in preservation of foods inside cold storages. High temperature destroys enzymes by causing their denaturation. This occurs at 50°C or so. In between the minimum and maximum temperatures, the reaction velocity doubles for every rise in 10°C (*i.e.*, Q_{10} = 2). Q_{10} or **temperature coefficient** is the increase in enzyme velocity when the temperature is increased by 10°C.

   The optimum temperature for most of the enzymes is between 35°C – 45°C. However, a few enzymes are active even at 100°C. This may be due to very stable structure and conformation of these enzymes.

4. **Effect of pH:** A change in the hydrogen ion concentration (pH) considerably influences the enzyme activity and a bell shaped curve is normally obtained (Fig 7.7). Each enzyme has an optimum pH at which the velocity is maximum. Below and above this

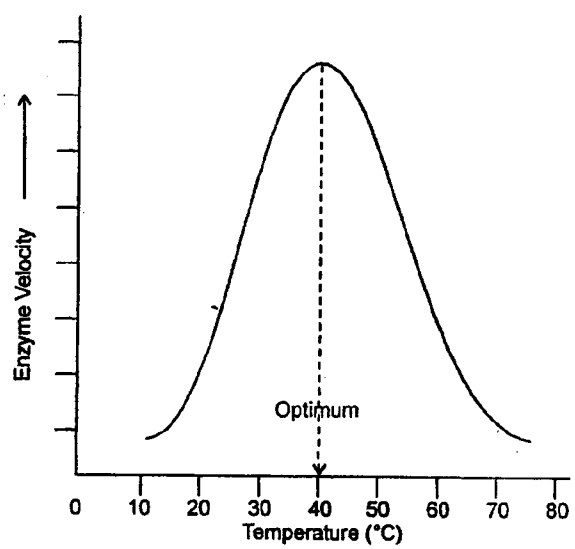


Fig. 7.7. *Effect of temperature on enzyme velocity*

pH, the enzyme activity is much lower and at extreme pH, the enzyme becomes totally inactive. Hydrogen ions influence the enzyme activity by altering the ionic charges on the amino acids (particularly at the active site), substrate, ES complex etc.

Most of the enzymes of higher organisms show optimum activity around neutral (pH 7–8). However, there are many exceptions like pepsin (pH = 1–2), acid phosphatase (pH = 4–5) and alkaline phosphatase (pH = 10–11). Enzymes from fungi and plants are most active in acidic pH (pH = 4–6).

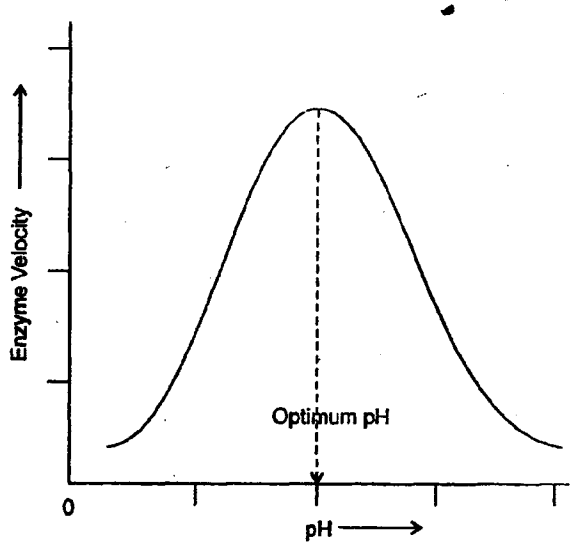


Fig. 7.8. *Effect of pH on enzyme velocity*

5. **Product Concentration:** The accumulation of reaction products generally decreases the enzyme velocity. For certain enzymes, the products combine with the active site of enzyme and form a loose complex and, thus, inhibit the enzyme activity. In the living system, this type of inhibition is generally prevented by a quick removal of product formed.
6. **Effect of Activators:** Some of the enzymes require certain inorganic metallic cations like Mg^{2+}, Mn^{2+}, Zn^{2+}, Ca^{2+}, Co^{2+}, Cu^{2+}, Na^{+}, K^{+} etc., for their optimum activity. Rarely, anions are also needed for enzyme activity. *e.g.,* chloride ion (Cl^{-}) for amylase. Metals function as activators of enzyme velocity through various mechanisms like combining with the substrate, formation of ES —metal complex, direct participation in the reaction and bringing a conformational change in the enzyme.

NOTES

7. **Effect of Light and Radiation:** The exposure of enzymes to UV, β, γ and X-rays inactivates certain enzymes due to the formation of peroxides. For example, the activity of enzyme salivary amylase is inhibited by UV rays.

7.9 ENZYME INHIBITION

Reduction or stoppage of enzyme activity due to presence of adverse conditions or chemicals is called enzyme inhibition. There are three broad categories of enzyme inhibition— reversible, irreversible suicide and allosteric inhibition.

1. Reversible Inhibition

The inhibitor binds non-covalently (temporarily) with enzyme and the enzyme inhibition can be reversed if the inhibitor is removed. The reversible inhibition is further sub-divided into—competitive and non-competitive inhibition.

(*i*) **Competitive Inhibition:** It is brought about by a substance which closely resembles the substrate in molecular structure. Such a substance is called **competitive inhibitor** or **substrate analogue.** The inhibitor competes with substrate and binds at the active site of the enzyme but does not undergo any catalysis. As long as the competitive inhibitor holds the active site, the enzyme is not available for the substrate to bind (Fig. 7.9 A)

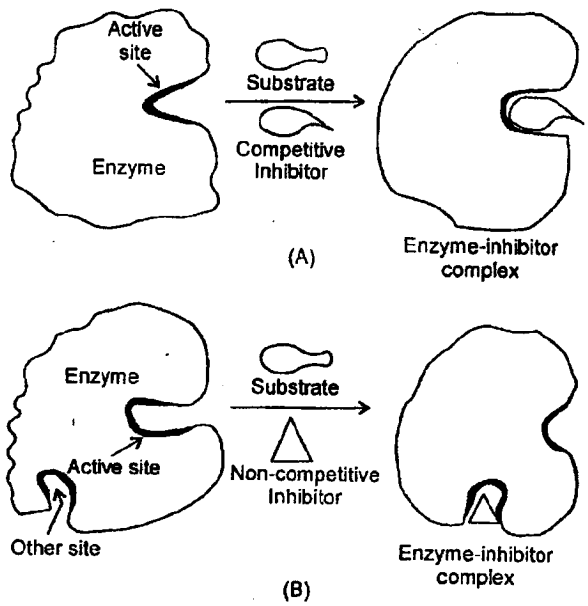


Fig. 7.9. *A diagrammatic representation of (A) Competitive, and (B) Non-competitive inhibition.*

The relative concentration of the substrate and inhibitor and their respective affinity with the enzyme determines the degree of competitive inhibition. The inhibition could be overcome by a high substrate concentration. In competitive inhibition, the K_m value increases whereas V_{max} remain unchanged (Fig. 7.10).

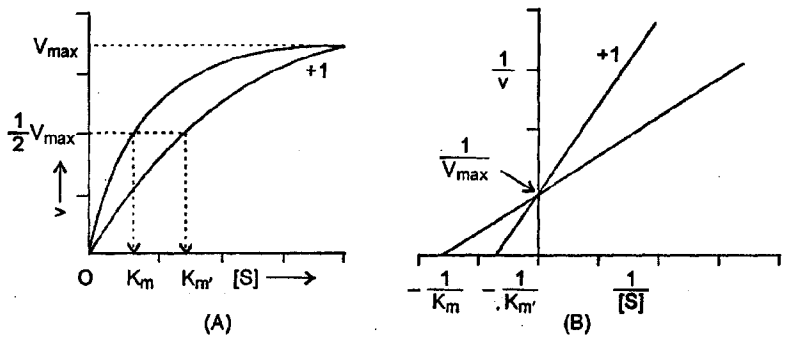
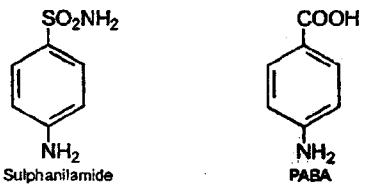


Fig. 7.10. *Effect of competitive inhibitor (i) on enzyme velocity. (A) Velocity (v) versus substrate (S) plot. (B) Lineweaver-Burk plot (Red lines with inhibitor; competitive inhibitor increases K_m, unalters V_{max}).*

Classical example of competitive inhibition is reduction of activity of succinate dehydrogenase by malonate, oxaloacetate and other anions which resemble succinate in their structure.

COO^-	COO^-	COO^-
|	|	|
CH_2	CH_2	C=O
|	|	|
CH_2	COO^-	CH_2
|		|
COO^-		COO^-
Succinate	Malonate	Oxaloacetate

Methanol poisoning is treated by ethanol on the principle of competitive inhibition. In the body methanol is converted into toxic formaldehyde by the enzyme *alcohol dehydrogenase (ADH).* Ethanol compete with methanol for ADH, and prevents the formation of formaldehyde. Similarly, control of bacterial pathogens has been affected through competitive inhibition. For example, sulpha drugs (like sulphanilamide) inhibit the synthesis of folic acid in bacteria by competing with *p*-amino benzoic acid (PABA) for the active site of enzyme. Humans obtain preformed folic acid in their diet. Therefore, sulpha drugs do not harm them.



Some more examples, of competitive inhibition of clinical importance are given in Table 7.2.

Table 7.2. Selected Examples of Competitive Inhibition of Clinical Importance

Enzyme	*Substrate*	*Inhibitor(s)*	*Significance of inhibitor(s)*
Xanthine oxidase	Hypoxanthine xanthine	Allopurinol	Used in the control of gout to reduce excess production of uric acid from hypoxanthine.
Monoamine oxidase	Catecholamines (epinephrine, noreinephrine)	Ephedrine, amphetamine	Useful for elevating catecholamine levels.
Dihydrofolate reductase	Dihydrofolic acid	Aminopterin, amethopterin, methotrexate	Employed in the treatment of leukemia and other cancers.
Acetylcholine esterase	Acetylcholine	Succinyl choline	Used in surgery for muscle relaxation, in anaesthetised patients.
Dihydropteroate synthase	Para amino-benzoic acid (PABA)	Sulfonilamide	Prevents bacterial synthesis of folic acid.
Vitamin K epoxide reductase	Vitamin K	Dicumarol	Acts as an anticoagulant.
HMG CoA reductase	HMG CoA	Lovastatin, compactin	Inhibit cholesterol biosynthesis

(*ii*) **Non-competitive Inhibition:** The inhibitor has no structural resemblance with the substrate. However, it has strong affinity to bind with the enzyme at the second site. The inhibitor binds at a site other than the active site on the enzyme surface. This binding impairs the enzyme function. The inhibitor does not interfere with the enzyme substrate binding. But the catalysis in prevented due to distortion in the enzyme confirmation (Fig. 7.9 B).

A number of heavy metal ions (*i.e.*, Pb^{2+}, Hg^{2+}, Ag^{+} etc.) can non-competitively inhibit enzyme action by binding with cysteinyl sulfhydryl groups as shown below:

$$E\text{-}SH + Pb^{2+} \rightleftharpoons E\text{-}S - Pb^{2+} + H^{+}$$

For non-competitive inhibition, the K_m value is unchanged while V_{max} is lowered (Fig 7.11)

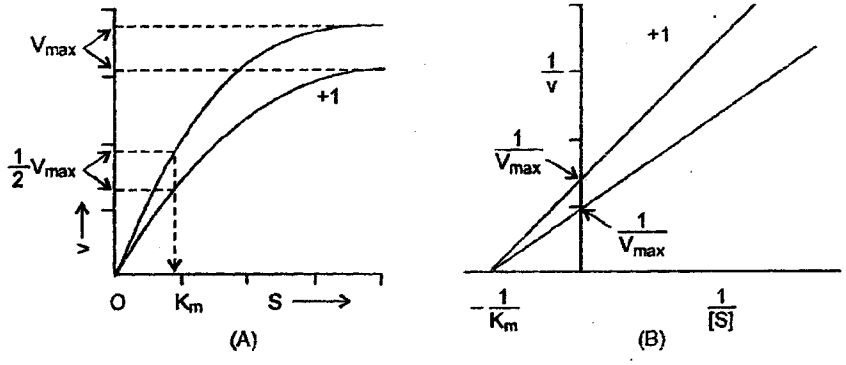


Fig. 7.11. *Effect of non-competitive inhibitor (i) on enzyme velocity. (A) Velocity (v) versus substrate (S). (B) Lineweaver-Burk plot (Red lines with ibhibitor, non-competitive inhibitor does not change K_m, but decreases V_{max}).*

2. Irreversible Inhibition

The irreversible inhibitors bind covalently with the enzymes or combines irreversibly with a functional group of enzyme that is essential for its catalytic function. Some example of irreversible inhibition are:

- The antibiotics—penicillin act as irreversible inhibitor of serine containing enzymes, and block the bacterial cell wall synthesis.
- Di-isopropyl fluorophosphate (DEP) is a nerve gas developed by the German during second World War. DEP prevents impulse transfer by combining irreversibly with amino acid serine of *acetylcholine esterase.*
- Many organophosphorus insecticides like melathion are toxic to animals (including man) as they block the activity of *acetylcholine esterase* (essential for nerve conduction), resulting in paralysis of vital body functions.
- The drug-Disulfiram (antabulase) is used for the treatment of alcohol addict. In the body, alcohol is first converted into acetaldehyde by the enzyme *alcohol dehydrogenase.* The acetadehyde is then metabolised into acetic acid by the enzyme *aldehyde dehydrogenase* Disulfiram irreversibly inhibits the enzyme *aldehyde dehydrogenase.* Alcohol addicts, when treated with disulfiram become sick due to the accumulation of acetaldehyde, making the alcohol addict to avoid the alcohol.

Suicide Inhibition

It is a specialized form of irreversible inhibition. In this case, the original inhibitor (*i.e.*, the structural analogue or competitive inhibitor) is converted

NOTES

to a more potent or effective form by the same enzyme that ought to be inhibited. The modified inhibitor binds irreversibly with the enzyme. This is in contrast to the original inhibitor which binds reversibly. For example,

- Allopurinol (used in the treatment of gout) is an inhibitor of *xanthine, oxidase*, gets converted to *alloxanthine*, a more effective inhibitor of this enzyme.
- 5-Fluorouracil (a pyrimidine analogue used in cancer therapy) gets converted to fluorodeoxyuridylate which inhibits the enzyme thymidylate synthase, and thus nucleotide synthesis.

Allosteric Inhibition

It is a type of reversible inhibition found in allosteric enzymes. The inhibitor is noncompetitive and is usually a low molecular intermediate or product of a metabolic pathway having a chain of reactions involving a number of enzymes. It is, therefore, also called **end product or feedback inhibition.** The inhibitor is also called **modulator.** Modulator is a substance that attaches with an allosteric enzyme at a site other than catalytic one but influences the latter, either inhibiting or activating the same. An example of feedback or allosteric inhibition is stoppage of activity of enzyme hexokinase (glucokinase) by glucose-6-phosphate, the product of reaction catalysed by it (Fig. 7.12).

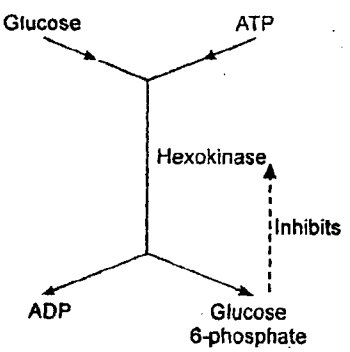


Fig. 7.12. Feedback or allosteric *inhibition of hexokinase.*

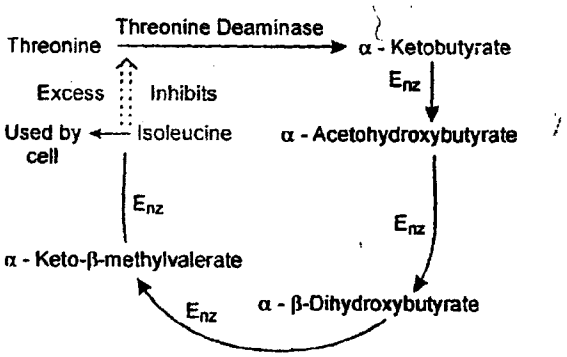


Fig. 7.13. *Feedback or end product in case of isoleucine.*

Another example is inhibition of threonine deaminase by isoleucine (Fig 7.13). Amino acid isoleucine is formed in bacterium *Escherichia coli* in a 5-step reaction from threonine. Each step requires a separate enzyme. When isoleucine accumulates beyond a **threshold value,** its further production stops. Isoleucine added to the medium of bacterium also stops its internal production showing that its excess prevents some step of the reaction. The latter was found out to be enzyme threonine

deaminase which is involved in the first step of the reaction (threonine to α-ketobutyrate).

Importance of Enzyme Inhibition

NOTES

- It has a regulatory role on enzyme activity.
- Enzyme inhibitors have been used in the study of metabolic pathways.
- Some inhibitors are used in controlling pathogenic activity, *e.g.*, sulpha drugs.
- Use of inhibitors have shown the mechanism of enzyme action.

STUDENT ACTIVITY

1. Who coined the term 'Enzyme'?

2. Explain how are enzymes named.

7.10 SUMMARY

- Enzymes are biocatalysists, mostly of protein nature synthesized by the living cells. They are classified into six major classes—oxidoreductases, transferases hydrolases, lyases, isomerases and ligases. Enzymes are specific in their action, possessing active site, where the substrate binds to form enzyme substrate complex before the product is formed. Many enzymes require the presence of non-protein substance called cofactors for their action.
- The substrate concentration to produce half maximal velocity is known as Michaelis constant (K_m value). Factors like concentration of enzyme, substrate, temperature, pH etc., influence enzyme activity.
- The mechanism of enzyme action is explained by lock and key model, more recently induced fit model and substrate strain theory have been proposed to explain enzyme action.
- The enzyme actions are inhibited by reversible (competitive and non-competitive) and irreversible and allosteric manner.

7.11 GLOSSARY

- **Active Site:** An area of the enzyme which attracts and holds particular substrate molecules and allow the chemical change.
- **Activation Energy:** Initial input of energy required to start a chemical reaction.
- **Allosteric Enzyme:** A regulatory enzyme whose catalytic activity is modulated by the binding of a specific metabolite at a site other than the active site.
- **Competitive Inhibition:** Inhibition of enzyme activity by a substance having molecular structure similar to the substrate.
- **Conjugated Enzyme:** An enzyme with a non-protein prosthetic group.
- **Feedback Inhibition:** Inhibition of an allosteric enzyme at the beginning of a metabolic sequences by the end product of the sequence.
- **Induced Fit:** A change in conformation of an enzyme in response to substrate binding that renders the enzyme catalytically active.
- **Isoenzymes:** Multiple molecular forms of an enzyme occurring in the same organism and having a similar substrate.
- **Non-competitive Inhibition:** Inhibition of enzyme activity by a substance at the site other than the active site, resulting in the destruction of some functional group of the enzyme.

NOTES

7.12 REVIEW QUESTIONS

I. Very Short Answer Type Questions:

1. Why are enzymes called 'the catalysts of life'?
2. Name the first enzyme obtained in crystalline form.
3. What is a substrate with reference to enzymes?
4. What does the abbreviation OTHLIL stand for?
5. What are proenzymes?

II. Short Answer Type Questions:

1. Write short notes on:
   (*i*) Active sits (*ii*) Coenzymes
   (*iii*) Allosteric enzyme (*iv*) Km value
   (*v*) Activation energy (*vi*) MM Equation.
2. Discuss the effect of substrate concentration on enzyme action.
3. What is irreversible inhibition? Give some examples of this type of inhibition.
4. What is competitive inhibition? Give some examples of competitive inhibition of clinical importance.

III. Long Answer Type Questions:

1. Describe the nomenclature and IUB classification of enzymes.
2. Discuss the mechanism of enzyme action.
3. Give an account of various factors affecting enzyme activity.
4. Discuss the inhibition of enzyme activity.

7.13 FURTHER READINGS

- **Outlines of Biochemistry;** Conn. E.E, Stumpf P.K., Bruening G. Doi R.H; Wiley india (P) Ltd. New delhi; 2007.
- **Biochemistry;** Satyanarayn U, Chakrapani U; Books and Allied (I) Ltd. Kolkata; 2008.
- **Biochemistry;** Powar, Chatwal; Himalayas Pub. House Bombay; 1988.

UNIT–V

CHAPTER 8 IMMUNOLOGY

OBJECTIVES

After going through this chapter, you should be able to:

- explain innate immunity
- know about acquired immunity
- describe the cells involved in acquired immunity
- know about active and passive immunity
- understand immune response.

STRUCTURE

8.1 INTRODUCTION

We are continuously exposed to various foreign particles including infectious agents like bacteria, viruses, fungi and parasites. However, only a few of these exposures result in disease. This is due to the fact that the body is able to defend itself from most of these foreign agents. This overall ability of the host to fight the disease-causing organisms is called **immunity.** In other words, immunity refers to the resistance of a host to pathogens and their toxic products. The system of our body,

NOTES

which protects us from various infectious agents and cancer is called **immune system.** The study of the immune system is know as **immunology.** Immunity is broadly classified into two types—innate (non-specific) immunity and acquired or adaptive (specific) immunity.

8.2 INNATE IMMUNITY (NON-SPECIFIC IMMUNITY)

Innate immunity is non-specific type of defence which an individual possesses by virtue of his/her genetic and constitutional make up. It comprises all those elements with which an individual is born, and which are always available to protect the living body. One kind of innate immunity consists of various types of barriers which prevent entry of foreign agents into our body. Innate immunity consists of four types of barriers: physical physiological, cellular and cytokine barriers.

1. Physical Barriers

Skin on our body is the main barrier which prevents entry of the microorganisms. Mucus secreted by the mucous membrane (epithelium lining) of the respiratory, gastro-intestinal and urogential tracts also help in trapping microbes entering our body.

2. Physiological Barriers

Body temperature, pH of the body fluids and various body secretions prevent growth of many disease causing microorganisms.

- The oil and sweat secreted by sebaceous and sudoriferous glands contain fatty acids and lactic acid which make the skin surface acidic (pH 3 to 5). This does not allow to microorganisms to establish on the skin.
- Acid of the stomach kills most of the microoranisms ingested with food.
- The enzyme, lysozyme, present in perspiration, saliva and tears kills many bacteria by destroying their cell walls.
- Cerumen (ear wax) traps dust particles, kills bacteria and repels insects.
- Certain bacteria living in vagina *e.g.*, (*Lactobacilli*) produce lactic acid which kills the foreign bacteria. The lactic acid bacteria of the vagina constitute female's best natural defence against infection.
- A rise in temperature (fever) due to infection is a natural defence mechanism which help not only to accelerate physiological processes but may in some cases destroy the infecting pathogens.

3. Cellular Barriers

Certain types of leukocytes (WBCs) such as Polymorphonuclear Leukocytes (PMNL), neutrophils, monocytes and natural killer cells (a type of lymphocytes) in the blood as well as macrophages in tissues can phagocytose and destroy microbes.

Besides to phagocytes there are **natural killer cells (NK cells)** in the body which kill virus infected and some tumour cells. The killer cells produce perforins which create pores in the plasma membrane of the target cells. These pores allow entry of water into the target cells, which then swell and burst. The cellular remains are taken by phagocytes.

4. Cytokine Barriers

The cells which are invaded by virus produce an anti-viral proteins called **interferons (IFNs).** The latter protect non-infected cells from further viral infection.

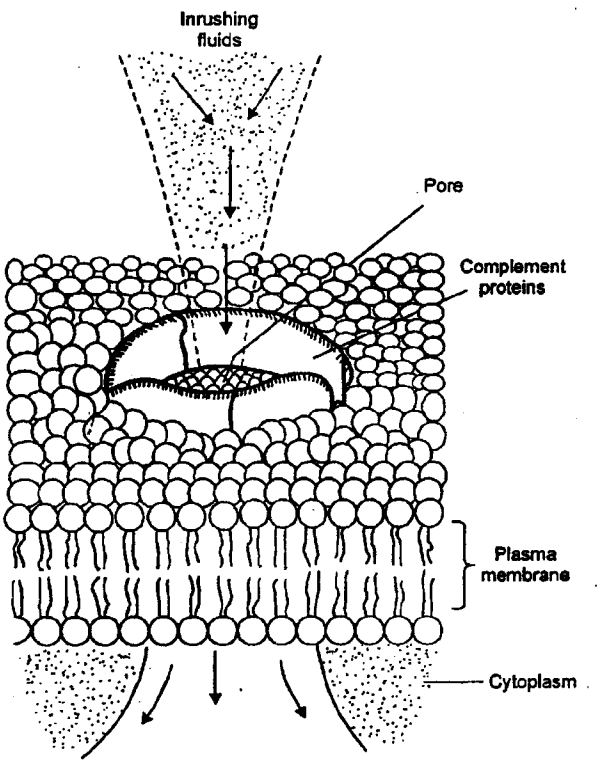


Fig. 8.1. *Complement proteins creating a hole in the plasma membrane.*

Fever may be brought about by toxins produced by pathogens and a protein called **endogenous pyrogen** (fever producing substance), also called **interleukin** released by macrophages. When enough pyrogens reach the brain, the body's thermostat is reset at a higher temperature. Moderate fever aids defence by stimulating the phagocytes and by inhibiting

NOTES

growth of microorganisms. However, a very high temperature is dangerous. It must be bring down quickly by giving anti-pyretics and by applying cold packs.

Complement System. The complement system participates in both innate and acquired immunities. It consists of over 30 proteins that act in various way to protect the individual from foreign invaders. The member proteins of the complement system function is an orderly manner. Ultimately, there is formation of transmembrane pores in the microbes, which leads to their lysis (Fig. 8.1). Some components of the complement system coat the invading microbes. This coating enables phagocytes to readily attach to the microbes and destroy them.

The various types of barriers which prevent the entry of foreign agents into the body constitute the first line of defence. The phagocytes, interferons, inflammatory reactions, fever, natural killer cells and complement system constitute the second line of defence. The Third line of defence is provided by specific defence mechanism which include antibodies and lymphocytes.

8.3 ACQUIRED IMMUNITY (ADAPTIVE OR SPECIFIC IMMUNITY)

The acquired immunity is the resistance that an individual acquires during his life. It is developed by individual in response to a disease caused by infection of microbes. Acquired immunity is found only in vertebrates. It supplements the protection provided by innate immunity. Acquired or adaptive immunity has the following unique features.

- **Specificity.** It is the ability to differentiate among various foreign molecules.
- **Diversity.** It is the ability to recognise a vast variety of foreign molecules.
- **Discrimination between self and non-self.** The acquired immunity is able to recognise and respond to foreign molecules (non-self) and can avoid response to those molecules that are present within the body (self) of the animal.
- **Memory.** When the immune system encounters a specific foreign agent, *e.g.,* a microbe for the first time, it generates immune response and eliminates the invader. The immune system retains the memory of this encounter. As a result second encounter with the same microbe evokes a heightened immune response.

8.4 CELLS INVOLVED IN ACQUIRED IMMUNITY

Acquired immunity involves two major groups of cells; Lymphocytes and antigen presenting cells.

NOTES

1. Lymphocytes

The major cells of the immune system are the WBC's called **lymphocytes.** A healthy human being possesses about a trillion lymphocytes. The lymphocytes are of two types: **T lymphocytes** or **T cells** and **B lymphocytes** or **B cells.** Both the types of lymphocytes as well as the other cells of the immune response are produced in bone marrow. The process of production of the cells of immune system in the bone marrow is called **haematopoiesis.**

(a) T Lymphocytes (T cells)

They, like the other blood corpuscles, arise from the stem cells (the **haemacytoblasts**) in the bone marrow in the adult and in the liver in the foetus. Some immature lymphocytes migrate via blood to the thymus. On entering the thymus, these cells are called ***thymocytes.*** In the thymus, these cells mature into T lymphocytes or T cells.

How T cells Respond to Antigens. T cells respond to antigens by producing a clone of T cells. Each T cell recognises a specific antigen. Therefore, the body contains separate T cells for every antigen that the body encounters. Apparently T cells remain alive for 4–5 years or even longer. The clone of T cells produced in the presence of an antigen are similar, but they perform different functions.

(*i*) **Helper T cells.** They regulate the overall immunity by forming a series of protein mediators called **lymphokines** that act on other cells of the immune system as well as on bone marrow cells. Helper T cells stimulate the B cell to produce antibodies. They also stimulate the killer T cells to destroy the non-self cells.

(*ii*) **Cytotoxic T cells (Killer cells or K cells).** These cells directly attack the foreign cells. The cytotoxic T cells secrete a protein perforin which punctures the invaders cell membrane. Water and ions flow into the non-self cells. The cyhotoxic cells are responsible for cell mediated immunity.

(*iii*) **Suppressor T cells.** They suppress the functions of cytotoxic and helper T cells. They also inhibit the immune system from attacking the body's own cells.

(*iv*) **Memon T cells.** They are stored in the lymphatic tissue *i.e.,* spleen, lymph nodes, and recognise original invading antigens ever years after the first encounter. These cells are ready to attack as soon as the same pathogen infect the body again. This explains why some childhood diseases are not contacted a second time.

(b) B Lymphocytes (B cells)

Certain lymphocytes produced by the stem cells remain in the bone marrow. These cells mature in the bone marrow itself. B lymphocytes

NOTES

produce an army of proteins in response to pathogens into the blood to fight with them. These proteins are called **antibodies.** B cells generate antibody mediated or humoral immunity.

How B cells Respond to Antigens. When antigens invade a tissue fluid, B cells are stimulated to produce antibodies. The body has thousands of antigen specific B cells. The membrane of each type would have been sensitised by previous contact with the antigen. If this does not happen, the B cells dies quickly. However, there is a constant supply of new B cells. Once an antigen specific B cell is triggered by the antigen, it gives rise to a clone of **plasma cells** or **effector B cells** of quick multiplication. Most members of this clone produce antibody at the rate of about 2,000 molecules per second. The capacity to produce a specific antibody is acquired by the B cells during its development without prior exposure to antigen. However, an antigen is necessary to trigger antibody production.

Some activated B cells do not differentiate into plasma cells but rather remain as memory cells. They are called **memory B cells.** The memory B cells have a longer life span. They remain dormant until activated once again by the same type of antigen.

Antigen Presenting Cells (APCs)

These are specialised cells which include **macrophages** (monocytes as blood macrophages and histocytes as tissue macrophages), **B lymphocytes** and **dendritic cells** (*e.g.*, Languorous cells of epidermis of skin). They are characterised by two properties (*i*) the express class II MIIC *n* (major histocompatibility complex.) molecules on the membrane and (*ii*) they are able to deliver a co-stimulatory signal necessary for helper T cell activation.

Major Histocompatibility Complex

The Major Histocompatibility Complex (MHC) represents a special group of **Proteins, present on the cell surfaces of T-lymphocytes.** MHC is involved in the recognition of antigens of T-cells. It may be noted here that the B-cell receptors (antibodies) can recognize antigens on their own, while T-cells can do so through the medication of MHC.

In humans, the MHC proteins are encoded by a cluster of genes located on chromosome 6 (it is on chromosome 17 for mice). The major histocompatibility complex in humans is referred to ***as human leukocyte antigen (HLA).*** Three classes of MHC molecules (chemically glycoproteins) are known in human. Class I molecules are found on almost all the nucleated cells of the body. Class II molecules are associated only with leukocytes involved in cell-mediated immune response. Class III molecules are the secreted proteins possessing immune functions *e.g.*, complement components (C_2, C_4), tumor necrosis factor.

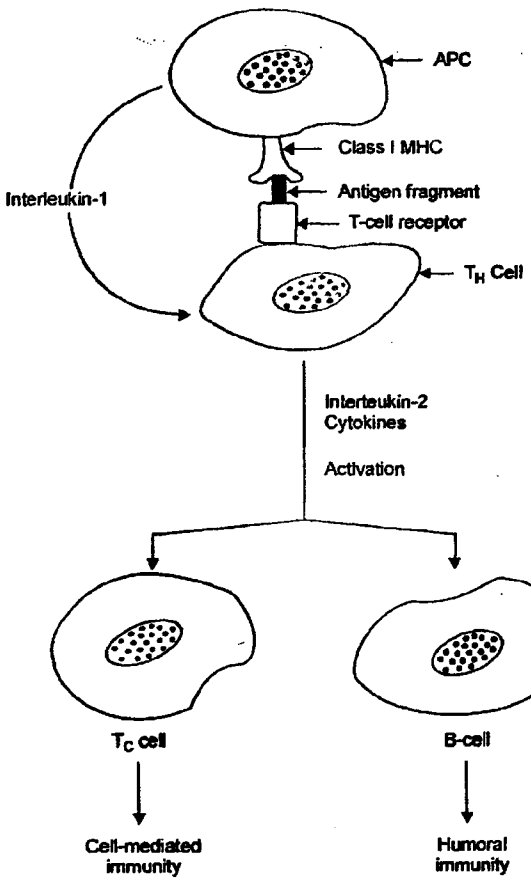


Fig. 8.2. *A diagrammatic representation of the central role of helper T Cells (T_H cells) in immune response (APC—Antigen presenting cell; T_C cell—Cytotoxic T-Cell).*

8.5 ACTIVE AND PASSIVE IMMUNITY (INFECTION AND IMMUNITY INTERACTION)

1. Active Immunity

It develops due to the immune response generated when a person suffers from a disease or gets vaccination for a disease. It involves the active functioning of the preson's own immune system leading to the synthesis of antibodies and/or the production of immunologically active cells.

The active immunity which develops due to infections of a pathogen is called **natural active immunity,** while the immunity which is induced by vaccines is called **artificial active immunity.** Vaccines are preparations of live or killed pathogens or their products. These are inoculated in a person's body to generate immunity. This process is called immunisation. Some commonly used vaccines are as follows: **Bacterial vaccines** (*a*) live-BCG vaccine for tuberculosis (*b*) killed-TAB vaccine for enteric

NOTES

fever. (*c*) **Bacterial products** toxoid for diphtheria and tetanus. **Viral vaccines** (*a*) Live-Sabin vaccine for poliomyelitis, MMR vaccine for measles, mumps, rubella (*b*) Killed-salk vaccine for poliomyelitis, neural and non-neural vaccines for rabies.

2. Passive Immunity

It is conferred by transfer of immune products, like antibodies etc., from another individual into a non-immune individual.

The yellowish fluid **colostrum** (first milk) secreted by mother during the initial days of lactation has abundant antibodies (IgA) to protect the infant. This is the reason, that mother's milk is considered very essential for the new born infants. The foetus also receives some antibodies from mother through placenta during pregnancy. Such passive immunity is called **natural passive immunity.** The passive immunity which is conferred in an individual by administration of antibodies is called **artificial passive immunity.** It is achieved through administration of hyperimmune sera (sing, serum) of man or animals. Serum contains antibodies. Some common examples of sera are as follows: (*a*) Antitetanus Serum (ATS) is prepared in horses by active immunisation of horses with tetanus toxoid, withdrawal of blood and separation of the serum. ATS is used for passive immunisation against tetanus. Similarly, antidiphtheric (ADS) and Anti-Gas Gangrene Serum (AGS) are also prepared.

Activation of Adaptive Immunity

Every antigen is processed by antigen presenting cells, like macrophages, B lymphocytes etc. The processed antigen is presented on the surface of these cells. The T helper cells specifically interacts with the presented antigen and become activated. The activated T helper cells then activate B cells and T cytotoxic cells in a specific manner. The activated B and T cytotoxic cells proliferate to produce clones. All the cells of a clone recognise the same antigen and eliminate it.

Antigens and Antibodies (Antigen-Antibody Interaction)

Antigens (Immunogens)

The term 'antigen' is the shortened form of antibody-generating material. Antigen may be defined as *'the substance which, when introduced into the body, stimulate the production of antibodies.'* Usually protein molecules present on the surface of foreign bodies (viruses, bacteria) act as antigens, but some carbohydrate and lipid molecules can also act as antigens.

The sites on antigens that are recognised by antibodies and receptors present of T and B cells are called **antigenic determinants** or **epitopes.** In fact the smallest unit of antigenicity are the antigenic determinants. Each determinant can stimulate the formation of a particular kind of

antibody or effector cell. Thus a pure protein antigen may give rise to many distinct antibodies and effector cells. Based on the ability of antigens to carry out their functions, antigens are of two types, (*i*) **Complete antigens.** These are able to induce antibody formation and produce a specific and observable reaction with the antibody so produced. (*ii*) **Partial Antigens (Haptens).** These are incapable of inducing antibody formation by themselves, but can be capable of inducing antibodies on combining with larger molecules (normally proteins) which serve as carriers.

The RBCs of all ABO blood groups possess a common antigen, the **H antigen,** which is a precursor for the formation of A and B antigens. Due to universal distribution, H antigen is not ordinarily important in grouping or blood transfusion.

Antibodies

Antibodies are a class of proteins called **immunoglobulins** (Igs), which are produced in response to antigenic stimulation. Antibodies are produced by B lymphocytes and plasma cells. In fact B lymphocytes give rise to plasma cells. The mature plasma cells produce antibody at the rate of about 2,000 molecules per second.

Each immunoglobulin molecule is made up of 4 polypeptide chains. There are two long chains called **heavy** or **H chains** and two short chains called **light** or **L chains.** The four polypeptide chains are held together by disulphide bond to form a Y shaped molecule (Fig. 8.3). The top two tips of this Y-shaped molecule bind to the specific antigens in a lock and key fashion, forming an **antigen-antibody complex.** Each antigen has many different antigenic determinants, each of which matches a specific antibody and binds to it (Fig. 8.4). The B cells, thus direct the **antibody-mediated immunity** (also called **humoral immunity).**

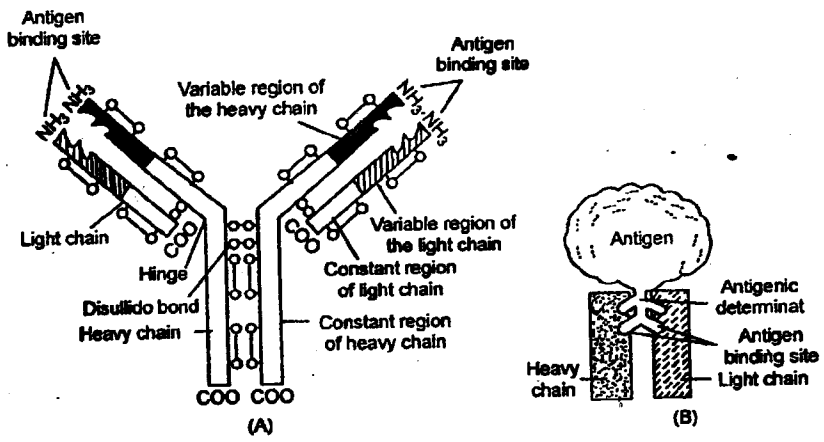


Fig. 8.3. *(A) Structures of an antibody molecule. (B) Antigen binding site.*

NOTES

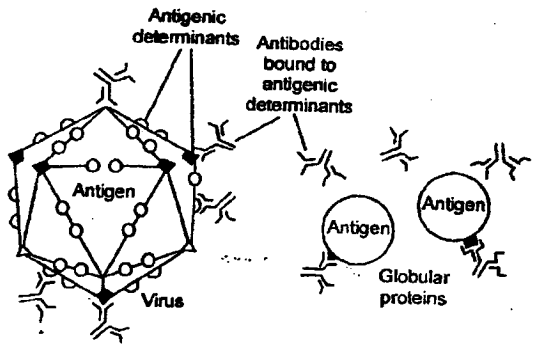


Fig. 8.4. *Formation of antigen-antibody complex.*

The antibody molecules may be bound to a cells membrane or they may remain free. The free antibodies have following three main functions:

(*i*) **Agglutination** clumping of particulate matter including bacteria and viruses,

(*ii*) **Opsonisation** coating of bacteria to facilitate their subsequent phagocytosis by cells (such antibioties are called **opsonins**), and

(*iii*) **Neutralisation**—to neutrilise the toxins released by bacteria *e.g.*, tetanus toxin.

Classes of Immunoglobulins

Based on physiochemical and antigenic structure, human immunoglobulins are grouped into five classes:

IgA (= alpha, α), **IgD** (= delta δ), **IgE** (= espilon, ε) **IgG** (= gamma, γ) and **Igm** (= mu, μ).

Immunoglobulin G (IgG)

IgG is the most abundant (75–80%) class of immunoglobulins. IgG is composed of a single Y-shaped unit (monomer). It can traverse blood vessels readily. IgG is the only immunoglobulin that can cross the placenta and transfer the mother's immunity to the developing fetus. IgG triggers **foreign cells destruction** mediated by complement system.

Immunoglobulin A (IgA)

IgA occurs as a single (monomer) or double unit (dimer) held together by J chain. It is mostly found in the body secretions such as saliva, tears, sweat, milk and the walls of intestine. IgA is the most predominant antibody in the colostrum, the initial secretion from the mother's breast after a baby is born. The IgA molecules bind with bacterial antigens present on the body (outer epithelial) surfaces and remove them. In this way, IgA ***prevents the foreign substances*** from entering the body cells.

Immunoglobulin M (IgM)

IgM is the largest immunoglobulin composed of 5 Y-shaped units (IgG type) held together by a J polypeptide chain. Thus IgM is a pentamer. Due to its large size, IgM cannot traverse blood vessels, hence it is restricted to the blood stream. IgM is the first antibody to be produced in response to an antigen and is the most ***effective against invading microorganisms.*** It may be noted that IgM can simultaneously combine with 5 antigenic sites due to its pentameric structure.

Immunoglobulin D (IgD)

IgD is composed of a single Y-shaped unit and is present in a low concentration in the circulation. IgD molecules are present on the surface of B cells. Their functions, however, is not known for certain. Some workers believe that IgD may function as ***B-cell receptor.***

Immunoglobulin E (IgE)

IgE is a single Y-shaped monomer. It is normally present in minute concentration in blood. IgE levels are elevated in individuals with ***allergies*** are it is associated with the body's allergic responses. The IgE molecules tightly bind with mast cells which release histamine and cause allergy.

Different classes of immunoglobulins and their functions are summarised in Table 8.1.

Table 8.1. Different Classes of Immunoglobulins and their Functions

Immunoglobulin Class	*Presence*	*Functions*
1. IgA	(*i*) Second most abundant class, constituting about 10 per cent of serum immunoglobulins.	(*i*) Protects from inhaled and ingested pathogens.
	(*ii*) Present in colostrum, saliva and tears.	(*ii*) Protects eyes.
	(*iii*) Also in gastro-intestinal tract and respiratory tract.	
2. IgD	Present on the surface of B lymphocytes which are destined to differentiate into antibody producing plasma cells.	Activates B cells to secrete antibody.
3. IgE	Remain free, Heat labile (inactivated at 56°C in one hour).	Acts as mediator in allergic response, also mediates type I hypersensitivity (anaphylaxis).
4. IgG	(*i*) Most abundant class constituting about	(*i*) Stimulates phagocytes and complement system

	75 per cent of the total immunoglobulins.	
	(*ii*) Only antibody that can cross placenta.	(*ii*) Provides natural passive immunity in the foetus and new born.
	(*iii*) Present in milk.	(*iii*) Protects the body fluids.
5. IgM	(*i*) Largest immunoglobulin (at least five times larger than IgG).	(*i*) Activates B cells.
	(*ii*) Earliest Ig to be synthe -sised by the foetus.	(*ii*) Protects the blood serum.

8.6 IMMUNE RESPONSE

the specific reactivity induced in a host by an antigenic stimulus is known as the immune response. Immune response is of two types: Humoral or Antibody Mediated Immunity (AMI) and Cell Mediated Immunity (CMI).

(*i*) Humoral Immunity or Antibody-Mediated Immunity (AMI)

The B lymphocytes from the humoral or antibody mediated immunity. B lymphocytes are preprocessed in the liver during midfoetal life and in the bone marrow during late foetal life and after birth. Later, the preprocessed B lymphocytes migrate to the lymphoid tissues.

Each B lymphocyte displays on its surface is specific receptor. This receptor is the antibody produced by the B lymphocyte. When this receptor interacts with the antigenic determinant specific to it, the lymphocyte becomes activated and divides to form clone of cells. These cells are also transformed into effector cells *i.e.*, antibody producing B cells. This phenomenon is called **clonal selection,** where all the cells in a given B cell clone are derived from a single parental cell, and exhibit the same specificity for antigenic determinant. But some of the activated lymphocytes develop into long lived **memory cells.** They do not produce antibodies or infected cells. The memory cells have a long life span and serve to recognise the same antigen when introduced subsequently. Antibodies produced by a single antibody forming cell or clone and directed against a single antigenic determinant are called **Monoclonal Antibodies (MCA).**

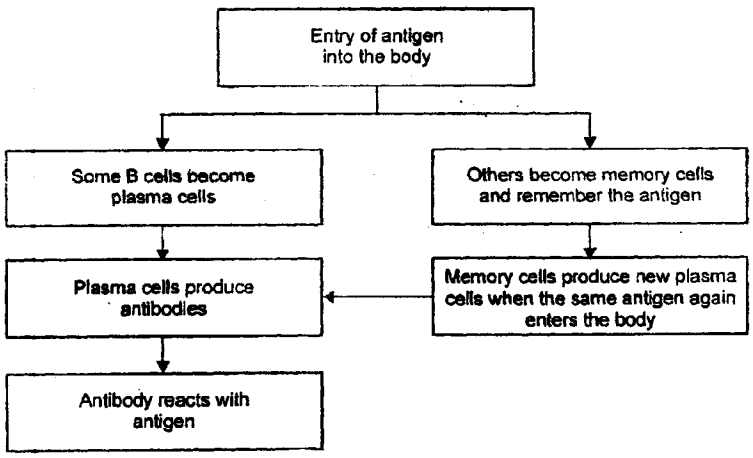


Fig. 8.5. *A summary of antibody-mediated immunity.*

(*ii*) Cell Mediated-Immunity (CMI)

The T lymphocytes form the cell mediated immunity. The T lymphocytes are produced in the bone marrow and migrate to the thymus. Here, they divide rapidly and develop extreme diversity for reacting against different specific antigens.

The cell mediated immunity is the responsibility of a sub-group of T cells, called T cytotoxic cells. Like B cells, each T cytotoxic cells displays on its surface a specific receptor. When this recaptor interacts with the antigenic determinant specific to it, the lymphocyte becomes activated and divides to form a clone of cells. An activated T cytotoxic cell is specific to a target cell which has been infected and kill the target cell by a variety of mechanisms. This prevents the completion of life cycle of the pathogen, since it depends on an intact host cell. Cell mediated immunity is also involved in killing of cancer cells.

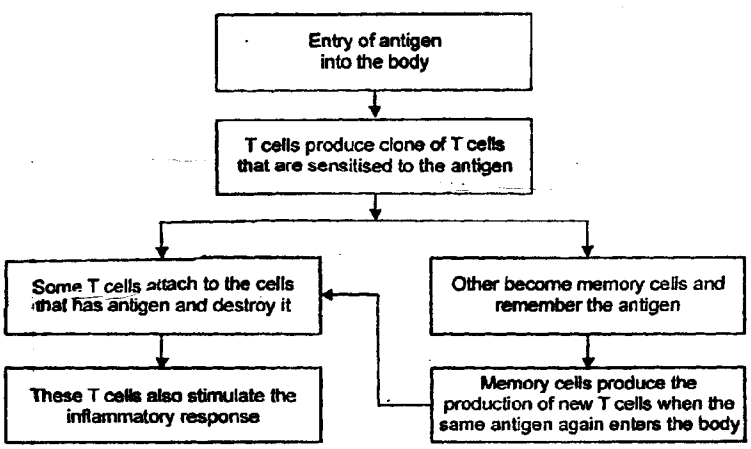


Fig. 8.6. *A aummary of cells mediated immunity.*

NOTES

T lymphocytes also release some regulatory proteins called **lymphokines.** The lymphokines play a role in regulation of immune response and growth and functions of reticuloendothelial system, *i.e.*, macrophages, phagocytic macrophages which are present in linings of sinuses and in reticulum of various organs and tissue (spleen, live, lymph nodes, bone marrow connective tissue, etc.).

Cell mediated immunity provide protection against most of the viruses, fungi, intracellular bacteria (*e.g., Mycobacterium laprae, M. tuberculosis* and *Salmonella*) and parasites (*e.g., Leishmania,* and *Trypanosomas*). It is also involved in allograft rejection, graft versus host reaction, delayed hypersensitivity and certain auto immune diseases.

8.7 PRIMARY AND SECONDARY IMMUNE RESPONSES

The immune response mounted as a result of the first encounter of an animal with an antigen is called **primary immune response.** It takes relatively longer time, is feeble and declines rapidly. But a subsequent encounter of this animal with the same antigen results in a hightened immune response. It is called **secondary immune response.** The secondary immune response is due to the memory cells that were produced during the primary response. This response occurs more rapidly and lasts much more longer than primary immune response. This is the reason that a person who had been suffering from disease like measles, smallpox or chickenpox become immune to subsequent attacks of these disease. The secondary immune response is also called **booster response.**

8.8 IMMUNE SYSTEM IN THE BODY

The immune system comprises **lymphoid organs, tissues, cells** and soluble molecules like antibodies. This system is unique as it recognises foreign bodies (antigen) respond to these antigens and remembers them. Besides, it plays a role in allergic reactions, autoimmune diseases and organ transplantation.

Lymphoid Organs

These organs are those organs where the maturation and proliferation of lymphocytes takes place (Fig. 8.7). Lymphoid organs are classified into two types: Primary lymphoid organs and secondary lymphoid organs.

1. Primary Lymphoid Organs (Central Lymphoid Organs)

These are the lymphoid organs where T lymphocytes and B lymphocytes, mature and acquire their antigen specific receptors. The primary lymphoid

organs include **bone marrow** and **thymus.** Bone marrow is the main lymphoid organ where all blood cells including lymphocytes are formed. Thymus is the site of T lymphocyte maturation and bone marrow is the site of B lymphocyte maturation. In birds **bursa of Fabricius** is the primary lymphoid organ and is considered equivalent to bone marrow of mammals.

2. Secondary Lymphoid Organs (Peripheral Lymphoid Organs)

These include **spleen, lymph nodes, tonsils, Peyer's patches** of small intestine, **appendix** and **Mucosal Associated Lymphoid Tissues (MALT).** Spleen is bean shaped organ which mainly contains lymphocytes, phagocytes and larger number of erythrocytes. Lymph nodes are small solid structures located as specific sites along the lymphatic system. Antigens that happen to enter in the lymph and tissue fluid activate the lymphocytes present inside the lymph nodes leading to the immune responses. MALT is located within the lining of the major tracts (digestive respiratory and urinogenital). It constitutes about 50 per cent of the lymphoid tissue is human body. The lymphoid tissue of the gut is called **Gut Associated Lymphoid Tissue (GALT).**

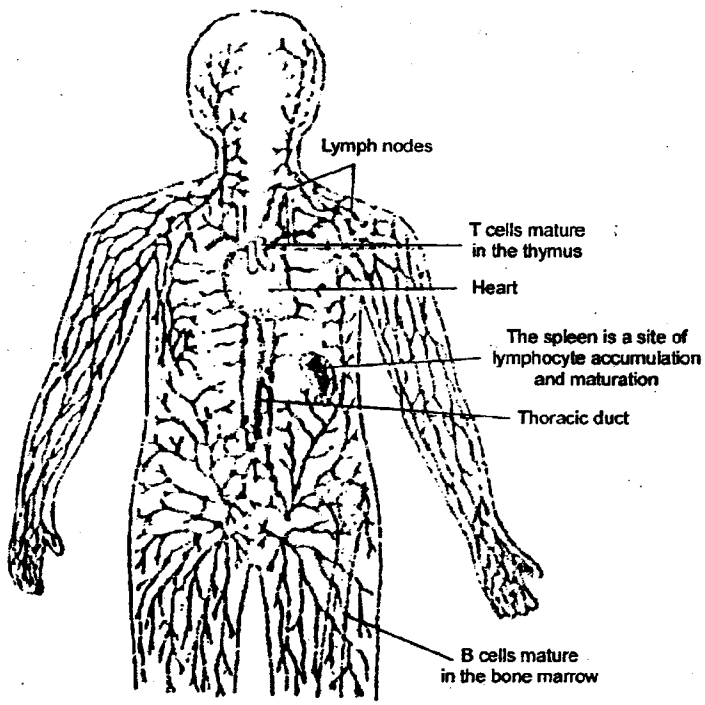


Fig. 8.7. *Human lymphatic system.*

After maturation B lymphocytes and T lymphocytes migrate via blood vascular and lymphatic system to the secondary lymphoid organs where they undergo proliferation and differentiation. The acquired immune

NOTES

response to antigens usually develops in these organs. The lymphocytes move from one lymphoid organ to another through blood and lymph.

Immunity in Health and Disease

The prime function of immune system is to protect the host against the invading pathogens. The body tries its best to overcome various strategies of infectious agents (bacteria, viruses), and provides immunity.

Some of the important immunological aspects in human health and disease are briefly described.

Autoimmune Diseases

In general, the immune system is self-tolerant *i.e.*, not responsive to cells or proteins of self. Sometimes, for various reasons, the ***immune system fails to discriminate between self and non-self.*** As a consequence, the cells or tissues of the body are attacked. This phenomenon is referred to as ***autoimmunity*** and the diseases are regarded as autoimmune diseases. The antibodies produced to self molecules are regarded as ***autoantibodies.*** Some examples of autoimmune diseases are listed.

- Insulin-dependent diabetes (pancreatic β-cells autoreactive T-cells and antibodies).
- Rheumatoid arthritis (antibodies against proteins present in joints).
- Myasthenia gravis (acetylcholine receptor autoantibodies).
- Autoimmune homolytic anemia (erythrocyte autoantibodies).

Mechanism of Autoimmunity

It is widely accepted that autoimmunity generally occurs as a consequence of ***body's response against bacterial, viral or any foreign antigen.*** Some of the epitopes of foreign antigens are similar (homologous) to epitopes present on certain host proteins. This results in cross reaction of antigens and antibodies which may lead to autoimmune diseases.

Cancers

Growth of tumors is often associated with the formation of novel antigens. These ***tumor antigens*** (also referred to as oncofetal antigens *e.g.*, α-fetoprotein) are recognized as non-self by the immune systems. However, tumors have developed several mechanisms to evade immune responses.

AIDS

Acquired Immunodeficiency Syndrome (AIDS), caused by human immunodeficiency virus, is characterized by immunosuppression, secondary neoplasma and neurological manifestations. AIDS primarily affects the cell-mediated immune system which protects the body from intracellular parasites such as viruses, and bacteria. Most of the immunodeficiency symptoms of AIDS are associated with a reduction in CD_4 (cluster determinant antigen 4) cells.

NOTES

8.9 VACCINATION AND IMMUNISATION

The principle of immunisation or vaccination is based on the property of "memory" of the immune system. Vaccine (L, *vacca* = cow) is a preparation of antigenic proteins of pathogens or inactivated/attenuated (weakened) pathogens, which on inoculation (injection) into a healthy person provides temporary/permanent active immunity by inducing antibodies formation. Thus, antibody provoking agents' are called vaccines.

Vaccines generate the primary immune response, and the memory B and T cells. When the vaccinated person is attacked by the same pathogen, the existing memory T or B cells recognise the antigen quickly and overwhelm the invaders with a massive production of lymphocytes and antibodies. In **passive immunisation** preformed antibodies are administered in the body. For example, in snake bites the injection which is given to the patient contains proformed antiboides against the snake venom.

Vaccination and immunisation are two different process. Vaccination refer to the administration of any vaccine, while immunisation is the process by which the body produces antibodies against pathogens through administration of specific vaccines.

History of Vaccine and Vaccination

The idea of vaccination was conceived by **Edward Jenner** (1748–1823) an English physician. During a dreadful small pox epidemic in England, the found that the epidemic killed large numbers of people in cities, but seldom affected rural people who worked around cattle. Most of the dairy men suffered from a similar but milder form of disease called cowpox, had recovered. This led him to think that an attack of cowpox had made these people immune to smallpox.

Dr. Jenner tested his vaccination theory in May, 1796 on **James Phipps,** a healthy boy of about 8 years of age. He scratched the skin of the boy to introduce into his body the fluid of a milkmaid, who was suffering from cowpox. When the boy was later exposed to smallpox, he showed resistance to the disease. Dr. Jenner's vaccination was successful. James Phipps had certainly become immune to smallpox. The antigen of small-pox and cowpox are so similar that the same antibodies work against both of them. Jenner proposed that inducing the mild form of a disease stimulates the body to develop an active immunity for protection against the virulent form. He used the term **'vaccine'** for the immunity producing preparation, and the term ***'vaccination'*** for the process of innoculating the preparation into the body. Edward Jenmer is regarded as ***'Father of immunology'***. The use of vaccine for the treatment of a disease is called **vaccino therapy.**

STUDENT ACTIVITY

1. What is acquired immunity?

2. Explain the structure of an antibody molecule.

8.10 SUMMARY

- The over all ability of a person to fight the disease causing organisms is called immunity. Immunity is broadly divided into two type innate and acquired immunity.
- Innate immunity represents the inherent capability of a person to offer resistance of the organism to offer resistance against diseases. It consists of defensive barrier like physical, physiological, cellular and cycloline barriers. The acquired immunity is the resistance that an individual acquires during his/her life.
- Acquired immunity involves two type of cells-lymphocytes and antigen presenting cells. Lymphocytes that mature in thymus are called T-cells. They destroy the foreign cells. The lymphocytes that mature in bone marrow are called B-cells. They produce antibodies against specific antigen. Antigen presenting cells express class II HMC molecules on the membrane and also deliver a co-stimulatory signal necessary for T-cell activation.
- Active immunity is generated when a person suffers from a disease or gets vaccination for a disease. Passive immunity is conferred by the transfer of performed antibodies from some other animal or another individual (mother's body to a child).
- Antigens are molecules stimulates the production of antibodies. Antibodies are immunoglobulins, which are produced in response to antigenic stimulation, and are responsible for humoral immunity. B-lymphocytes are involved in humoral immunity, which T-lymphocytes. The immune system give body consists of lymphoid organs, tissues, cells and soluble molecules like antibodies.
- Vaccination is based on the property of memory of immune system, and involves the administration of inactivated pathogen or a preparation of antigenic protein of the pathogen in the body. The idea of vaccination was concieved by Edward Jenner.

8.11 GLOSSARY

- **Allergen:** A substance (generally a weak antigen) that cause allergic reaction.
- **Allergy:** An exaggerated response of the immune system to certain antigens present in the environment.
- **Immunity:** Ability of an individual to fight the disease causing agents.
- **Immunization:** Process of making a person immune to a disease.

NOTES

- **Interferon (IFN):** An antiviral protein produced by the viral infected cells.
- **Cytokines:** Non-antibody soluble proteins released by the cells of immune system.

8.12 REVIEW QUESTIONS

I. Very Short Answer Type Questions:

1. What is meant by immunity?
2. What structure/mechanisms constitute the first line of defence?
3. Expand the following:
   (*i*) APCs
   (*ii*) CMI
   (*iii*) AMI
4. What is meant by primary immune response?
5. What is vaccination?
6. Who is called Father of Immunology?

II. Short Answer Type Questions:

1. What are physiological barriers of immune system? How do they defend our body against infection?
2. Name the various types of T-lymphocytes and discuss their role in immunity.
3. Differentiate between active immunity and passive immunity.
4. Give a brief, account of immune system in the body.
5. Explain the main functions of antibodies.

III. Long Answer Type Questions:

1. Give an account of innate immunity.
2. Discuss the role of different types of cells involved in acquired immunity.
3. Give an account of different classes of immunoglobulins.
4. Give an account of AMI and CMI

8.13 FURTHER READINGS

- **Biochemistry;** Satyanarayan U., Chakrapani U.; Books and Allied (P) Ltd, Kolkata; 2007.
- **Outline of Biochemistry;** Conn. E.E., Stumpf P.K., Bruening G., Doi. R.H., Wiley India (P) Ltd. New Delhi; 2008.

CHAPTER 9 HIGH ENERGY COMPOUNDS

NOTES

OBJECTIVES

After going through this chapter, you should be able to:

- describe the energy rich compounds
- define high energy and low energy compounds
- know about redox potential (E_0)
- explain the electron transport chain
- understand the structural organisation of electron transport.

STRUCTURE

9.1 INTRODUCTION

In order to maintain living processes, all organisms must get free energy from their environment. The autotrophic organisms get supply of free energy by coupling their metabolism to some exergonic process in their surroundings. For example, green plants utilise the energy of sunlight. On the other hand, heterotrophic organisms get free energy by coupling their metabolism to the breakdown of complex organic molecules.

A large number of coupled reactions occur in the biological systems, which involve high energy compounds or energy rich compounds.

NOTES

9.2 ENERGY RICH COMPOUNDS

In the biological systems, there are certain compounds which possess sufficient free energy to liberate (*i.e.*, at least 7 cal/mole at pH 7.0). Such compounds are called **high energy compounds** or **energy rich compounds.** Certain other compounds which release less than 7.0 cal/mole (*i.e.*, less than the energy liberated on hydrolysis of ATP to ADP + Pi) are referred to as low energy compounds.

Free Energy of Hydrolysis

The standard free energy of hydrolysis of a number of biochemically important compounds is given in Table 9.1.

Table 9.1. Standard Free Energy of Hydrolysis of Some Biochemically Important Compounds.

Compounds	*Free Energy or $\Delta G°$ (cal/mol)*
High-energy phosphates	
Phosphoenol pyruvate	–14.8
Carbamoyl phosphate	–12.3
Cyclic AMP	–12.0
1,3-Bisphosphoglycerate	–11.8
Phosphocreatine	–10.3
Acetyl phosphate	–10.3
S-Adenosylmethionine*	–10.0
Pyrophosphate	–8.0
Acetyl CoA	–7.7
ATP → ADP + Pi	–7.3
Low-energy phosphates	
ADP → AMP + Pi	–6.6
Glucose 1-phosphate	–5.0
Fructose 6-phosphate	–3.8
Glucose 6-phosphate	–3.3
Glycerol 3-phosphate	–2.2

* In fact K Cal, which is also written as cal.

Based on free energy, these compounds are classified into two groups.

(*i*) High Energy Compounds

The compounds of this group has free energy (ΔG°) values higher than that of ATP. These include phosphoenol pyruvate, 1, 3-bisphosphoglycerate, phosphocreatine etc. Most of the energy rich compounds contain phosphate group (exception acetyl CoA), hence they are called high energy phosphate compounds. There are at least 5 groups of high energy compounds.

NOTES

- Pyrophosphates, *e.g.*, ATP.
- Acyl phosphate, *e.g.*, 1, 3-bisphosphoglycerate.
- Enol phosphates, *e.g.*, phosphoenolpyruvate.
- Thioesters, *e.g.*, acetyl CoA.
- Phosphagens, *e.g.*, phosphocreatine.

(*ii*) Low Energy Compounds

The compounds of this group has free energy (ΔG°) values lower than that of ATP. This group of compounds is examplified by the ester phosphates produced as intermediates in glycolysis.

9.3 HIGH ENERGY BONDS

The high energy compounds possess acid anhydride bonds (mostly phosphoanhydride bonds), which are formed by the condensation of two acidic groups or related compounds. These bonds are referred to as **high energy bonds,** since the free energy is liberated when these bonds are hydrolysed. **Lipmann** employed the symbol ~ to incidate high energy bond. For instance, ATP is written as AMP ~ P ~ P. The symbol ~ is indicative of the fact that the group attached to the bond, when transferred to an appropiate acceptor, results in transfer of large quantity of energy. Because of this reason, **'group transfer potential'** is used to indicate high energy bond.

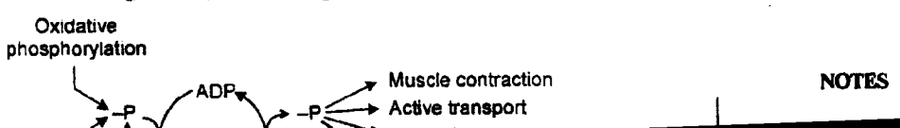
9.4 ATP—THE MOST IMPORTANT HIGH ENERGY COMPOUNDS

Adenosine triphosphate (ATP) is a unique and the most important high energy molecule in the living cells. It is known as **energy carrier** or energy currency of the cells, because it can trap, store and release small packet of energy with ease.

Chemically, ATP is made of a purine-**adenine** linked to a five carbon **sugar-ribose,** which in turn, is attached to a string of three phosphate radicals (Fig. 9.1). Out of three phosphate radicals, the last two are

link between the catabolism (degradation of molecules) and anabolism (synthesis) in the biological system (Fig. 9.2).

NOTES



NOTES

attached by means of **phosphoanhydride** or **energy rich bonds (~)** because of two reasons:

(*i*) Mutual repulsion of positive charges gained by adjacent phosphorus atoms due to dipole formation with oxygen.

(*ii*) Loss of resonance by phosphate radicals in the combined state.

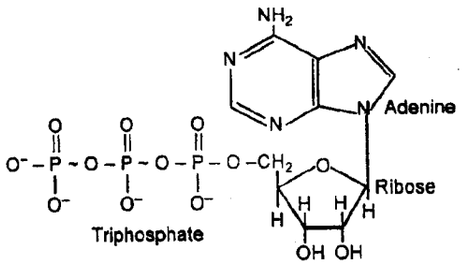


Fig. 9.1. *Structure of ATP.*

The bond between the second and the third phosphates can be easily broken through hydrolysis, which is associated with release of large amount of energy.

$$ATP + H_2O \xrightarrow{ATPase} ADP + Pi + 7.3 \text{ k cal}$$

Latest estimates indicate that the breakage of the last energy rich bond releases 8.6 k cal, while the second energy rich bond has an energy equivalent of 6.5 k cal per mole. The energy of an ordinary bond is about 0.3 k cal per mole. After liberation of the last phosphate radical, ATP is changed to ADP (adenosine diphosphate).

9.5 ATP-ADP CYCLE (ROLE OF ATP IN BIOCHEMICAL ENERGETICS)

ATP acts as a donor of high energy phosphate to the compounds which are low energy phosphates. Similarly, ADP can accept high energy phosphate to form ATP from compounds, which are energy phosphates. In fact ATP-ADP cycle connects the processes which generate ~ P to those processes which utilize ~ P.

After liberation of the last phosphate radical, ATP is changed ADP. The latter can again be changed to ATP in the presence of inorganic phosphate provided energy is available. The last phosphate radical which can be easily released or build up is said to possess a high transfer potential. ATP serves as an immediately available energy currency of the cell which is constantly being utilized and regenerated. The liberated energy is utilized for various processes like muscle contraction, active transport, biosynthesis etc. Thus, ATP-ADP cycle represents, the fundamental basis of energy exchange reactions in living system. ATP acts as an energy

NOTES

9.7 BIOLOGICAL OXIDATION

Oxidation is defined as the loss of electrons and reduction as the gain of electrons. This can be illustrated by the interconversion of ferrous (Fe^{2+}) to ferric (Fe^{3+}).

NOTES

Table 9.2. Standard Redox Potential (E_0) of Some Oxidation-reduction Systems

Redox Pair	*E_0 Volts*
Succinate/α-ketoglutarate	–0.67
$2H^+/H_2$	–0.42
$NAD^+/NADH$	–0.32
$NADP^+/NADPH$	–0.32
$FMN/FMNH_2$ (enzyme bound)	–0.30
Lipoate (ox/red)	–0.29
$FAD/FADH_2$	–0.22
Pyruvate/lactate	–0.19
Fumarate/succinate	+0.03
Cytochrome b (Fe^{3+}/Fe^{2+})	+0.07
Coenzyme Q (ox/red)	+0.10
Cytochrome c_1 (Fe^{3+}/Fe^{2+})	+0.23
Cytochrome c (Fe^{3+}/Fe^{2+})	+0.25
Cytochrome a (Fe^{3+}/Fe^{2+})	+0.29
$\frac{1}{2}O_2/H_2O$	+0.82

9.9 MITOCHONDRIA—THE POWERHOUSE OF CELL

The mitochondria are the centres for oxidative reactions to generate reduced coenzymes (NADH and $FADH_2$), which in turn, are utilized in ETC to liberate energy in the form of ATP. For this reason, mitochondrion is regarded as the **powerhouse of the cell.**

A mitochondrion is enclosed by a double membrane envelope. The two membranes of the envelope are reparated by a narrow fluid filled space called peri-mitochondrial space or intermembrane space. The outer membrane is smooth and is permeable to small molecules. The inner membrane surrounds a fluid filled central cavity called matrix. The matrix is rich in enzymes responsible for the citric acid cycle, β-oxidation of fatty acids and oxidation of amino acids. The inner membrane is infolded into matrix as incomplete partitions called cristae. The surface area of the inner mitochondrial membrane possesses specialized particles called **F_0-F_1 particles** (Fig. 9.3). These particles look like lollipops. $F_0 - F_1$ particles are the phosphorylating subunits which act as centres for ATP production.

NOTES

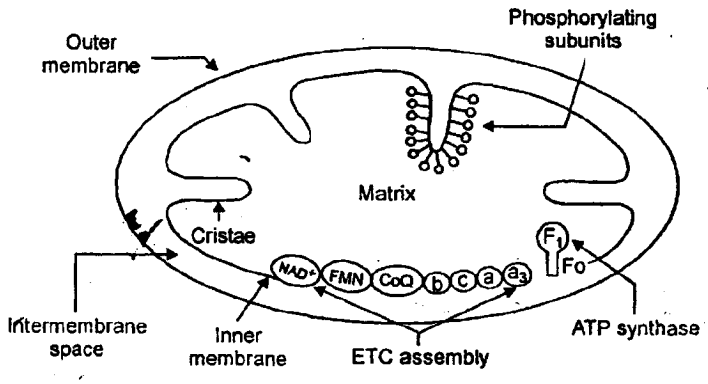


Fig. 9.3. *Structure of mitochondrion depicting electron transport Chain (ETC) F_0–F_1 Protein subunits.*

9.10 ELECTRON TRANSPORT CHAIN (ETC)

The energy rich carbohydrates, fatty acids and amino acids undergo series of metabolic reactions and finally, get oxidized to CO_2 and H_2(The reducing equivalents from various metabolic intermediates a transferred to enzymes NAD^+ and FAD to produce NADH + H^+ ar $FADH_2$ respectively. The reduced coenzymes (NADH + H^+ and FADH pass through the electron transport chain (ETC) and finally redu oxygen to water. The passage of electrons through the ETC is associat with the loss of free energy. A part of this free energy is utilized generate ATP from ADP and Pi (Fig. 9.4)

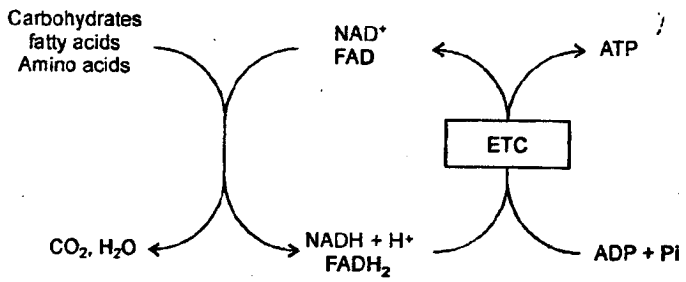


Fig. 9.4. *An overview of ETC.*

Inner mitochondrial membrane contains groups of electron and prot transporting enzymes. In each group the enzymes are arranged in specific series called **electron transport chain** (ETC) or **mitochondri respiratory chain or electron transport system (ETS). An electr** transport chain or system is a series of coenzymes and cytochrom that take part in the passage of electrons from a chemical to its ultima acceptor. The passage of electrons from one enzyme or cytochrome

NOTES

the next is a downhill journey with a loss of energy at each step. At each step the electron carriers include flavins, iron sulphur complexes, quinones and cytochromes. Most of them are prosthetic groups of proteins. Quinones are highly mobile electron carriers. Inner mitochondrial membrane possesses five complexes. Complexes I to IV are involved in electron transport (Fig. 9.5):

(*i*) NADH-Q reductase or NADH-dehydrogenase complex,

(*ii*) Succinate Q-reductase complex,

(*iii*) QH_2^- cytochrome *c* reductase complex,

(*iv*) Cytochrome *c* oxidase complex, *NADH-Q reductase* (or NADH-dehydrogenase) has two prosthetic groups, flavin mononucleotide (FMN) and iron sulphur (Fe-S) complexes.

(*v*) Complex V is connected with ATP synthesis (F_0–F_1 particle).

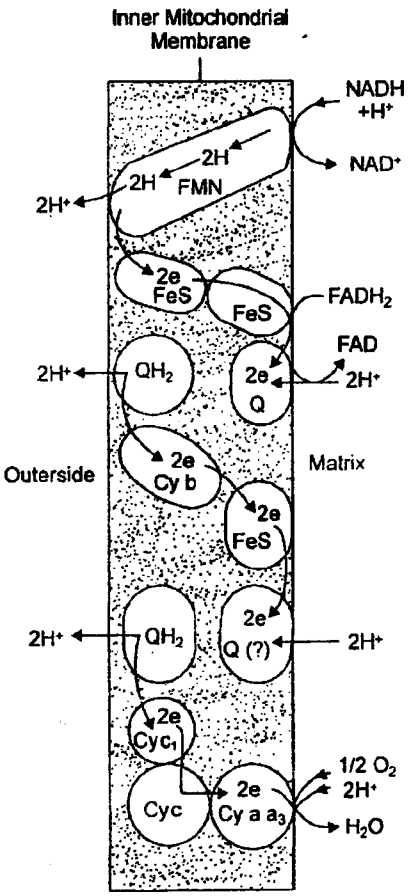


Fig. 9.5. *Mitochondrial electron transport chain.*

Both electrons and protons pass from NADH to FMN. The latter is reduced. However, $FMNH_2$ breaks to release protons (H^+) and electrons.

$$NADH + H^+ + FMN \longrightarrow FMNH_2 + NAD^+$$

$$FMNH_2 \longrightarrow FMN + 2H^+ + 2e^-$$

Electron now moves to the FeS complex and from there to a quinone. The common quinone is coenzyme Q, also called ubiquinone (UQ).

$$2e^- + 2\ Fe^{3+}\ S \longrightarrow 2\ Fe^{2+}\ S$$

$$2\ Fe^{2+}\ S + Q \longrightarrow 2\ Fe^{3+}\ S + Q^{2-}$$

Charged ubiquinone picks up protons and passes it into the outer chamber with the help of Cyt *b*.

NOTES

$FADH_2$ produced during reduction of succinate also hands over its electrons and protons to ubiquinone or coenzyme Q through FeS complex. The enzyme is succinate-Q reductase complex.

$$FADH_2 + 2\ Fe^{3+}\ S \longrightarrow 2\ Fe^{2} + S + 2H^{+} + FAD$$

$$2\ Fe^{2+}\ S + Q + 2H^{+} \longrightarrow 2\ Fe^{3+}\ S + QH_2$$

QH_2-cytochrome *c* reductase complex has three components—cytochrome *b*, FeB complex and cytochrome c_1. Coenzyme Q may also be involved between FeS complex and cytochrome c_1. Reduced ubiquinone or ubiquinol (QH_2) is oxidised with the passage of protons to the outside and handing over the electrons to cytochrome *c* via cytochrome b–c_1 complex.

$$QH_2 + 2\ Fe^{3+}\ \text{cyt. } b \longrightarrow Q + 2H^{+} + 2Fe^{2+}\ \text{cyt. } b$$

$$2\ Fe^{2+}\ \text{cyt, } b + 2\ Fe^{3+}\ S \longrightarrow 2\ Fe^{3+}\ \text{cyt. } b + 2\ Fe^{2+}\ S$$

$$2\ Fe^{2+}\ S + Q + 2H^{+} \longrightarrow 2\ Fe^{3+}\ S + QH_2\ (?)$$

$$QH_2 + 2\ Fe^{3+}\ \text{cyt . } c_1 \longrightarrow Q + 2H^{+} + 2\ Fe^{2+}\ \text{cyt . } c_1$$

Cytochrome c_1 hands over its electron to a small protein called cytochrome *c*. Like coenzyme Q, cytochrome *c* is also mobile carrier of electrons that transfers electrons between complex III and IV.

$$2\ Fe^{2+}\ \text{cyt. } c_1 + 2\ Fe^{3+}\ \text{cyt. } c \longrightarrow 2\ Fe^{3+}\ \text{cyt. } c_1 + 2\ Fe^{2+}\ \text{cyt . } c$$

Cytochrome *c* oxidase complex contains cytochrome *a* and cytochrome a_3. Cytochrome a_3 also possesses two copper centres. The latter help in transfer of electron to oxygen.

$$2\ Fe^{2+}\ \text{cyt.}c + 2\ Fe^{3+}\ \text{cyt . } a \longrightarrow 2\ Fe^{3+}\ \text{cyt. } c + 2\ Fe^{2+}\ \text{cyt. } a$$

$$2\ Fe^{2+}\ \text{cyt.}a + 2\ Fe^{3+}\ \text{cyt. } a_3\ Cu^{2+} \longrightarrow 2\ Fe^{3+}\ \text{cyt. } a + 2\ Fe^{2}\ \text{cyt. } a_3\ Cu^{2+}$$

$$2\ Fe^{2}\ \text{cyt. } a_3\ Cu^{2+} \longrightarrow 2\ Fe^{3}\ \text{cyt } a_3\ Cu^{1+}$$

$$2\ Fe^{3}\ \text{cyt.}a_3\ Cu^{1+} + [O] \longrightarrow 2\ Fe^{3}\ \text{cyt. } a_3\ Cu^{2+} + [O^{2-}]$$

Oxygen is the ultimate acceptor of electrons. It becomes reactive and combines with protons to form metabolic water.

$$2H^{+} + O^{2-} \longrightarrow 2H_2O$$

Energy released during passage of electrons from one carrier to the next is made available to specific transmembrane complexes, which pump protons (H^+) from the matrix side of the inner mitochondrial membrane to the outer chamber. There are three such sites corresponding to three enzymes present in the electron transport chain (NADH-Q reductase, QH_2-cytochrome *c* reductase and cytochrome *c*-oxidase). This increases proton concentration in the outer chamber or outer surface of the inner mitochondrial membrane. It creates a proton gradient. The difference in the proton concentration on the outer and inner sides of the inner mitochondrial membrane creates an electric potential across

the membrane with inner surface becoming negative as compared to outer surface. The electrochemical potential gradient created across the membrane due to high H^+ concentration on one side is called **proton motive force (PMF, Δp).**

NOTES

9.11 STRUCTURAL ORGANISATION OF ELECTRON TRANSPORT CHAIN (ETC)

The inner mitochondrial membrane can be disrupted into five distinct respiratory or enzyme complexes, denoted as ***complex I, II, III, IV and V*** (Fig. 9.6). The complexes I–IV are carriers of electrons while complex V is responsible for ATP synthesis. Besides these enzyme complexes, there are certain mobile electron carriers in the respiratory chain. These include NADH, coenzyme Q, cytochrome C and oxygen.

The enzyme complexes (I-IV) and the mobile carriers are collectively involved in the transport of electrons which, ultimately, combine with oxygen to produce water. The largest proportion of the oxygen supplied to the body is utilized by the mitochondria for the operation of electron transport chain.

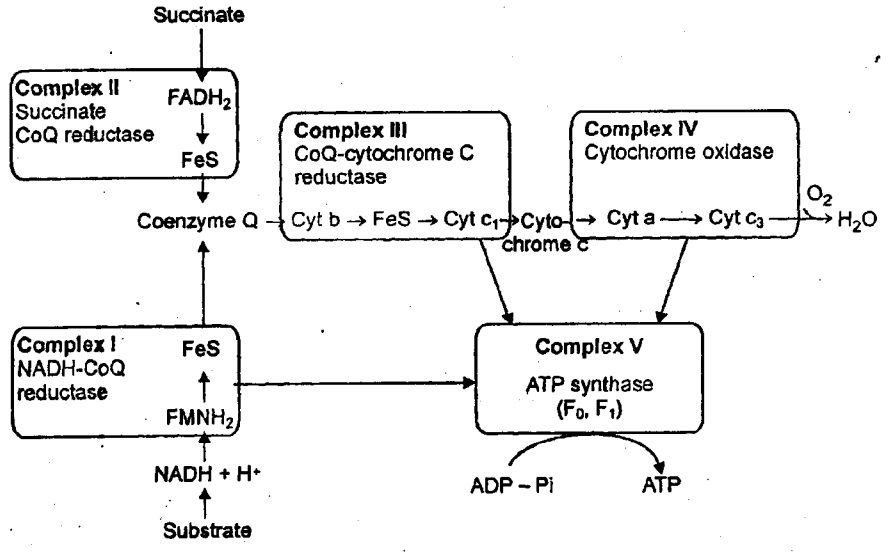


Fig. 9.6. *Multiprotein complexes in electron transport chain.*

Components and Reactions of the Electron Transport Chain

There are five distinct carriers that participate in the electron transport chain (ETC). These carriers are sequentially arranged and are responsible for the transfer of electrons from a given substrate to ultimately combine with proton and oxygen to form water (Fig. 9.7).

NOTES

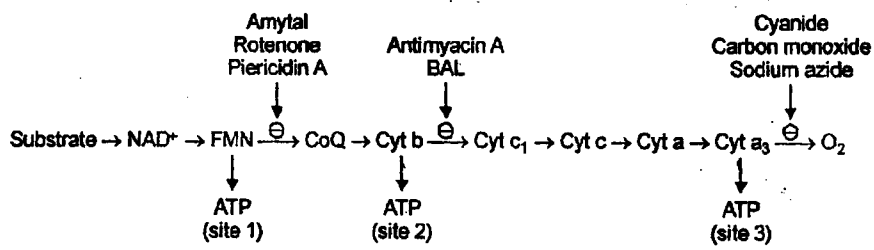


Fig. 9.7. *Electron transport chain with sites of ATP synthesis and inhibitors.*

I. Nicotinamide Nucleotides

Of the two coenzymes NAD^+ and $NADP^+$ derived from the vitamin niacin, NAD^+ is more actively involved in the ETC. NAD^+ is reduced to ***NADH*** + ***H***$^+$ by dehydrogenases with the removal of two hydrogen atoms from the substrate (AH_2). The substrates include glyceraldehyde-3 phosphate, pyruvate, isocitrate, α-ketoglutarate and malate.

$$AH_2 + NAD^+ \rightleftharpoons A + NADH + H^+$$

$NADPH + H^+$ produced by $NADP^+$-dependent dehydrogenase is not usually a substrate for ETC. NADPH is more effectively utilized for anabolic reactions (*e.g.*, fatty acid synthesis, cholesterol synthesis).

II., Flavoproteins

The enzyme NADH dehydrogenase (NADH-coenzyme Q reductase) is a flavoprotein with FMN as the prosthetic group. The coenzyme FMN accepts two electrons and a proton to form $FMNH_2$. ***NADH dehydrogenase*** is a complex enzyme closely associated with non-heme iron proteins (NHI) or iron-sulphur proteins (FeS).

$$NADH + H^+ + FMN \longrightarrow NAD^+ + FMNH_2$$

Succinate dehydrogenase (succinate-coenzyme Q reductase) is an enzyme found in the inner mitochondrial membrane. It is also a flavoprotein with FAD as the coenzyme. This can accept two hydrogen atoms ($2H^+ + 2e^-$) from succinate.

$$Succinate + FAD \longrightarrow Fumarate + FADH_2$$

III. Iron-sulfur Proteins

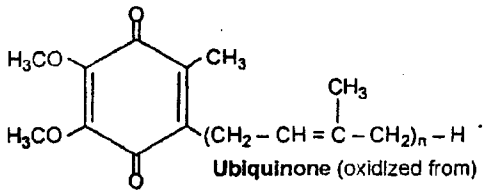
The iron-sulfur (FeS) proteins exist in the oxidized (Fe^{3+}) or reduced (Fe^{2+}) state. About half a dozen FeS proteins connected with respiratory chain have been identified. However, the mechanism of action of iron-sulfur proteins in the ETC is not clearly understood.

One FeS participates in the transfer of electrons from FMN to coenzyme Q. Other FeS proteins associated with cytochrome *b* and cytochrome c_1 participate in the transport of electrons.

NOTES

IV. Coenzyme Q

Coenzyme Q is also known as ***ubiquinone*** since it is ubiquitous in living system. It is a quinone derivative with a variable ***isoprenoid*** side chain. The mammalian tissues possess a quinone with 10 isoprenoid units which is known as coenzyme Q_{10} (CoQ_{10}).



Ubiquinone (oxidized from)

Coenzyme Q is a lipophilic electron carrier. It can accept electrons from $FMNH_2$ produced in the ETC by NADH dehydrogenase or $FADH_2$ produced outside ETC (*e.g.*, succinate dehydrogenase, acyl CoA dehydrogenase).

Coenzyme Q is not found in mycobacteria. Vitamin K performs similar function as coenzyme Q in these organisms. Coenzyme Q has no known vitamin precursor in animals. It is directly synthesized in the body.

V. Cytochromes

The cytochromes are conjugated proteins containing heme group. The latter consists of a porphyrin ring with iron atom. The heme group of cytochromes differ from that found in the structure of hemoglobin and myoglobin. The iron of heme in cytochromes is alternately oxidized (Fe^{3+}) and reduced (Fe^{2+}), which is essential for the transport of electrons in the ETC. This is in contrast to the heme iron of hemoglobin and myoglobin which remains in the ferrous (Fe^{2+}) state.

Three cytochromes were initially discovered from the mammalian mitochondria. They were designated as cytochrome *a*, *b* and *c* depending on the type of heme present and the respective absorption spectrum. Additional cytochromes such as c_1, b_1, b_2, a_3 etc., were discovered later.

The ***electrons*** are ***transported*** from ***coenzyme Q to cytochromes*** (in the order) ***b, c_1, c, a and a_3***. The property of reversible oxidation-reduction of heme iron $Fe^{2+} \rightleftharpoons Fe^{3+}$ present in cytochromes allows them to function as effective carriers of electrons in ETC.

Cytochrome c (mol. wt. 13,000) is a small protein containing 104 amino acids and a heme group. It is a central member of ETC with an intermediate redox potential. It is rather loosely bound to inner mitochondrial membrane and can be easily extracted.

Cytochrome *a* and a_3: The term ***cytochrome oxidase*** is frequently used to collectively represent cytochrome a and a_3 which is the terminal component of ETC. Cytochrome oxidase is the only electron carrier, the heme iron of which can directly react with molecular oxygen. Besides heme (with iron), this oxidase also contains copper that undergoes oxidation-reduction ($Cu^{2+} \rightleftharpoons Cu^{+}$) during the transport of electrons.

NOTES

In the final stage of ETC, the transported electrons, the free protons and the molecular oxygen combine to produce water.

Oxidative Phosphorylation

Oxidative phosphorylation is the synthesis of energy rich ATP molecules with the help of energy liberated during oxidation of reduced coenzymes (NADH, $FADH_2$) produced in respiration. The enzyme required for this synthesis is called **ATP synthase.** It is considered to be **fifth complex** of electron transport chain. ATP synthase is located in F_1 or head piece of F_0–F_1 or elementary particles. The particles are present in the inner mitochondrial membrane. ATP-synthase becomes active in ATP formation only where there is a **proton gradient** having higher concentration of H^+ or protons on the F_0 side as compared to F_1 side (chemiosmotic hypothesis of Peter Mitchel, 1961, Nobel Prize in 1978). Increased proton concentration is produced in the outer chamber or outer surface of inner mitochondrial membrane by the pushing of protons with the help of energy liberated by passage of electrons from one carrier to another. Transport of the electrons from NADH over ETC helps in pushing three pairs of protons to the outer chamber while two pairs of protons are sent outwardly during electron flow from $FADH_2$ (as the latter donates its electrons further down to the ETC).

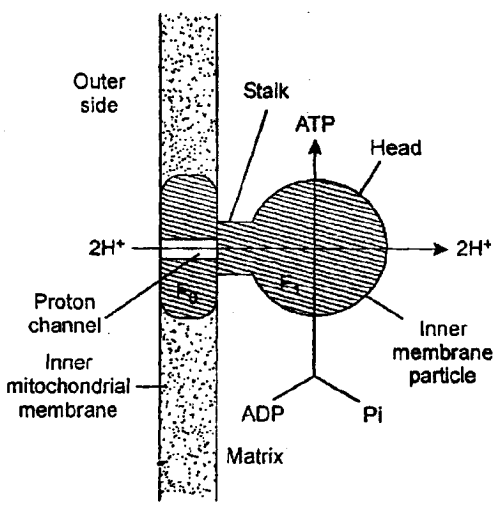


Fig. 9.8. *ATP synthesis by $F_0 - F_1$ particle.*

Higher proton concentration in the outer chamber causes the protons to pass inwardly into matrix to inner chamber through the inner membrane. The latter possesses special rotating proton channels in the region of F_0 (base) of the $F_0 - F_1$ particles (Paul Boyes, 1964). The flow of protons through the F_0 channel induces F_1 particle to function as ATP-synthase. The energy of the proton gradient is used in attaching a phosphate radicle to ADP by high energy bond. This produces ATP (Fig. 9.8). Oxidation of one molecule of $NADH_2$ produces 3 ATP molecules while a similar oxidation of $FADH_2$ forms 2 ATP molecules.

NOTES

Energetics of Oxidative Phosphorylation

The transport of electrons from redox pair $NAD^+/NADH$ ($E_0 = -0.32$) to finally the redox pair $\frac{1}{2}\ O_2/H_2O$ ($E_0 = +0.82$) can be represented in the following equation

$$\frac{1}{2}\ O_2 + NADH + H^+ \longrightarrow H_2O + NAD^+$$

The redox potential difference between these two redox pairs is 1.14 V, which is equivalent to an energy 52 cal/mol.

Three ATP are synthesized in the ETC when NADH is oxidized which equals to 21.9. cal (each ATP = 7.3 cal).

The efficiency of energy conservation is calculated as

$$\frac{21.9 \times 100}{52} = 42\%.$$

Therefore, when NADH is oxidized, about 42% of energy is trapped in the form of 3 ATP and the remaining is lost as heat. The heat liberation is not a wasteful process, since it allows ETC to go on continuously to generate ATP. Further, this heat is necessary to ***maintain body temperature.***

Chemiosmotic Hypothesis of ATP Synthesis

It was proposed by **Peter Mitchell** (1961), and is accepted widely. The inner mitochondrial membrane as such impermeable to proton (H^+) and hydroxylions (OH^-). The transport of electrons through ETC pumps protons (H^+) from matrix into the inter membrane space. The pumping of protons, results in an electrochemical or proton gradient. This is due to accumulation of more proton (H^+) on the under side of the inner mitochondrial membrane than the inner side. The proton gradient is sufficient to synthesize ATP from ADP and Pi. **ATP Synthase** (also called ATP) present in the complex V, utilizers the proton gradient for the synthesis of ATP. The protons that accumulate on the inter membrane space re-enter the mitochondrial matrix leading to the synthesis of ATP (Fig. 9.9).

Rotary Motor Model for ATP Generation

The model for ATP generation was proposed by **Paul Boyer** in 1964. According to this model a conformational change in the mitochondrial membrane protein leads to the synthesis of ATP.

The enzyme ATP synthase is F_0F_1 complex (of complex V). The F_0 subcomplex is composed of channel protein 'C' subunits to which F_1-ATP synthase is attached (Fig. 9.10). F_1-ATP synthase consists of a central γ subunit surrounded by alternating α and β subunits (α_3 and β_3).

In response to the proton flux, the γ subunit physically rotates. This induces conformational changes in the β_3 subunits that finally lead to the release of ATP.

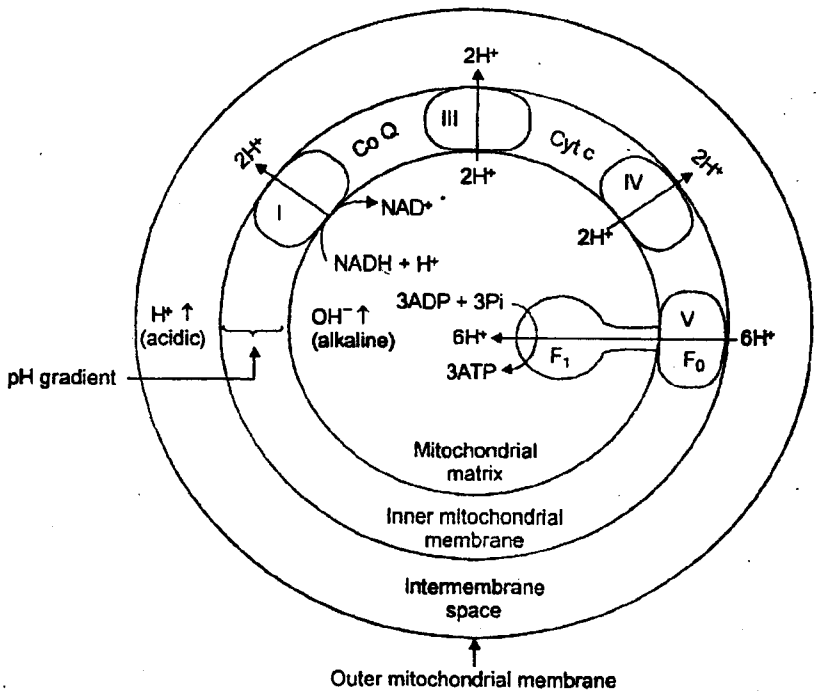


Fig. 9.9. *Diagrammatic representation of chemiosmotic hypothesis for oxidative phosphorylation (I, III, IV and V-Respiratory chain complexes; F_0 F_1-Protein subunits for phosphorylation).*

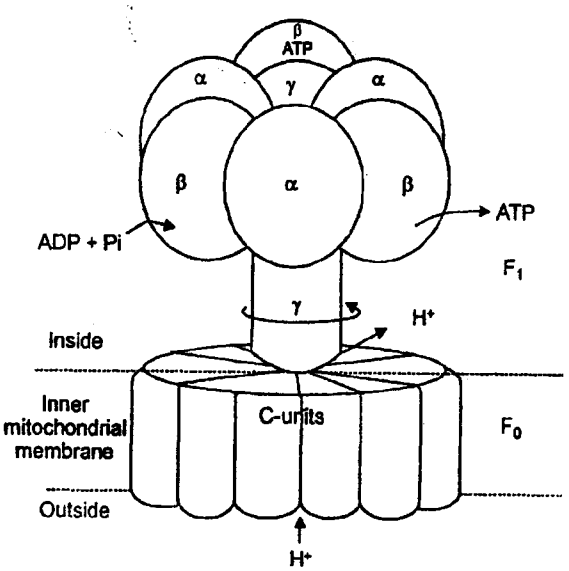


Fig. 9.10. *Structure of mitochondrial ATP synthase (F_0F_1) complex (C units-channel protein subunits; α, β, and γ are the subunits of F_1-ATP synthase).*

STUDENT ACTIVITY

1. Define high energy bonds.

2. Explain the rotary motor model of ATP generation.

NOTES

9.12 SUMMARY

- In biological systems, the compounds which possess sufficient energy to liberate are called high energy compounds. Certain other compounds which release energy less than the energy liberated on hydrolysis of ATP, are referred as low energy compounds. High energy compounds play a crucial role in the energy transfer of biochemical reactions.
- ATP is the energy currency of the cell. ATP-ADP cycle acts as a connecting energy link between catabolic and anabolic reactions. Electron transport chain (ETC) located in the inner mitochondrial membrane represents the final stage of oxidation of reducing equivalents (NADH + H^+ and $FADH_2$) formed during the metabolism to water. ETC is organised into five distinct complexes. The complex I to IV are electron carrier while complex V is responsible for the synthesis of ATP. The process of synthesis of ATP from ADP and Pi conpled with ETC is known as oxidative phosphorylation. Oxidation of one molecule of NAD + H^+ produces 3 ATP molecules. While similar oxidation of $FADH_2$ form 2 ATP molecules.
- Chemiosmotic hypothesis of Mitchell of ATP synthesis is accepted widely and states, that, the protons that accumulate on the inter membrane space re-enter the mitochondrial matrix leading to the synthesis of ATP.

9.13 GLOSSARY

- **Energy Rich Compounds.** Certain compounds in biological systems, which possess sufficient energy to liberate.
- **High Energy Compounds.** The compounds, which have free energy value higher than that of ATP.
- **Low Energy Compounds.** The compounds which have free energy value lower than that of ATP.
- **High Energy Bonds.** The bonds that liberate free energy on hydrolysis.
- **Phosphorylation.** Synthesis of ATP from ADP and inorganic phosphate, utilizing energy.
- **Redox-Potential.** Refers to oxidation-reduction potential.
- **Proton Motive Force.** Electrochemical potential gradient created across the membrane due to high H^+ concentration on one side.

NOTES

9.14 REVIEW QUESTIONS

I. Very Short Answer Type Questions:

1. What are high energy compounds?
2. What are low energy compounds?
3. Why ATP is called energy currency of cells?
4. In what forms high energy phosphates are stored in vertebrate and invertebrate animals?
5. Define biological oxidation.

II. Short Answer Type Questions:

1. Write short notes on:
   (*i*) Redox Potential
   (*ii*) High energy bonds
   (*iii*) ATP Synthase.
2. What are different ways of phosphorylation? Explain.
3. Explain, what is meant by biological oxidation?
4. Give the structural organisation of mitochondria.
5. Explain, chemiosmotic hypothesis of ATP synthesis.

III. Long Answer Type Questions:

1. Explain the components of electron transport chain.
2. Discuss the functioning of ETC in oxidation of NADH.
3. What is oxidative phosphorylation? Explain chemiosmotic hypothesis of ATP synthesis.
4. Explain the role of energy rich compounds in metabolism.

9.15 FURTHER READINGS

- **Biochemisty:** Powar and Chatwal; Himalayas Pub. House; Mumbai 1988.
- **Outlines of Biochemistry:** Conn. E.E., Stumpf P.K., Bruening G., Doi R.H.; Wiley India (P) Ltd. New Delhi; 2007.
- **Biochemistry:** Satyanarayan U., Chakrapani U.; Books and Allied (P) Ltd. Kolkata; 2008.

(కత్తిరించి పంపవలెను)

అధ్యాపకుల, విద్యార్థుల సలహాలు, సూచనలు :

అధ్యాపకులు, విద్యార్థులు ఈ స్టడీ మెటీరియల్‌కు సంబంధించిన సలహాలు, సూచనలు, ముద్రణ దోషాలు తెలియపరచినచో, పునర్ముద్రణలో తగు చర్యలు తీసుకొనగలము. తెలియపరచవలసిన చిరునామా : **డిప్యూటీ డైరెక్టర్, దూరవిద్యా కేంద్రం, ఆచార్య నాగార్జున విశ్వవిద్యాలయం, నాగార్జున నగర్ - 522 510.**

Course	Year	Paper No. &Title

(కత్తిరించి పంపవలెను)

(కత్తిరించి పంపవలెను)

(కత్తిరించి పంపవలెను)