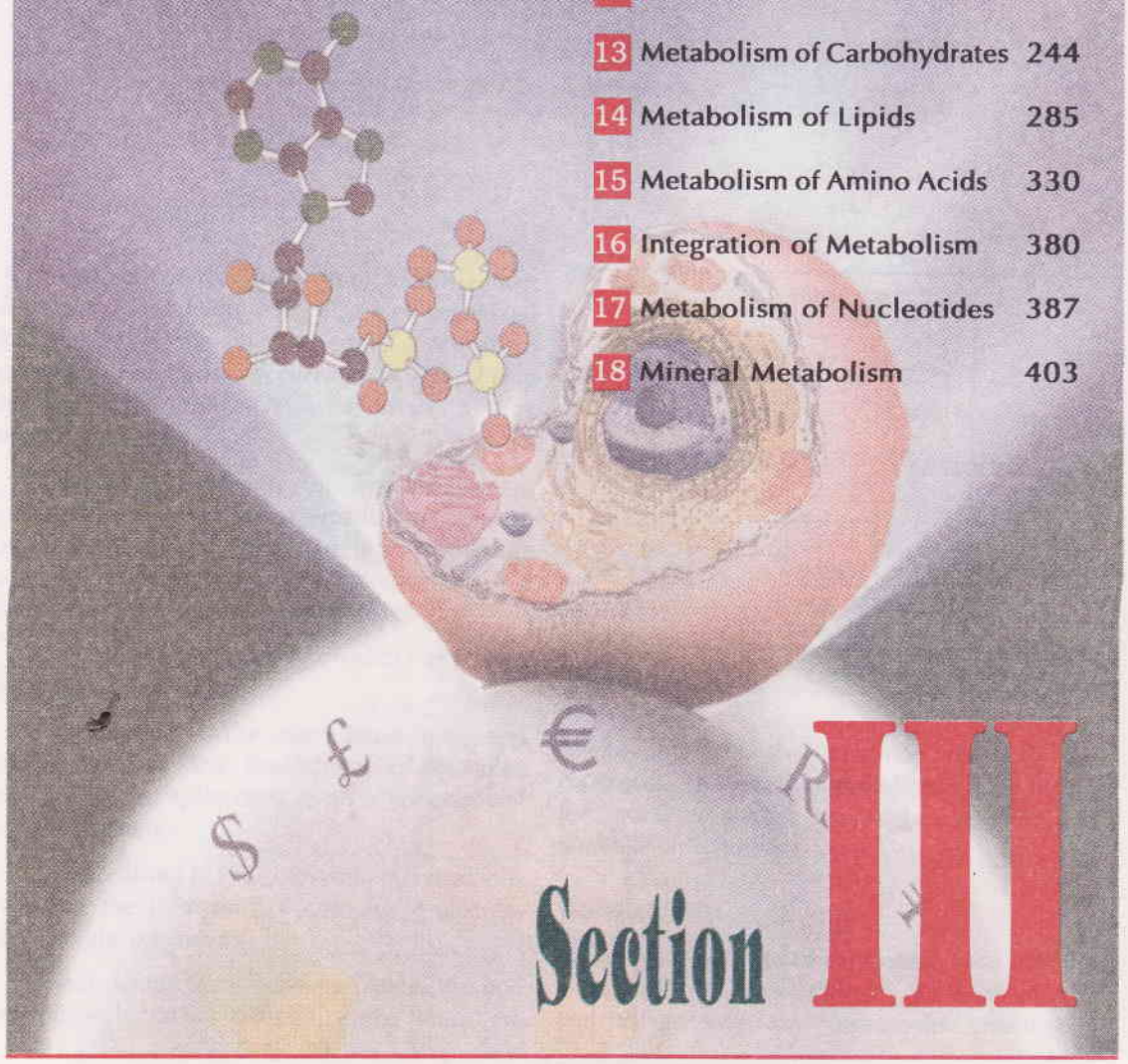
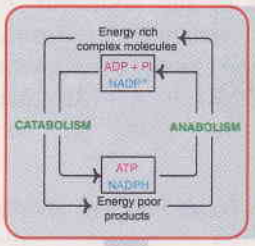
METABOLISMS



Section III

12 Introduction to Metabolism



The metabolism introduces itself :

"I represent the chemical reactions of life;
Composed of catabolism and anabolism;
Catabolism is degradative to generate energy;
Anabolism is synthetic that consumes energy."

Hundreds of reactions simultaneously take place in a living cell, in a well-organized and integrated manner. The entire spectrum of ***chemical reactions, occurring in the living system,*** are collectively referred to as ***metabolism.***

A ***metabolic pathway*** (or metabolic map) constitutes a series of enzymatic reactions to produce specific products. The term ***metabolite*** is applied to a substrate or an intermediate or a product in the metabolic reactions.

Metabolism is broadly divided into two categories (***Fig.12.1***).

1. **Catabolism :** The degradative processes concerned with the breakdown of complex molecules to simpler ones, with a concomitant release of energy.

2. **Anabolism :** The biosynthetic reactions involving the formation of complex molecules from simple precursors.

A clear demarcation between catabolism and anabolism is rather difficult, since there are several intermediates common to both the processes. The term ***amphibolism*** is also in use for reactions which are both catabolic and anabolic in nature.

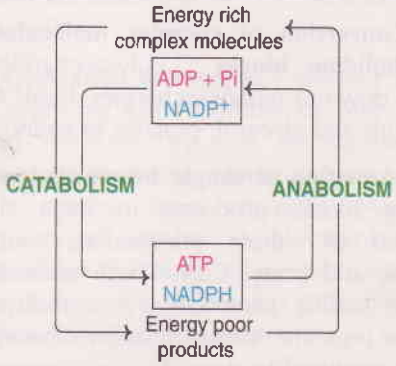


***Fig. 12.1** : An outline of catabolism and anabolism.*

Catabolism

The very purpose of catabolism is to trap the energy of the biomolecules in the form of ATP and to generate the substances (precursors)

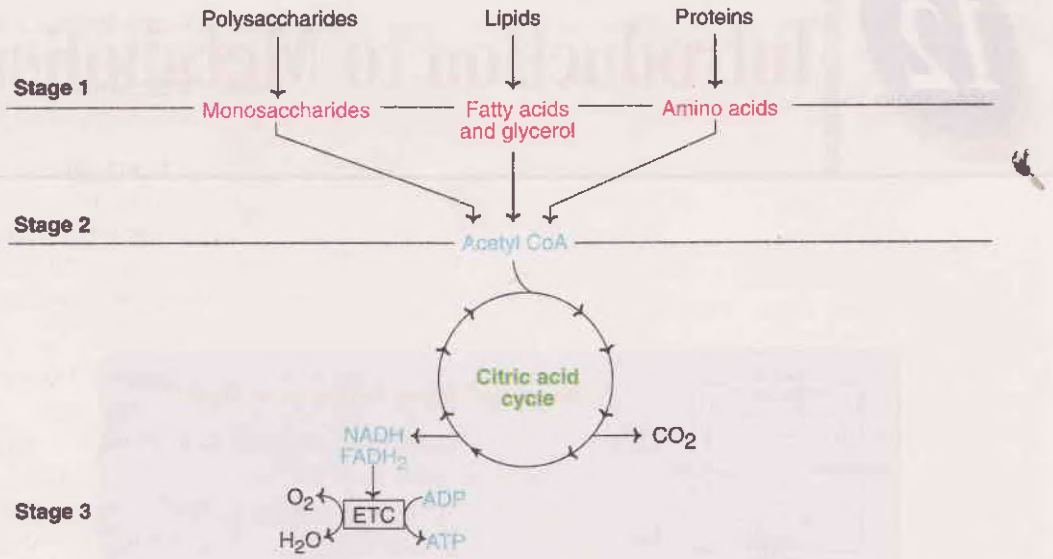


Fig. 12.2 : The three stages of catabolism (ETC–Electron transport chain).

required for the synthesis of complex molecules. Catabolism occurs in three stages (***Fig.12.2***).

1. **Conversion of complex molecules into their building blocks :** Polysaccharides are broken down to monosaccharides, lipids to free fatty acids and glycerol, proteins to amino acids.

2. **Formation of simple intermediates :** The building blocks produced in stage (1) are degraded to simple intermediates such as pyruvate and acetyl CoA. These intermediates are not readily identifiable as carbohydrates, lipids or proteins. A small quantity of energy (as ATP) is captured in stage 2.

3. **Final oxidation of acetyl CoA :** Acetyl CoA is completely oxidized to CO_2, liberating NADH and $FADH_2$ that finally get oxidized to release large quantity of energy (as ATP). ***Krebs cycle*** (or citric acid cycle) is the common metabolic pathway involved in the final oxidation of all energy-rich molecules. This pathway accepts the carbon compounds (pyruvate, succinate etc.) derived from carbohydrates, lipids or proteins.

Anabolism

For the synthesis of a large variety of complex molecules, the starting materials are relatively few. These include pyruvate, acetyl CoA and the intermediates of citric acid cycle. Besides the availability of precursors, the anabolic reactions are dependent on the ***supply of energy*** (as ATP or GTP) and reducing equivalents (as NADPH + H^+).

The anabolic and catabolic pathways are not reversible and operate independently. As such, the metabolic pathways occur in specific cellular locations (mitochondria, microsomes etc.) and are controlled by different regulatory signals.

The terms—intermediary metabolism and energy metabolism—are also in use. ***Intermediary metabolism*** refers to the entire range of catabolic and anabolic reactions, not involving nucleic acids. ***Energy metabolism*** deals with the metabolic pathways concerned with the storage and liberation of energy.

Types of metabolic reactions

The biochemical reactions are mainly of four types

1. Oxidation-reduction.
2. Group transfer.
3. Rearrangement and isomerization.

4. Make and break of carbon-carbon bonds.

These reactions are catalysed by specific enzymes—more than 2,000 known so far.

Methods employed to study metabolism

The metabolic reactions do not occur in isolation. They are interdependent and integrated into specific series that constitute ***metabolic pathways.*** It is, therefore, not an easy task to study metabolisms. Fortunately, the ***basic metabolic pathways in most organisms are essentially identical.*** For this reason, many organisms can be used to understand metabolisms.

Several methods are employed to elucidate biochemical reactions and the metabolic pathways. These experimental approaches may be broadly divided into 3 categories

1. Use of whole organisms or its components.
2. Utility of metabolic probes.
3. Application of isotopes.

The actual methods employed may be either ***in vivo*** (in the living system) or ***in vitro*** (in the test tube) or, more frequently, both.

1. **Use of whole organism or its components :**

   (a) **Whole organisms :** The ultimate aim of a biochemist is to know the metabolism in the organism as a whole. Glucose tolerance test (GTT), employed to measure the response of man (or other animals) towards carbohydrate metabolism is a good example of the use of whole organism.

   (b) **Isolated organs, tissue slices, whole cells,** subcellular organelles, cell-free systems and recently purified components are frequently used to elucidate biochemical reactions and metabolic pathways.

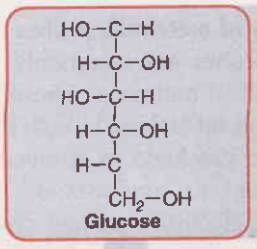
2. **Utility of metabolic probes :** Two types of metabolic probes are commonly used to trace out biochemical pathways. These are metabolic ***inhibitors*** and ***mutations.*** In both the cases, there is a specific blockade in a metabolic reaction which helps to understand the pathway. Inhibitors of electron transport chain have been largely responsible to elucidate the sequence of electron carriers (***Chapter 11***). The ***inborn errors of metabolism*** in higher organisms and the genetic manipulations in the microorganisms have also contributed a lot to the understanding of metabolisms.

3. **Application of isotopes :** Isotopes are the atoms with the same number of protons but different neutrons. By use of isotopes, the molecules of the living system can be labelled without altering their chemical properties. Application of isotopes in biochemistry has revolutionized the study of metabolisms. More details on the utility of isotopes in biochemistry are given elsewhere (***Chapter 41***).

SUMMARY

1. *The wide range of chemical reactions occurring in the living system are collectively known as metabolism. Catabolism is concerned with the degradation of complex molecules to simpler ones coupled with the liberation of energy (ATP). On the other hand, anabolism deals with the synthetic reactions converting simple precursors to complex molecules, coupled with the consumption of energy (ATP). A metabolic pathway constitutes a series of enzymatic reactions to produce specific products.*
2. *Several methods are employed to study metabolism. These include the use of the whole organism or its components (organ, tissue, cells, organelles etc.), utility of metabolic probes (inhibitors and mutations) and application of isotopes.*

13 Metabolism of Carbohydrates



The official spokesperson of carbohydrate metabolism, 'glucose', speaks :

"I burn myself to provide fuel to life!
Generated through gluconeogenesis by my friends;
Engaged in the synthesis of lipids, amino acids;
Deranged in my duties due to diabetes mellitus."

Carbohydrates are the major source of energy for the living cells. As such, carbohydrates are the first cellular constituents, synthesized by green plants during photosynthesis from carbon dioxide and water, on absorption of light. Thus, light is the ultimate source of energy for all biological processes.

The monosaccharide ***glucose is the central molecule in carbohydrate metabolism*** since all the major pathways of carbohydrate metabolism are connected with it (***Fig.13.1***). Glucose is utilized as a source of energy, it is synthesized from non-carbohydrate precursors and stored as glycogen to release glucose as and when the need arises. The other monosaccharides important in carbohydrate metabolism are fructose, galactose and mannose.

The ***fasting blood glucose*** level in normal individuals is ***70-100 mg/dl*** (4.5-5.5 mmol/l) and it is very efficiently maintained at this level (for details refer ***Chapter 36***). Liver plays a key role in monitoring and stabilizing blood glucose levels. Thus ***liver*** may be appropriately considered as ***glucostat monitor***.

Major pathways of carbohydrate metabolism

The important pathways of carbohydrate metabolism are listed

1. **Glycolysis** (Embden-Meyerhof pathway) : The oxidation of glucose to pyruvate and lactate.

2. **Citric acid cycle** (Krebs cycle or tricarboxylic acid cycle) : The oxidation of acetyl CoA to CO_2. Krebs cycle is the final common oxidative pathway for carbohydrates, fats or amino acids, through acetyl CoA.

3. **Gluconeogenesis** : The synthesis of glucose from non-carbohydrate precursors (e.g. amino acids, glycerol etc.).

4. **Glycogenesis** : The formation of glycogen from glucose.

5. **Glycogenolysis** : The breakdown of glycogen to glucose.

6. **Hexose monophosphate shunt** (pentose phosphate pathway or direct oxidative pathway) : This pathway is an alternative to glycolysis and

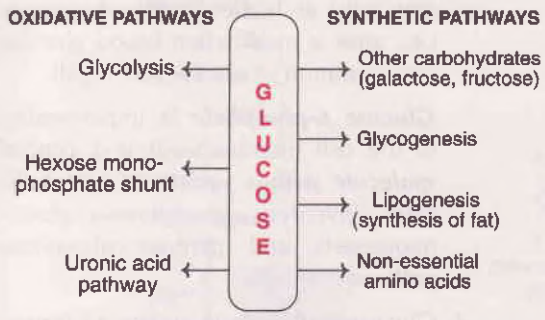


Fig. 13.1 : Overview of glucose metabolism. (Note : For majority of the pathways, glucose participates as glucose 6-phosphate).

TCA cycle for the oxidation of glucose (directly to carbon dioxide and water).

7. **Uronic acid pathway** : Glucose is converted to glucuronic acid, pentoses and, in some animals, to ascorbic acid (not in man). This pathway is also an alternative oxidative pathway for glucose.

8. **Galactose metabolism** : The pathways concerned with the conversion of galactose to glucose and the synthesis of lactose.

9. **Fructose metabolism** : The oxidation of fructose to pyruvate and the relation between fructose and glucose metabolism.

10. **Amino sugar and mucopolysaccharide metabolism** : The synthesis of amino sugars and other sugars for the formation of mucopolysaccharides and glycoproteins.

Entry of glucose into cells

Glucose concentration is very low in the cells compared to plasma (for humans < 100 mg/dl). However, glucose does not enter the cells by simple diffusion. Two specific transport systems are recognized for the entry of glucose into the cells

1. **Insulin-independent transport system of glucose** : This is a carrier mediated uptake of glucose which is not dependent on the hormone insulin. This is operative in hepatocytes, erythrocytes and brain.

2. **Insulin-dependent transport system** : This occurs in muscle and adipose tissue.

Glucose transporters : In recent years, at least six glucose transporters (GLUT-1 to GLUT-5 and GLUT-7) in the cell membranes have been identified. They exhibit tissue specificity. For instance, GLUT-1 is abundant in erythrocytes whereas GLUT-4 is abundant in skeletal muscle and adipose tissue.

Insulin increases the number and promotes the activity of GLUT-4 in skeletal muscle and adipose tissue. In type 2 diabetes mellitus, insulin resistance is observed in these tissues. This is due to the reduction in the quantity of GLUT-4 in insulin deficiency.

GLYCOLYSIS

Glycolysis is derived from the *Greek* words (glycose—sweet or sugar; lysis—dissolution). It is a universal pathway in the living cells. The complete pathway of glycolysis was elucidated in 1940. This pathway is often referred to as ***Embden-Meyerhof pathway (E.M. pathway)*** in honour of the two biochemists who made a major contribution to the knowledge of glycolysis.

Glycolysis is defined as the sequence of reactions converting glucose (or glycogen) to pyruvate or lactate, with the production of ATP.

Salient features

1. Glycolysis takes place in all cells of the body. The ***enzymes*** of this pathway are present in the ***cytosomal fraction*** of the cell.

2. Glycolysis occurs in the absence of oxygen (anaerobic) or in the presence of oxygen (aerobic). Lactate is the end product under anaerobic condition. In the aerobic condition, pyruvate is formed, which is then oxidized to CO_2 and H_2O.

3. Glycolysis is a major pathway for ATP synthesis in tissues lacking mitochondria, e.g. erythrocytes, cornea, lens etc.

4. Glycolysis is very ***essential for brain*** which is dependent on glucose for energy. The glucose in brain has to undergo glycolysis before it is oxidized to CO_2 and H_2O.

5. Glycolysis (anaerobic) may be summarized by the net reaction

Glucose + 2ADP + 2Pi ⟶ 2Lactate + 2ATP

6. Glycolysis is a central metabolic pathway with many of its intermediates providing branch point to other pathways. Thus, the intermediates of glycolysis are useful for the synthesis of amino acids and fat.

7. Reversal of glycolysis along with the alternate arrangements at the irreversible steps, will result in the synthesis of glucose (gluconeogenesis).

Reactions of glycolysis

The sequence of reactions of glycolysis is given in ***Fig.13.2***. The pathway can be divided into three distinct phases

A. Energy investment phase or priming stage

B. Splitting phase

C. Energy generation phase.

The sequence of reactions are discussed below.

A. Energy investment phase

1. Glucose is phosphorylated to glucose 6-phosphate by ***hexokinase*** or ***glucokinase*** (both are ***isoenzymes***). This is an irreversible reaction, dependent on ATP and Mg^{2+}. The enzyme hexokinase is present in almost all the tissues. It catalyses the phosphorylation of various hexoses (fructose, mannose etc.), has low K_m for substrates (about 0.1 mM) and is inhibited by glucose 6-phosphate.

   Glucokinase present in liver, catalyses the phosphorylation of only glucose, has high K_m for glucose (10 mM) and is not inhibited by glucose 6-phosphate.

   Due to high affinity (low K_m), glucose is utilized by hexokinase even at low concentration, whereas glucokinase acts only at higher levels of glucose i.e., after a meal when blood glucose concentration is above 100 mg/dl.

   Glucose 6-phosphate is impermeable to the cell membrane. It is a ***central molecule with a variety of metabolic fates***—glycolysis, glycogenesis, gluconeogenesis and pentose phosphate pathway.

2. Glucose 6-phosphate undergoes isomerization to give fructose 6-phosphate in the presence of the enzyme phosphohexose isomerase and Mg^{2+}.

3. Fructose 6-phosphate is phosphorylated to fructose 1,6-bisphosphate by phosphofructokinase (PFK). This is an irreversible and a regulatory step in glycolysis.

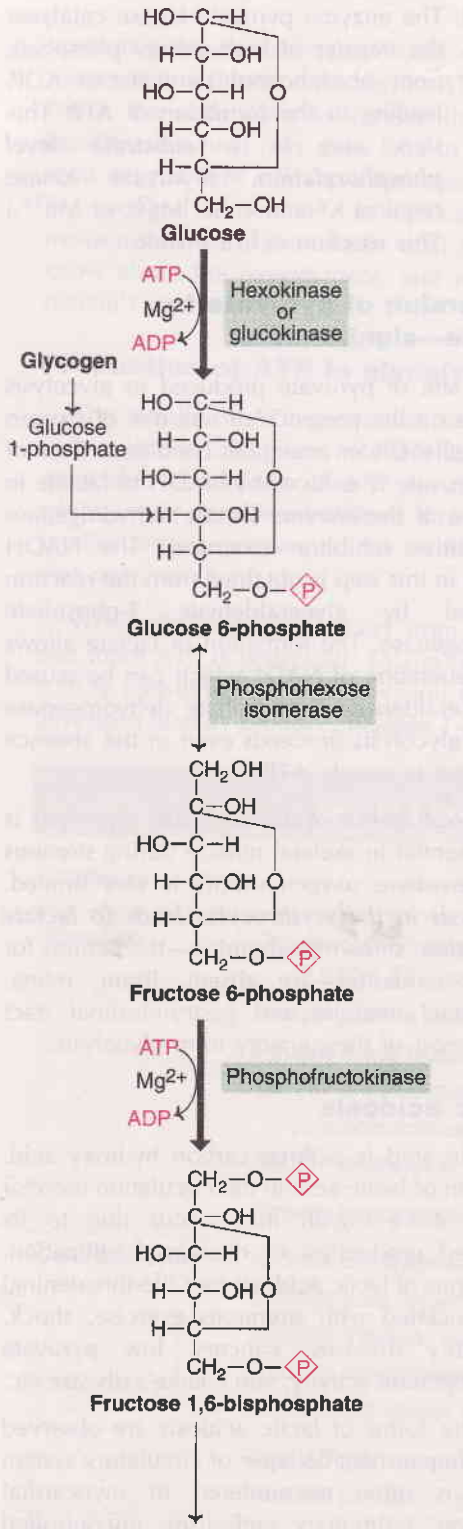
B. Splitting phase

4. The six carbon fructose 1,6-bisphosphate is split (hence the name glycolysis) to two three-carbon compounds, glyceraldehyde 3-phosphate and dihydroxyacetone phosphate by the enzyme aldolase (fructose 1,6-bisphosphate aldolase).

5. The enzyme phosphotriose isomerase catalyses the reversible interconversion of glyceraldehyde 3-phosphate and dihydroxyacetone phosphate. Thus, two molecules of glyceraldehyde 3-phosphate are obtained from one molecule of glucose.

C. Energy generation phase

6. Glyceraldehyde 3-phosphate dehydrogenase converts glyceraldehyde 3-phosphate to 1,3-bisphosphoglycerate. This step is important as it is involved in the formation of NADH + H^+ and a high energy compound 1,3-bisphosphoglycerate. ***Iodoacetate*** and ***arsenate inhibit*** the enzyme glyceraldehyde 3-phosphate dehydrogenase. In aerobic condition, NADH passes through the electron transport chain and 6 ATP (2 × 3 ATP) are synthesized by oxidative phosphorylation.



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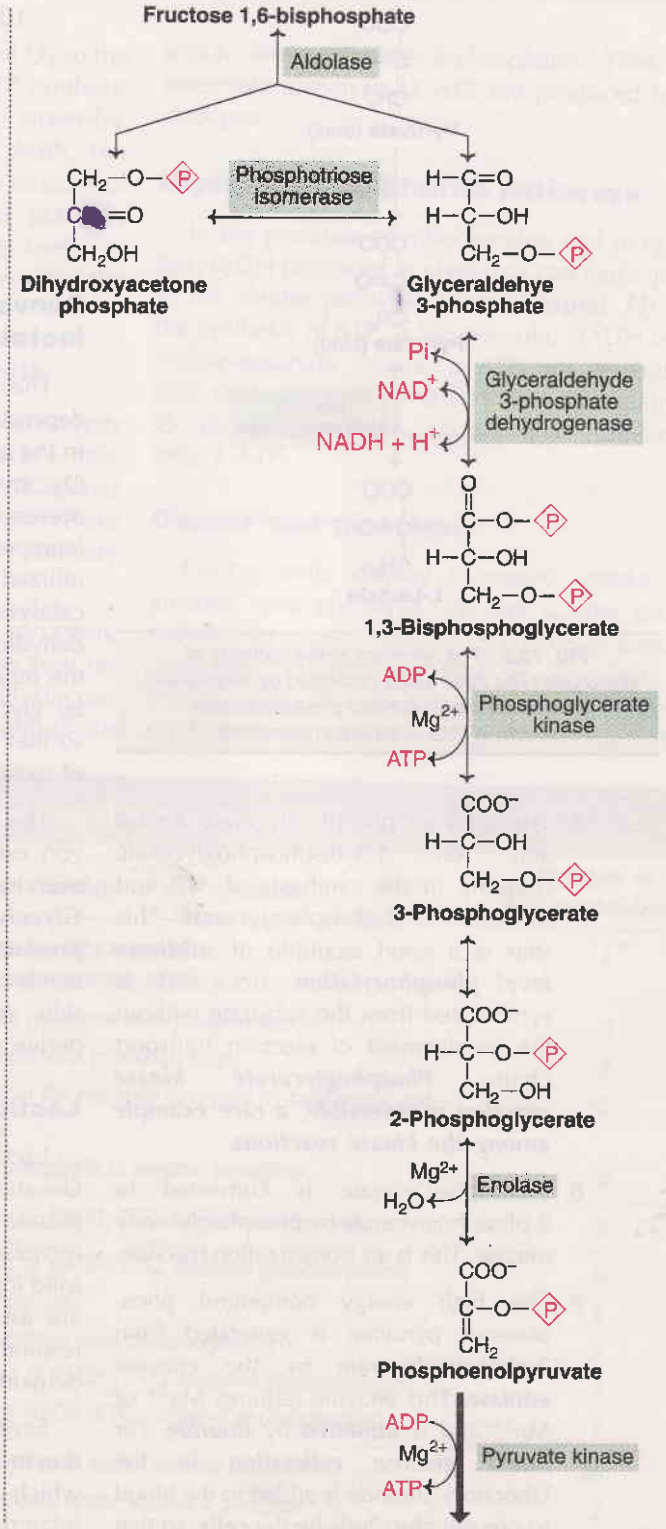

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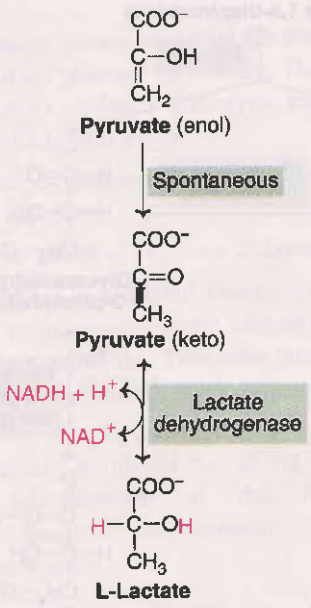


Fig. 13.2 : The reactions in the pathway of glycolysis (The three steps catalysed by hexokinase, phosphofructokinase and pyruvate kinase, shown in thick lines are irreversible).

7. The enzyme phosphoglycerate kinase acts on 1,3-bisphosphoglycerate resulting in the synthesis of ATP and formation of 3-phosphoglycerate. This step is a good example of ***substrate level phosphorylation***, since ATP is synthesized from the substrate without the involvement of electron transport chain. ***Phosphoglycerate kinase reaction is reversible, a rare example among the kinase reactions.***
8. 3-Phosphoglycerate is converted to 2-phosphoglycerate by phosphoglycerate mutase. This is an isomerization reaction.
9. The high energy compound phosphoenol pyruvate is generated from 2-phosphoglycerate by the enzyme ***enolase***. This enzyme requires Mg^{2+} or Mn^{2+} and is ***inhibited*** by ***fluoride.*** For blood ***glucose estimation*** in the laboratory, fluoride is added to the blood to prevent glycolysis by the cells, so that blood glucose is correctly estimated.
10. The enzyme pyruvate kinase catalyses the transfer of high energy phosphate from phosphoenol pyruvate to ADP, leading to the formation of ATP. This step also is a ***substrate level phosphorylation.*** (Pyruvate kinase requires K^+ and either Mg^{2+} or Mn^{2+}.) This reaction is irreversible.

Conversion of pyruvate to lactate—significance

The fate of pyruvate produced in glycolysis depends on the presence or absence of oxygen in the cells. Under anaerobic conditions (lack of O_2), pyruvate is reduced by NADH to lactate in presence of the enzyme lactate dehydrogenase (competitive inhibitor—oxamate). The NADH utilized in this step is obtained from the reaction catalysed by glyceraldehyde 3-phosphate dehydrogenase. The formation of lactate allows the regeneration of NAD^+ which can be reused by glyceraldehyde 3-phosphate dehydrogenase so that glycolysis proceeds even in the absence of oxygen to supply ATP.

The occurrence of uninterrupted glycolysis is very essential in skeletal muscle during strenous exercise where oxygen supply is very limited. ***Glycolysis in the erythrocytes leads to lactate production***, since mitochondria—the centres for aerobic oxidation—are absent. Brain, retina, skin, renal medulla and gastrointestinal tract derive most of their energy from glycolysis.

Lactic acidosis

Lactic acid is a three carbon hydroxy acid. Elevation of lactic acid in the circulation (normal plasma 4–15 mg/dl) may occur due to its increased production or decreased utilization. Mild forms of lactic acidosis (not life-threatening) are associated with strenuous exercise, shock, respiratory diseases, cancers, low pyruvate dehydrogenase activity, von Gierke's disease etc.

Severe forms of lactic acidosis are observed due to impairment/collapse of circulatory system which is often encountered in myocardial infarction, pulmonary embolism, uncontrolled hemorrhage and severe shock. This type of lactic

acidosis is due to inadequate supply of O_2 to the tissues with a drastic reduction in ATP synthesis (since the cells have to survive in anaerobic conditions) which may even lead to death. The term ***oxygen debt*** refers to the excess amount of O_2 required to recover. In clinical practice, measurement of plasma lactic acid is useful to know about the oxygen debt, and monitor the patient's recovery.

Production of ATP in glycolysis

The details of ATP generation in glycolysis (from glucose) are given in ***Table 13.1***. Under ***anaerobic conditions, 2 ATP*** are synthesized while, under ***aerobic conditions, 8*** or ***6 ATP*** are synthesized—depending on the shuttle pathway that operates.

When the glycolysis occurs from glycogen, one more ATP is generated. This is because no ATP is consumed for the activation of glucose (glycogen directly produces glucose 1-phosphate which forms glucose 6-phosphate). Thus, in anaerobic glycolysis, 3 ATP are produced from glycogen.

Glycolysis and shuttle pathways

In the presence of mitochondria and oxygen, the NADH produced in glycolysis can participate in the shuttle pathways (***Refer Chapter 11***) for the synthesis of ATP. If the cytosolic NADH uses malate-aspartate shuttle, 3 ATP are generated from each molecule of NADH. This is in contrast to glycerolphosphate shuttle that produces only 2 ATP.

Cancer and glycolysis

Cancer cells display increased uptake of glucose, and glycolysis. As the tumors grow rapidly, the blood vessels are unable to supply adequate oxygen, and thus a condition of hypoxia exists. Due to this, anaerobic glycolysis predominantly occurs to supply energy. The

Table 13.1 Generation of ATP in glucose metabolism

Pathway	*Enzyme (method of ATP synthesis)*	*Number of ATP synthesized*
Glycolysis	Glyceraldehyde 3-phosphate dehydrogenase (2 NADH, ETC, oxidative phosphorylation)	6*
	Phosphoglycerate kinase (substrate level phosphorylation)	2
	Pyruvate kinase (substrate level phosphorylation)	2
	Two ATP are consumed in the reactions catalysed by hexokinase and phosphofructokinase	–2
	Net ATP synthesis in glycolysis in aerobic condition	***8***
	Pyruvate dehydrogenase (2 NADH, ETC, oxidative phosphorylation)	6
Citric acid cycle	Isocitrate dehydrogenase (2 NADH, ETC, oxidative phosphorylation)	6
	α-Ketoglutarate dehydrogenase	6
	Succinate thiokinase (substrate level phosphorylation)	2
	Succinate dehydrogenase (2 $FADH_2$, ETC, oxidative phosphorylation)	4
	Malate dehydrogenase (2 NADH, ETC, oxidative phosphorylation)	6
	Total ATP per mole of glucose under aerobic condition	***38***
	Total ATP per mole of glucose under anaerobic condition	2

* *6 ATP are produced if NADH uses malate shuttle; only 4 ATP are produced if glycerol-phosphate shuttle operates, in which case total ATP synthesized per mole of glucose oxidation is 36 and not 38.*

cancer cells get adapted to hypoxic glycolysis through the involvement of a transcription factor namely hypoxia-inducible transcription factor (HIF). HIF increases the synthesis of glycolytic enzymes and the glucose transporters. However, the cancer cells cannot grow and survive without proper vascularization. One of the modalities of cancer treatment is to use drugs that can inhibit vascularization of tumors.

Irreversible steps in glycolysis

Most of the reactions of glycolysis are reversible. However, the three steps catalysed by the enzymes ***hexokinase*** (or glucokinase), ***phosphofructokinase*** and ***pyruvate kinase,*** are irreversible. These three stages mainly regulate glycolysis. The reversal of glycolysis, with alternate arrangements made at the three irreversible stages, leads to the synthesis of glucose from pyruvate (gluconeogenesis).

Regulation of glycolysis

The three enzymes namely hexokinase (and glucokinase), phosphofructokinase and pyruvate kinase, catalysing the irreversible reactions regulate glycolysis.

Hexokinase is inhibited by glucose 6-phosphate. This enzyme prevents the accumulation of glucose 6-phosphate due to product inhibition. Glucokinase, which specifically phosphorylates glucose, is an inducible enzyme. The substrate glucose, probably through the involvement of insulin, induces glucokinase.

Phosphofructokinase (PFK) is the most important regulatory enzyme in glycolysis. This enzyme catalyses the ***rate limiting committed step.*** PFK is an allosteric enzyme regulated by allosteric effectors. ATP, citrate and H^+ ions (low pH) are the most important allosteric inhibitors, whereas, fructose 2,6-bisphosphate, ADP, AMP and Pi are the allosteric activators.

Role of fructose 2,6-bisphosphate in glycolysis

Fructose 2,6-bisphosphate (F2,6-BP) is considered to be the most important ***regulatory factor*** (activator) for controlling PFK and, ultimately, ***glycolysis*** in the liver. F2,6-BP is synthesized from fructose 6-phosphate by the enzyme phosphofructokinase called PFK-2 (PFK-1 is the glycolytic enzyme). F2,6-BP is hydrolysed by fructose 2,6-bisphosphatase. The function of synthesis and degradation of F2,6-BP is brought out by a single enzyme (same polypeptide with two active sites) which is referred to as ***bifunctional enzyme (Fig.13.3)***. In fact, the combined name of phosphofructokinase-2/ fructose 2,6-bisphosphatase is used to refer to the enzyme that synthesizes and degrades F2,6-BP.

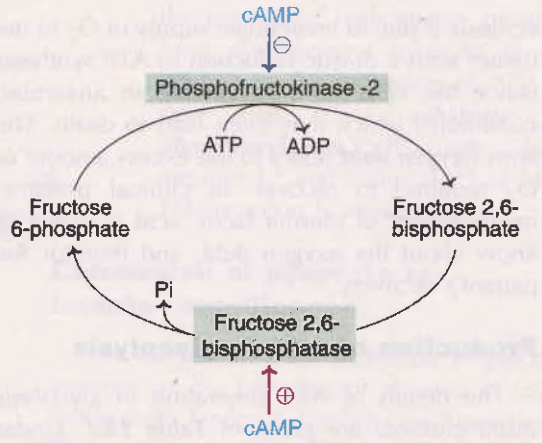


Fig. 13.3 : Regulation of fructose 2,6-bisphosphatase.

The activity of PFK-2 and fructose 2,6-bisphosphatase is controlled by covalent modification which, in turn, is regulated by cyclic AMP (cAMP is the second messenger for certain hormones). Cyclic AMP brings about dephosphorylation of the bifunctional enzyme, resulting in inactivation of active site responsible for the synthesis of F2,6-BP but activation of the active site responsible for the hydrolysis of F2,6-BP.

Pyruvate kinase also regulates glycolysis. This enzyme is inhibited by ATP and activated by F1,6-BP. Pyruvate kinase is active (a) in dephosphorylated state and inactive (b) in phosphorylated state. Inactivation of pyruvate kinase by phosphorylation is brought about by cAMP-dependent protein kinase. The hormone—***glucagon inhibits*** hepatic glycolysis by this mechanism (***Fig.13.4***).

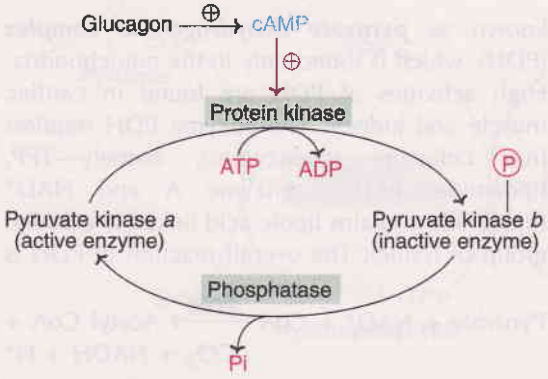


Fig. 13.4 : Regulation of pyruvate kinase.

Pasteur effect

The ***inhibition of glycolysis by oxygen*** (aerobic condition) is known as Pasteur effect. This effect was discovered by Louis Pasteur, more than a century ago, while studying fermentation by yeast. He observed that when anaerobic yeast cultures (metabolizing yeast) were exposed to air, the utiliziation of glucose decreased by nearly seven fold.

In the aerobic condition, the levels of glycolytic intermediates from fructose 1,6-bisphosphate onwards decrease while the earlier intermediates accumulate. This clearly indicates that Pasteur effect is due to the inhibition of the enzyme phosphofructokinase. The inhibitory effect of citrate and ATP (produced in the presence of oxygen) on phosphofructokinase explains the Pasteur effect.

Crabtree effect

The phenomenon of inhibition of oxygen consumption by the addition of glucose to tissues having high aerobic glycolysis is known as Crabtree effect. Basically, this is ***opposite*** to that of ***Pasteur effect.*** Crabtree effect is due to increased competition of glycolysis for inorganic phosphate (Pi) and NAD^+ which limits their availability for phosphorylation and oxidation.

RAPAPORT-LEUBERING CYCLE

This is a supplementary pathway to glycolysis which is operative in the erythrocytes of man and other mammals. Rapaport-Leubering cycle is mainly concerned with the synthesis of ***2,3-bisphosphoglycerate (2,3-BPG)*** in the RBC. 1,3-Bisphosphoglycerate (1,3-BPG) produced in glycolysis is converted to 2,3-BPG by the enzyme 2,3-bisphosphoglycerate mutase (***Fig.13.5***). 2,3-BPG is hydrolysed to 3-phosphoglycerate by bisphosphoglycerate phosphatase. [**Note :** There is a difference between the usages—bisphosphate and diphosphate. A bisphosphate has two phosphates held separately (e.g. 2,3-BPG), in contrast to diphosphate (e.g. ADP) where the phosphates are linked together].

It is now believed that bisphosphoglycerate mutase is a bifunctional enzyme with mutase and phosphatase activities catalysed by two different sites present on the same enzyme.

About 15-25% of the glucose that gets converted to lactate in erythrocytes goes via 2,3-BPG synthesis.

Significance of 2,3-BPG

1. Production of 2,3-BPG allows the glycolysis to proceed without the synthesis of ATP. This is advantageous to erythrocytes since glycolysis occurs when the need for ATP is minimal. Rapaport-Leubering cycle is, therefore, regarded as ***a shunt pathway of glycolysis*** to dissipate or waste the energy not needed by erythrocytes.

2. 2,3-BPG, however, is not a waste molecule in RBC. It combines with hemoglobin (Hb) and reduces Hb affinity with oxygen. Therefore, in the presence of 2,3-BPG, ***oxyhemoglobin unloads more oxygen to the tissues.***

Increase in erythrocyte 2,3-BPG is observed in hypoxic condition, high altitude, fetal tissues, anemic conditions etc. In all these cases, 2,3-BPG will enhance the supply of oxygen to the tissues.

3. Glycolysis in the erythrocytes is linked with 2,3-BPG production and oxygen transport. In the deficiency of the enzyme hexokinase, glucose is not phosphorylated, hence the synthesis and concentration of 2,3-BPG are low in RBC. The

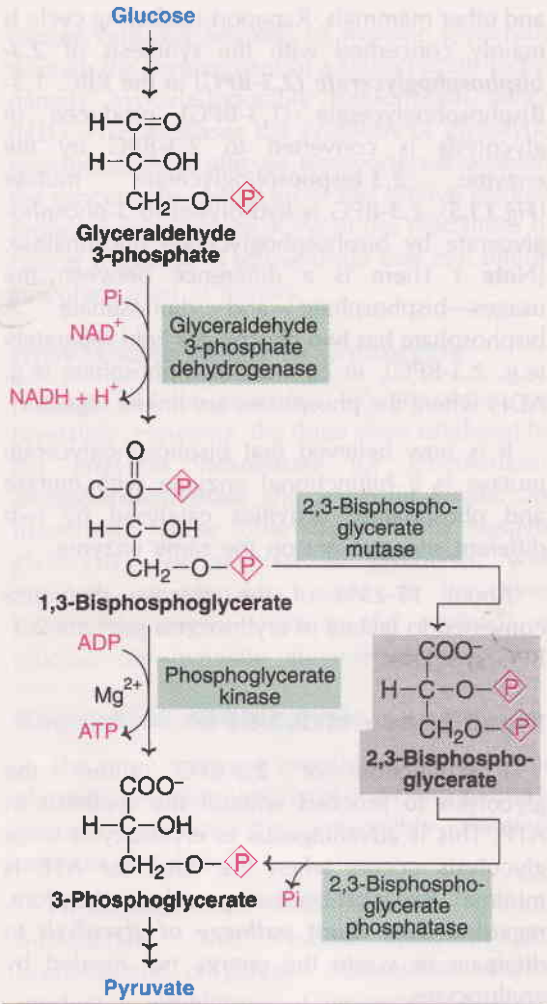


Fig. 13.5 : Rapaport-Leubering cycle for the synthesis of 2,3-bisphosphoglycerate (2,3-BPG).

hemoglobin exhibits high oxygen affinity in hexokinase-defective patients. On the other hand, in the patients with pyruvate kinase deficiency, the level of 2,3-BPG in erythrocytes is high, resulting in low oxygen affinity.

For a more detailed discussion on 2,3-BPG, refer ***Chapter 10***.

CONVERSION OF PYRUVATE TO ACETYL CoA

Pyruvate is converted to acetyl CoA by ***oxidative decarboxylation***. This is an irreversible reaction, catalysed by a multienzyme complex, known as ***pyruvate dehydrogenase complex*** (PDH), which is found only in the mitochondria. High activities of PDH are found in cardiac muscle and kidney. The enzyme PDH requires five cofactors (coenzymes), namely—TPP, lipoamide, FAD, coenzyme A and NAD^+ (lipoamide contains lipoic acid linked to ε-amino group of lysine). The overall reaction of PDH is

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

Reactions of PDH complex

The sequence of reactions brought about by different enzymes of PDH complex in association with the coenzymes is depicted in ***Fig.13.6***. Pyruvate is decarboxylated to give hydroxyethyl TPP, catalysed by PDH (decarboxylase activity). Dihydrolipoyl transacetylase brings about the formation of acetyl lipoamide (from hydroxethyl-TPP) and then catalyses the transfer of acetyl group to coenzyme A to produce acetyl CoA. The cycle is complete when reduced lipoamide is converted to oxidized lipoamide by dihydrolipoyl dehydrogenase, transferring the reducing equivalents to FAD. $FADH_2$, in turn, transfers the reducing equivalents to NAD^+ to give NADH + H^+, which can pass through the respiratory chain to give 3 ATP (6 ATP from 2 moles of pyruvate formed from glucose) by oxidative phosphorylation.

The intermediates of PDH catalysed reaction are not free but bound with enzyme complex. ***In mammals, the PDH complex has an approximate molecular weight of 9×10^6. It contains 60 molecules of dihydrolipoyltransacetylase and about 20–30 molecules each of the other two enzymes (pyruvate dehydrogenase and dihydrolipoyl dehydrogenase).***

A comparable enzyme with PDH is ***α-ketoglutarate dehydrogenase complex*** of citric acid cycle which catalyses the oxidative decarboxylation of α-ketoglutarate to succinyl CoA.

Arsenic poisoning : The enzymes PDH and α-ketoglutarate dehydrogenase are inhibited by arsenite. Arsenite binds to thiol (−SH) groups of

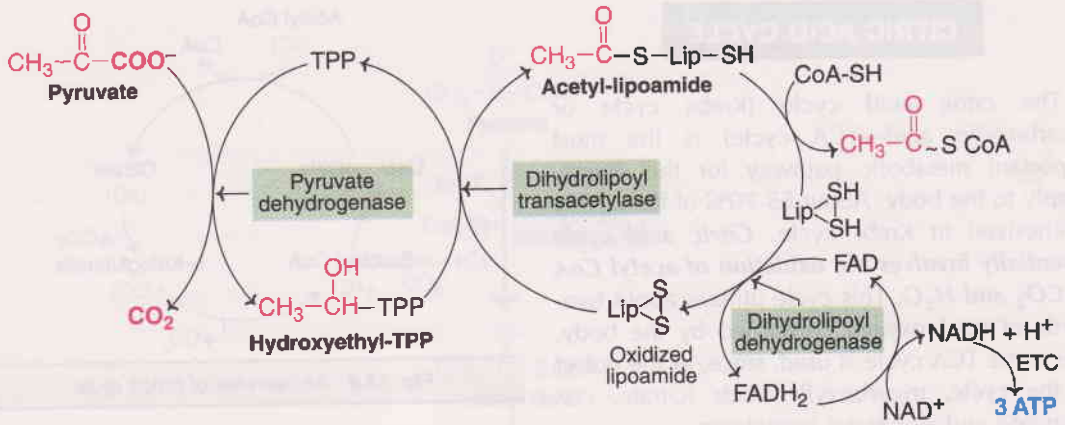


*Fig. 13.6 : The mechanism of action of pyruvate dehydrogenase complex. (**Note** : The reaction involving the conversion of pyruvate to acetyl CoA requires five coenzymes—TPP, lipoamide, CoASH, FAD and NAD+).*

lipoic acid and makes it unavailable to serve as cofactor.

Regulation of PDH

Pyruvate dehydrogenase is a good example for ***end product*** (acetyl CoA, NADH) ***inhibition.*** Besides this, PDH is also regulated by phosphorylation and dephosphorylation (***Fig.13.7***) PDH is active as a dephosphoenzyme while it is inactive as a phosphoenzyme. PDH phosphatase activity is promoted by Ca^{2+}, Mg^{+} and insulin (in adipose tissue). It is of interest to note that calcium released during muscle contraction stimulates PDH (by increasing phosphatase activity) for energy production.

PDH kinase (responsible to form inactive PDH) is promoted by ATP, NADH and acetyl CoA, while it is inhibited by NAD^{+}, CoA and pyruvate. The net result is that in the presence of high energy signals (ATP, NADH), the PDH is turned off.

Biochemical importance of PDH

1. Lack of TPP (due to deficiency of thiamine) inhibits PDH activity resulting in the accumulation of pyruvate.

2. In the thiamine deficient alcoholics, pyruvate is rapidly converted to lactate, resulting in lactic acidosis.

3. In patients with inherited deficiency of PDH, lactic acidosis (usually after glucose load) is observed.

4. PDH activity can be inhibited by arsenic and mercuric ions. This is brought about by binding of these ions with –SH groups of lipoic acid.

Metabolic importance of pyruvate

Pyruvate is a key metabolite. Besides its conversion to acetyl CoA (utilized in a wide range of metabolic reactions-citric acid cycle, fatty acid synthesis etc.), pyruvate is a good substrate for gluconeogenesis.

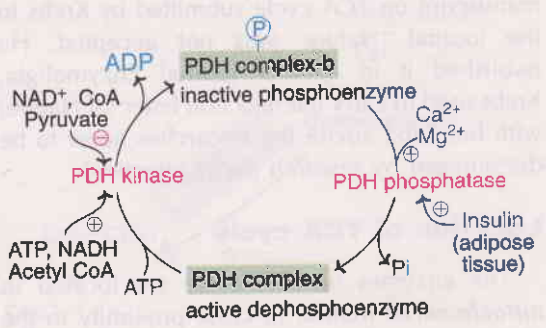


Fig. 13.7 : Regulation of pyruvate dihydrogenase (PDH) complex.

CITRIC ACID CYCLE

The citric acid cycle (Krebs cycle or tricarboxylic acid—TCA cycle) is the most important metabolic pathway for the energy supply to the body. About 65-70% of the ATP is synthesized in Krebs cycle. ***Citric acid cycle essentially involves the oxidation of acetyl CoA to CO_2 and H_2O.*** This cycle utilizes about two-thirds of total oxygen consumed by the body. The name TCA cycle is used, since, at the outset of the cycle, tricarboxylic acids (citrate, cis-aconitate and isocitrate) participate.

TCA cycle—the central metabolic pathway

The citric acid cycle is the final common oxidative pathway for carbohydrates, fats and amino acids. This cycle not only supplies energy but also provides many intermediates required for the synthesis of amino acids, glucose, heme etc. Krebs cycle is the most important central pathway ***connecting almost all the individual metabolic pathways*** (either directly or indirectly).

Brief history

The citric acid cycle was proposed by Hans Adolf Krebs in 1937, based on the studies of oxygen consumption in pigeon breast muscle. The cycle is named in his honour (Nobel Prize for Physiology and Medicine in 1953.)

[**Note :** It is of interest to note that the original manuscript on TCA cycle submitted by Krebs to the journal 'Nature' was not accepted. He published it in another journal Enzymoligia. Krebs used to carry the rejection letter (of Nature) with him, and advise the researches never to be discouraged by research paper rejection].

Location of TCA cycle

The enzymes of TCA cycle are located in ***mitochondrial matrix***, in close proximity to the electron transport chain. This enables the synthesis of ATP by oxidative phosphorylation without any hindrance.

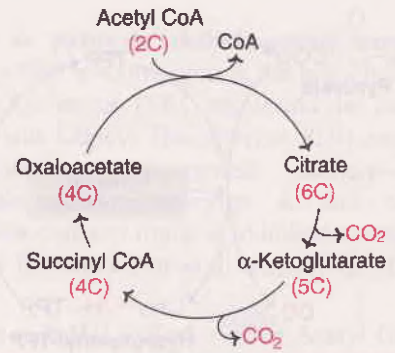


Fig. 13.8 : An overview of Krebs cycle.

TCA cycle—an overview

Krebs cycle basically involves the combination of a two carbon acetyl CoA with a four carbon oxaloacetate to produce a six carbon tricarboxylic acid, citrate. In the reactions that follow, the two carbons are oxidized to CO_2 and oxaloacetate is regenerated and recycled. ***Oxaloacetate is considered to play a catalytic role in citric acid cycle.*** An overview of Krebs cycle is depicted in ***Fig.13.8.***

TCA cycle—an open cycle

Krebs cycle is a cyclic process. However, it should not be viewed as a closed circle, since many compounds enter the cycle and leave. TCA cycle is comparable to a heavy traffic circle in a national highway with many connecting roads. Each intermediate of the cycle connecting another pathway is a road!

Reactions of citric acid cycle

Oxidative decarboxylation of pyruvate to acetyl CoA by pyruvate dehydrogenase complex is discussed above. This step is a connecting link between glycolysis and TCA cycle. A few authors, however, describe the conversion of pyruvate to acetyl CoA along with citric acid cycle. The events of TCA cycle are described hereunder (***Fig.13.9***).

1. **Formation of citrate :** Krebs cycle proper starts with the condensation of acetyl CoA and oxaloacetate, catalysed by the enzyme citrate synthase.

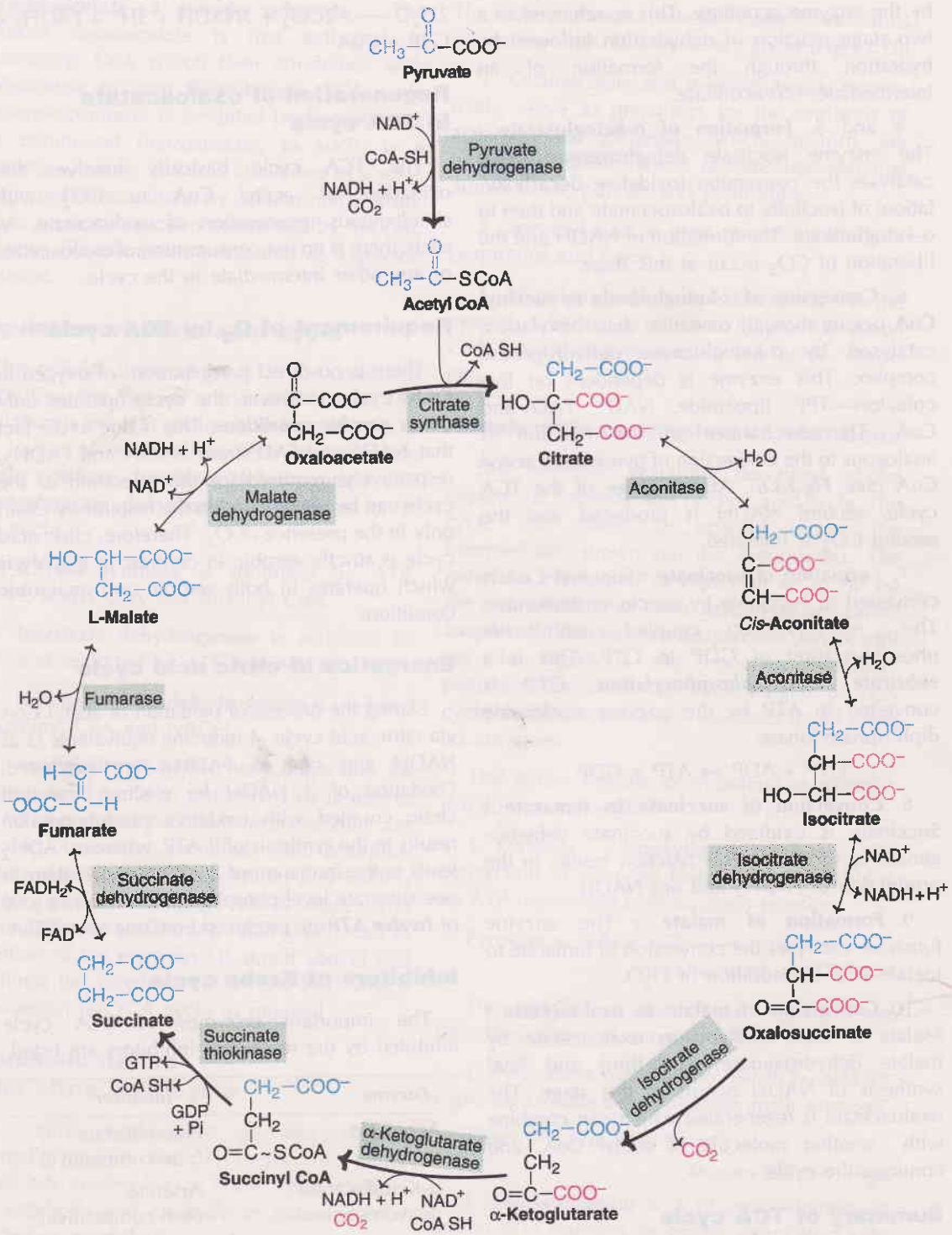


Fig. 13.9 : The citric acid (Krebs) cycle. (Irreersible reactions shown by thick arrows)

2. and 3. **Citrate is isomerized to isocitrate** by the enzyme aconitase. This is achieved in a two stage reaction of dehydration followed by hydration through the formation of an intermediate—*cis*-aconitate.

4. and 5. **Formation of α-ketoglutarate :** The enzyme isocitrate dehydrogenase (ICD) catalyses the conversion (oxidative decarboxylation) of isocitrate to oxalosuccinate and then to α-ketoglutarate. The formation of NADH and the liberation of CO_2 occur at this stage.

6. **Conversion of α-ketoglutarate to succinyl CoA** occurs through oxidative decarboxylation, catalysed by α-ketoglutarate dehydrogenase complex. This enzyme is dependent on five cofactors—TPP, lipoamide, NAD^+, FAD and CoA. The mechanism of the reaction is analogous to the conversion of pyruvate to acetyl CoA (***See Fig.13.6***). At this stage of the TCA cycle, second NADH is produced and the second CO_2 is liberated.

7. **Formation of succinate :** Succinyl CoA is converted to succinate by succinate thiokinase. This reaction is coupled with the phosphorylation of GDP to GTP. This is a ***substrate level phosphorylation***. GTP is converted to ATP by the enzyme nucleoside diphosphate kinase.

$$GTP + ADP \leftrightarrow ATP + GDP$$

8. **Conversion of succinate to fumarate :** Succinate is oxidized by succinate dehydrogenase to fumarate. This reaction results in the production of $FADH_2$ and not NADH.

9. **Formation of malate :** The enzyme fumarase catalyses the conversion of fumarate to malate with the addition of H_2O.

10. **Conversion of malate to oxaloacetate :** Malate is then oxidized to oxaloacetate by malate dehydrogenase. The third and final synthesis of NADH occurs at this stage. The oxaloacetate is regenerated which can combine with another molecule of acetyl CoA, and continue the cycle.

Summary of TCA cycle

The events of Krebs cycle may be summarized as given in the next column

$$\text{Acetyl CoA} + 3\ NAD^+ + FAD + GDP + Pi + 2H_2O \longrightarrow 2CO_2 + 3NADH + 3H^+ + FADH_2 + GTP + CoA$$

Regeneration of oxaloacetate in TCA cycle

The TCA cycle basically involves the oxidation of acetyl CoA to CO_2 with simultaneous regeneration of oxaloacetate. As such, there is no net consumption of oxaloacetate or any other intermediate in the cycle.

Requirement of O_2 by TCA cycle

There is no direct participation of oxygen in Krebs cycle. However, the cycle operates only under ***aerobic conditions.*** This is due to the fact that NAD^+ and FAD (from NADH and $FADH_2$, respectively) required for the operation of the cycle can be regenerated in the respiratory chain only in the presence of O_2. Therefore, citric acid cycle is strictly aerobic in contrast to glycolysis which operates in both aerobic and anaerobic conditions.

Energetics of citric acid cycle

During the process of oxidation of acetyl CoA via citric acid cycle, 4 reducing equivalents (3 as NADH and one as $FADH_2$) are produced. Oxidation of 3 NADH by electron transport chain coupled with oxidative phosphorylation results in the synthesis of 9 ATP, whereas $FADH_2$ leads to the formation of 2 ATP. Besides, there is one substrate level phosphorylation. Thus, a total of ***twelve ATP*** are produced from one acetyl CoA.

Inhibitors of Krebs cycle

The important enzymes of TCA cycle inhibited by the respective inhibitors are listed

Enzyme	*Inhibitor*
Aconitase	Fluoroacetate (non-competitive)
α-Ketoglutarate dehydrogenase	Arsenite (non-competitive)
Succinate dehydrogenase	Malonate (competitive)

Fluoroacetate – a suicide substrate : The inhibitor fluoroacetate is first activated to fluoroacetyl CoA which then condenses with oxaloacetate to form ***fluorocitrate***. TCA cycle (enzyme-aconitase) is inhibited by fluorocitrate. The compound fluoroacetate, as such, is a harmless substrate. But it is converted to a toxic compound (fluorocitrate) by cellular metabolism. This is a suicide reaction committed by the cell, and thus fluoroacetate is regarded as a suicide substrate.

Regulation of citric acid cycle

The cellular demands of ATP are crucial in controlling the rate of citric acid cycle. The regulation is brought about either by enzymes or the levels of ADP. Three enzymes—namely ***citrate synthase, isocitrate dehydrogenase*** and ***α-ketoglutarate dehydrogenase***—regulate citric acid cycle.

1. **Citrate synthase** is inhibited by ATP, NADH, acetyl CoA and succinyl CoA.

2. **Isocitrate dehydrogenase** is activated by ADP, and inhibited by ATP and NADH.

3. **α-Ketoglutarate dehydrogenase** is inhibited by succinyl CoA and NADH.

4. **Availability of ADP** is very important for the citric acid cycle to proceed. This is due to the fact that unless sufficient levels of ADP are available, oxidation (coupled with phosphorylation of ADP to ATP) of NADH and $FADH_2$ through electron transport chain stops. The accumulation of NADH and $FADH_2$ will lead to inhibition of the enzymes (as stated above) and also limits the supply of NAD^+ and FAD which are essential for TCA cycle to proceed.

Amphibolic nature of the citric acid cycle

The citric acid cycle provides various intermediates for the synthesis of many compounds needed by the body. Krebs cycle is ***both catabolic and anabolic in nature***, hence regarded as ***amphibolic***.

TCA cycle is actively involved in gluconeogenesis, transamination and deamination. The most important synthetic (anabolic) reactions connected with TCA cycle are given (***Fig.13.10***)

1. Oxaloacetate and α-ketoglutarate, respectively, serve as precursors for the synthesis of aspartate and glutamate which, in turn, are required for the synthesis of other non-essential amino acids, purines and pyrimidines.

2. Succinyl CoA is used for the synthesis of porphyrins and heme.

3. Mitochondrial citrate is transported to the cytosol, where it is cleaved to provide acetyl CoA for the biosynthesis of fatty acids, sterols etc.

Anaplerosis or anaplerotic reactions

The synthetic reactions described above deplete the intermediates of citric acid cycle. The cycle will cease to operate unless the intermediates drawn out are replenished. ***The reactions concerned to replenish or to fill up the intermediates of citric acid cycle are called anaplerotic reactions or anaplerosis*** (*Greek* : fill up). In ***Fig.13.10***, the important synthetic pathways that draw the intermediates of TCA cycle and the anaplerotic reactions to fill them up are given.

The salient features of important anaplerotic reactions are described

1. Pyruvate carboxylase catalyses the conversion of pyruvate to oxaloacetate. This is an ATP dependent carboxylation reaction.

$$\text{Pyruvate} + CO_2 + \text{ATP} \longrightarrow \text{Oxaloacetate} + \text{ADP} + \text{Pi}$$

The details of the above reaction are described under gluconeogenesis.

2. Pyruvate is converted to malate by $NADP^+$ dependent malate dehydrogenase (malic enzyme).

$$\text{Pyruvate} + CO_2 + \text{NADPH} + H^+ \rightleftharpoons \text{Malate} + NADP^+ + H_2O$$

3. Transamination is a process wherein an amino acid transfers its amino group to a keto acid and itself gets converted to a keto acid. The formation of α-ketoglutarate and oxaloacetate occurs by this mechanism.

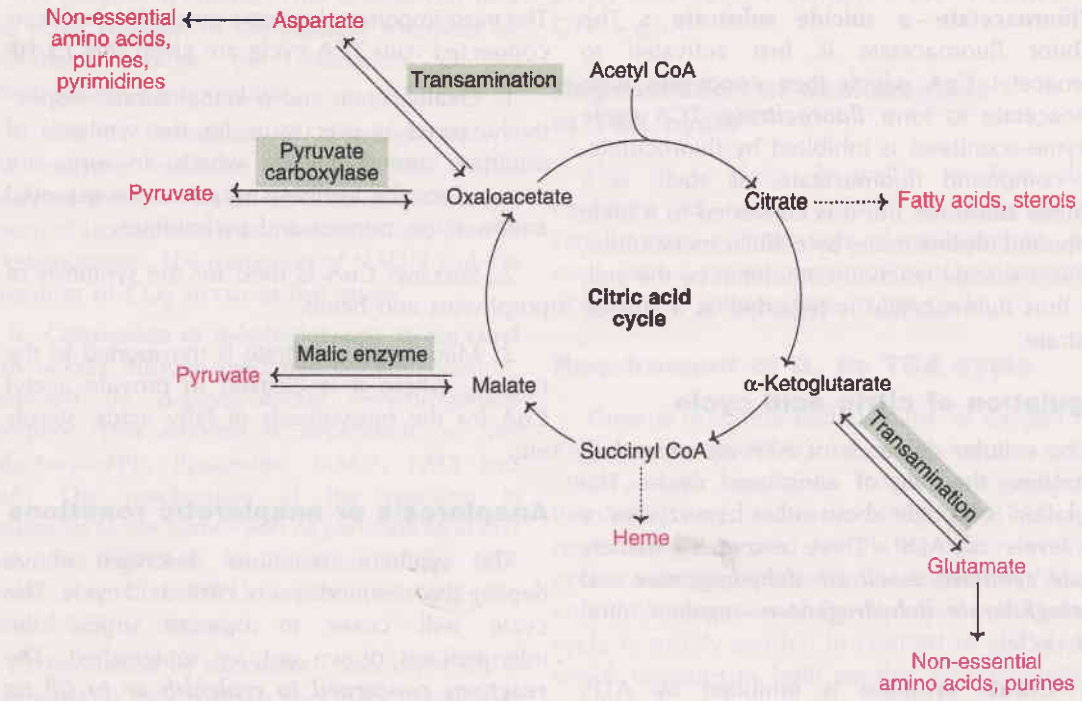


Fig. 13.10 : Major synthetic and anaplerotic pathways of the intermediates of citric acid cycle.

4. α-Ketoglutarate can also be synthesized from glutamate by glutamate dehydrogenase action.

$$\text{Glutamate} + NAD(P)^+ + H_2O \leftrightarrow \alpha\text{-Ketoglutarate} + NAD(P)H + H^+ + NH_4^+$$

Energetics of glucose oxidation

When a molecule of glucose (6 carbon) undergoes glycolysis, 2 molecules of pyruvate or lactate (3 carbon) are produced. Pyruvate is oxidatively decarboxylated to acetyl CoA (2 carbon) which enters the citric acid cycle and gets completely oxidized to CO_2 and H_2O. The overall process of glucose being completely oxidized to CO_2 and H_2O via glycolysis and citric acid cycle is as follows

$$C_6H_{12}O_6 + 6O_2 + 38ADP + 38Pi \longrightarrow 6CO_2 + 6H_2O + 38ATP$$

The enzymes of glucose metabolism responsible for generating ATP are given in ***Table 13.1.***

When a molecule of glucose is burnt in a calorimeter, 2,780 KJ of heat is liberated. In the living system, energy is trapped leading to the synthesis of 38 ATP which is equivalent to 1,159 KJ (1 ATP has high energy bond equivalent to 30.5 KJ). That is, about 48% of the energy in glucose combustion is actually captured for ATP generation.

GLUCONEOGENESIS

The synthesis of glucose from non-carbohydrate compounds is known as gluconeogenesis. The major ***substrates***/precursors for gluconeogenesis are ***lactate, pyruvate, glucogenic amino acids, propionate*** and ***glycerol.***

Location of gluconeogenesis

Gluconeogenesis occurs mainly in the ***cytosol,*** although some precursors are produced in the mitochondria. Gluconeogenesis mostly takes place in liver (about 1 kg glucose synthesized everyday) and, to some extent, in kidney matrix (about one-tenth of liver capacity).

Importance of gluconeogenesis

Glucose occupies a key position in the metabolism and its continuous supply is absolutely essential to the body for a variety of functions

1. Brain and central nervous system, erythrocytes, testes and kidney medulla are dependent on glucose for continuous supply of energy. Human brain alone requires about 120 g of glucose per day, out of about 160 g needed by the entire body.

2. Glucose is the only source that supplies energy to the skeletal muscle, under anaerobic conditions.

3. In fasting even more than a day, gluconeogenesis must occur to meet the basal requirements of the body for glucose and to maintain the intermediates of citric acid cycle. This is essential for the survival of humans and other animals.

4. Certain metabolites produced in the tissues accumulate in the blood, e.g. lactate, glycerol, propionate etc. Gluconeogenesis effectively clears them from the blood.

Reactions of gluconeogenesis

Gluconeogenesis closely resembles the reversed pathway of glycolysis, although it is not the complete reversal of glycolysis. Essentially, 3 (out of 10) reactions of glycolysis are irreversible. The seven reactions are common for both glycolysis and gluconeogenesis (***Fig.13.11***). The ***three irreversible steps*** of glycolysis are catalysed by the enzymes, namely hexokinase, phosphofructokinase and pyruvate kinase. These three stages—bypassed by alternate enzymes specific to gluconeogenesis—are discussed

1. **Conversion of pyruvate to phosphoenol-pyruvate :** This takes place in two steps (***Fig.13.12***). ***Pyruvate carboxylase*** is a biotin—dependent mitochondrial enzyme that converts pyruvate to oxaloacetate in presence of ATP and

BIOMEDICAL / CLINICAL CONCEPTS

- *Glycolysis is an important source of energy supply for brain, retina, skin and renal medulla.*
- *The crucial significance of glycolysis is its ability to generate ATP in the absence of oxygen.*
- *Skeletal muscle, during strenous exercise, requires the occurrence of uninterrupted glycolysis. This is due to the limited supply of oxygen.*
- *The cardiac muscle cannot survive for long in the absence of oxygen since it is not well adapted for glycolysis under anaerobic conditions.*
- *Glycolysis in erythrocytes is associated with 2, 3-bisphosphoglycerate (2,3-BPG) production. In the presence of 2, 3-BPG, oxyhemoglobin unloads more oxygen to the tissues.*
- *The occurrence of glycolysis is very much elevated in rapidly growing cancer cells.*
- *Lactic acidosis is also observed in patients with deficiency of the enzyme pyruvate dehydrogenase. It could also be due to collapse of circulatory system encountered in myocardial infarction and pulmonary embolism.*
- *Citric acid cycle is the final common oxidative pathway for carbohydrates, fats and amino acids. It utilizes (indirectly) about 2/3 of the total oxygen consumed by the body and generates about 2/3 of the total energy (ATP).*
- *Unlike the other metabolic pathways/cycles, very few genetic abnormalities of Krebs cycle are known. This may be due to the vital importance of this metabolic cycle for the survival of life.*

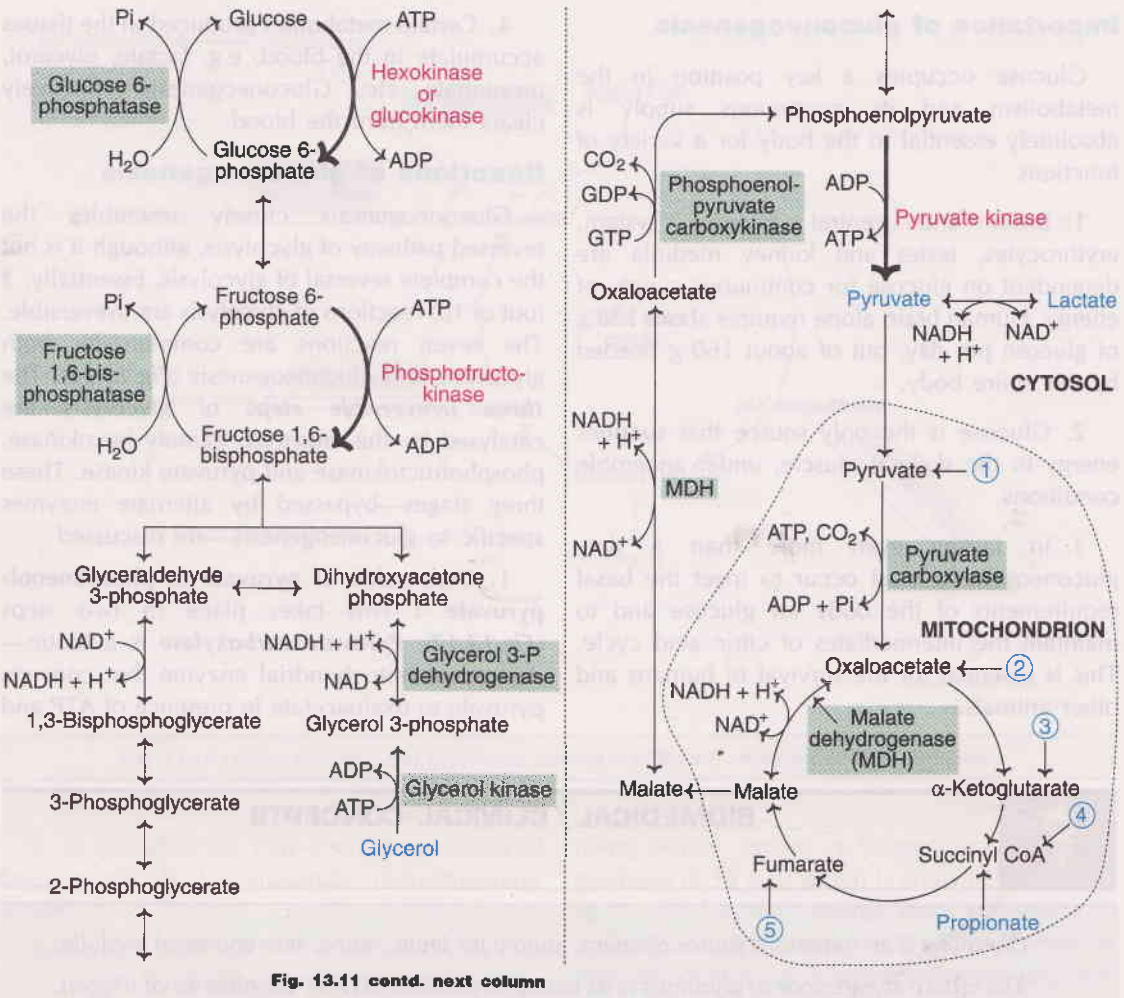


Fig. 13.11 : *The pathway of gluconeogenesis. [The enzymes catalysing irreversible steps in glycolysis are shown in red. The important enzymes participating in gluconeogenesis are shown in shaded green. The substrates for gluconeogenesis are in blue. The numbers represent the entry of glucogenic amino acids : (1) Alanine, glycine, serine, cysteine, threonine and tryptophan; (2) Aspartate and asparagine; (3) Arginine, glutamate, glutamine, histidine, proline; (4) Isoleucine, methionine, valine; (5) Phenylalanine, tyrosine].*

CO_2. This enzyme regulates gluconeogenesis and requires acetyl CoA for its activity.

Oxaloacetate is synthesized in the mitochondrial matrix. It has to be transported to the cytosol to be used in gluconeogenesis, where the rest of the pathway occurs. Due to membrane impermeability, oxaloacetate cannot diffuse out of the mitochondria. It is converted to malate and then transported to the cytosol. Within the cytosol, oxaloacetate is regenerated. The reversible conversion of oxaloacetate and malate is catalysed by malate dehydrogenase, an enzyme present in both mitochondria and cytosol.

In the cytosol, ***phosphoenolpyruvate carboxykinase*** converts oxaloacetate to phosphoenolpyruvate. GTP or ITP (not ATP) is used in this reaction and the CO_2 (fixed by carboxylase) is liberated. For the conversion of pyruvate to phosphoenol pyruvate, 2 ATP equivalents are utilized. This is in contrast to only one ATP that is liberated in glycolysis for this reaction.

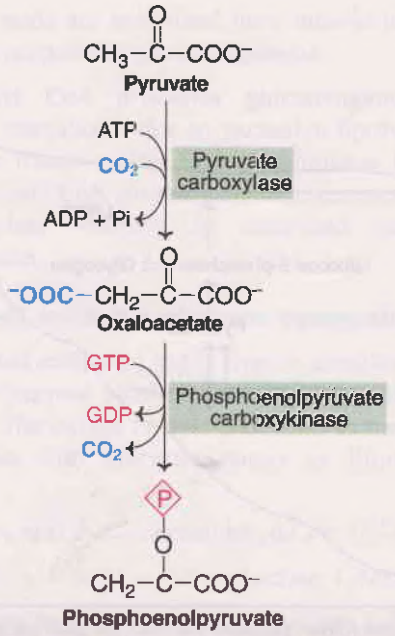


Fig. 13.12 : Conversion of pyruvate to phosphoenolpyruvate.

2. **Conversion of fructose 1,6-bisphosphate to fructose 6-phosphate :** Phosphoenolpyruvate undergoes the reversal of glycolysis until fructose 1,6-bisphosphate is produced. The enzyme ***fructose 1,6-bisphosphatase*** converts fructose 1,6-bisphosphate to fructose 6-phosphate. This enzyme requires Mg^{2+} ions. Fructose 1,6-bisphosphatase is absent in smooth muscle and heart muscle. This enzyme is also regulatory in gluconeogenesis.

3. **Conversion of glucose 6-phosphate to glucose :** ***Glucose 6-phosphatase*** catalyses the conversion of glucose 6-phosphate to glucose. The presence or absence of this enzyme in a tissue determines whether the tissue is capable of contributing glucose to the blood or not. It is mostly present in liver and kidney but ***absent in muscle***, brain and adipose tissue.

The overall summary of gluconeogenesis for the conversion of pyruvate to glucose is shown below

$$2\ \text{Pyruvate} + 4\text{ATP} + 2\text{GTP} + 2\text{NADH} + 2\text{H}^+ + 6\text{H}_2\text{O} \longrightarrow \text{Glucose} + 2\text{NAD}^+ + 4\text{ADP} + 2\text{GDP} + 6\text{Pi} + 6\text{H}^+$$

Gluconeogenesis from amino acids

The carbon skeleton of glucogenic amino acids (all except leucine and lysine) results in the formation of pyruvate or the intermediates of citric acid cycle (***Fig.13.11***) which, ultimately, result in the synthesis of glucose.

Gluconeogenesis from glycerol

Glycerol is liberated mostly in the adipose tissue by the hydrolysis of fats (triacylglycerols). The enzyme glycerokinase (found in liver and kidney, absent in adipose tissue) activates glycerol to glycerol 3-phosphate. The latter is converted to dihydroxyacetone phosphate by glycerol 3-phosphate dehydrogenase. Dihydroxyacetone phosphate is an intermediate in glycolysis which can be conveniently used for glucose production.

Gluconeogenesis from propionate

Oxidation of odd chain fatty acids and the breakdown of some amino acids (methionine, isoleucine) yields a three carbon propionyl CoA. Propionyl CoA carboxylase acts on this in presence of ATP and biotin and converts to methyl malonyl CoA which is then converted to succinyl CoA in presence of B_{12} coenzyme (***Refer Fig.7.38***). Succinyl CoA formed from propionyl CoA enters gluconeogenesis via citric acid cycle.

Gluconeogenesis from lactate (Cori cycle)

Lactate produced by active skeletal muscle is a major precursor for gluconeogenesis. Under anaerobic conditions, pyruvate is reduced to lactate by lactate dehydrogenase (LDH)

$$\text{Pyruvate} + \text{NADH} + \text{H}^+ \underset{}{\overset{\text{LDH}}{\rightleftharpoons}} \text{Lactate} + \text{NAD}^+$$

Lactate is a dead end in glycolysis, since it must be reconverted to pyruvate for its further metabolism. The very purpose of lactate production is to regenerate NADH so that glycolysis proceeds uninterrupted in skeletal muscle. Lactate or pyruvate produced in the muscle cannot be utilized for the synthesis of

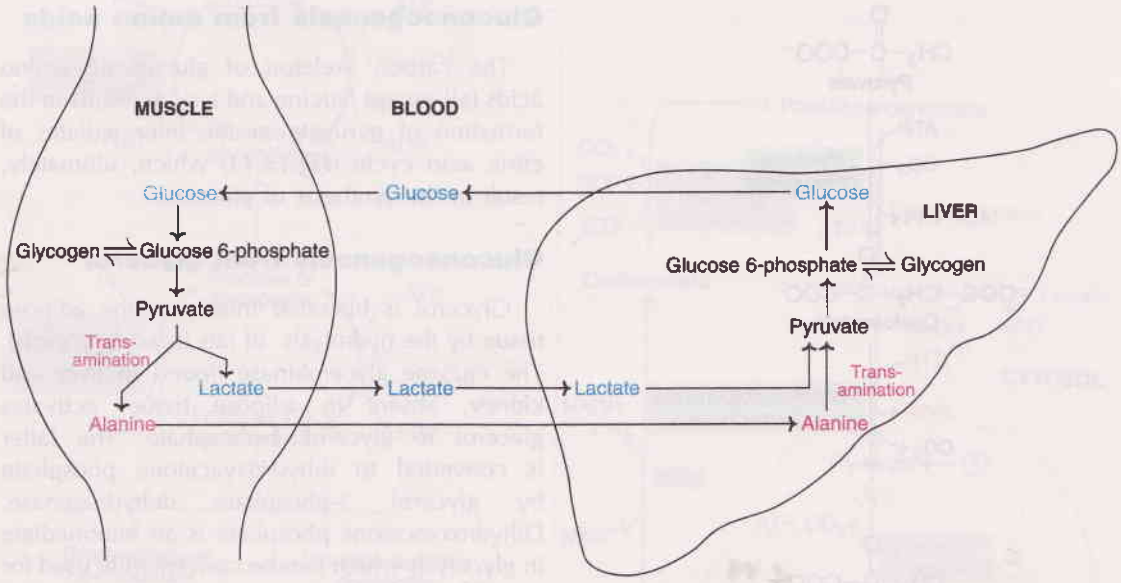


Fig. 13.13 : The Cori cycle (blue) and glucose-alanine (red) cycle (other reactions common for both cycles).

glucose due to the absence of the key enzymes of gluconeogenesis (glucose 6-phosphatase and fructose 1,6-bisphosphatase).

The plasma membrane is freely permeable to lactate. Lactate is carried from the skeletal muscle through blood and handed over to liver, where it is oxidized to pyruvate. Pyruvate, so produced, is converted to glucose by gluconeogenesis, which is then transported to the skeletal muscle.

The cycle involving the ***synthesis of glucose in liver from the skeletal muscle lactate*** and the reuse of glucose thus synthesized by the muscle for energy purpose is known as Cori cycle (***Fig.13.13***).

Glucose-alanine cycle

There is a continuous transport of amino acids from muscle to liver, which predominantly occurs during starvation. Alanine dominates among the transported amino acids. It is postulated that pyruvate in skeletal muscle undergoes transamination to produce alanine. Alanine is transported to liver and used for gluconeogenesis. This cycle is referred to as glucose-alanine cycle (***Fig.13.13***).

Regulation of gluconeogenesis

The hormone ***glucagon*** and the availability of substrates mainly regulate gluconeogenesis, as discussed hereunder.

Influence of glucagon : This is a hormone, secreted by α-cells of the pancreatic islets. Glucagon stimulates gluconeogenesis by two mechanisms

1. Active form of pyruvate kinase is converted to inactive form through the mediation of cyclic AMP, brought about by glucagon. ***Decreased pyruvate kinase*** results in the reduced conversion of phosphoenol pyruvate to pyruvate and the former is diverted for the synthesis of glucose.

2. Glucagon reduces the concentration of fructose 2,6-bisphosphate. This compound allosterically inhibits phosphofructokinase and activates fructose 1,6-bisphosphatase, both favour increased gluconeogenesis.

Availability of substrates : Among the various substrates, glucogenic amino acids have stimulating influence on gluconeogenesis. This is particularly important in a condition like diabetes mellitus (decreased insulin level) where

amino acids are mobilized from muscle protein for the purpose of gluconeogenesis.

Acetyl CoA promotes gluconeogenesis : During starvation—due to excessive lipolysis in adipose tissue—acetyl CoA accumulates in the liver. Acetyl CoA allosterically activates pyruvate carboxylase resulting in enhanced glucose production.

Alcohol inhibits gluconeogenesis

Ethanol oxidation in the liver to acetaldehyde by the enzyme alcohol dehydrogenase utilizes NAD^+. The excess NADH produced in the liver interferes with gluconeogenesis as illustrated below.

Ethanol + NAD^+ ⟶ Acetaldehyde + NADH + H^+

Pyruvate + NADH + H^+ ↔ Lactate + NAD^+

Oxaloacetate + NADH + H^+ ↔ Malate + NAD^+

It is evident from the above reactions that pyruvate and oxaloacetate, the predominant substrates for gluconeogenesis, are made unavailable by alcohol intoxication. This happens due to overconsumption of NAD^+ and excessive production of NADH by alcohol.

Alcohol consumption increases the risk of hypoglycemia (reduced plasma glucose) due to reduced gluconeogenesis. This is particularly important in diabetic patients who are on insulin treatment.

Gluconeogenesis from fat?

It is often stated that glucose cannot be synthesized from fat. In a sense, it is certainly true, since the fatty acids (most of them being even chain), on oxidation, produce acetyl CoA which cannot be converted to pyruvate. Further, the two carbons of acetyl CoA disappear as 2 moles of CO_2 in TCA cycle. Therefore, even chain fatty acids cannot serve as precursors for glucose formation. The prime reason why animals cannot convert fat to glucose is the ***absence of glyoxylate cycle*** (described later).

However, the glycerol released from lipolysis and the propionate obtained from the oxidation of odd chain fatty acids are good substrates for gluconeogenesis, as discussed above.

GLYCOGEN METABOLISM

Glycogen is the storage form of glucose in animals, as is starch in plants. It is stored mostly in liver (6-8%) and muscle (1-2%). Due to more muscle mass, the quantity of glycogen in muscle (250 g) is about three times higher than that in the liver (75 g). Glycogen is stored as granules in the cytosol, where most of the enzymes of glycogen synthesis and breakdown are present.

Functions of glycogen

The prime function of liver glycogen is to maintain the blood glucose levels, particularly between meals. Liver glycogen stores increase in a well-fed state which are depleted during fasting. Muscle glycogen serves as a fuel reserve for the supply of ATP during muscle contraction.

Why store glycogen as a fuel reserve?

As such, fat is the fuel reserve of the body. However, fat is not preferred, instead glycogen is chosen for a routine, and day to day use of energy for the following reasons

- Glycogen can be rapidly mobilized
- Glycogen can generate energy in the absence of oxygen
- Brain depends on continuous glucose supply (which mostly comes from glycogen.)

On the other hand, fat mobilization is slow, needs O_2 for energy production and cannot produce glucose (to a significant extent). Thus, fat may be considered as a fixed deposit while glycogen is in the current/saving account in a bank!

GLYCOGENESIS

The ***synthesis of glycogen*** from glucose is glycogenesis (***Fig.13.14***). Glycogenesis takes place in the cytosol and requires ATP and UTP, besides glucose.

1. **Synthesis of UDP-glucose :** The enzymes hexokinase (in muscle) and glucokinase (in liver) convert glucose to glucose 6-phosphate. Phosphoglucomutase catalyses the conversion of

glucose 6-phosphate to glucose 1-phosphate. Uridine diphosphate glucose (UDPG) is synthesized from glucose 1-phosphate and UTP by UDP-glucose pyrophosphorylase.

2. **Requirement of primer to initiate glycogenesis :** A small fragment of pre-existing glycogen must act as a '***primer***' to initiate glycogen synthesis. It is recently found that in the absence of glycogen primer, a specific protein—namely '***glycogenin***'—can accept glucose from UDPG. The hydroxyl group of the amino acid tyrosine of glycogenin is the site at which the initial glucose unit is attached. The enzyme glycogen initiator synthase transfers the first molecule of glucose to glycogenin. Then glycogenin itself takes up a few glucose residues to form a fragment of primer which serves as an acceptor for the rest of the glucose molecules.

3. **Glycogen synthesis by glycogen synthase :** Glycogen synthase is responsible for the formation of 1,4-glycosidic linkages. This enzyme transfers the glucose from UDP-glucose to the non-reducing end of glycogen to form α-1,4 linkages.

4. **Formation of branches in glycogen :** Glycogen synthase can catalyse the synthesis of a linear unbranched molecule with 1,4 α-glycosidic linkages. Glycogen, however, is a branched tree-like structure. The formation of branches is brought about by the action of a branching enzyme, namely ***glucosyl α-4-6 transferase***. (amylo α 1,4 → 1,6 transglucosidase). This enzyme transfers a small fragment of five to eight glucose residues from the non-reducing end of glycogen chain (by breaking α-1,4 linkages) to another glucose residue where it is linked by α-1,6 bond. This leads to the formation of a new non-reducing end, besides the existing one. Glycogen is further elongated and branched, respectively, by the enzymes glycogen synthase and glucosyl 4-6 transferase.

The overall reaction of the glycogen synthesis for the addition of each glucose residue is

$$(Glucose)_n + Glucose + 2ATP \longrightarrow (Glucose)_{n+1} + 2\ ADP + Pi$$

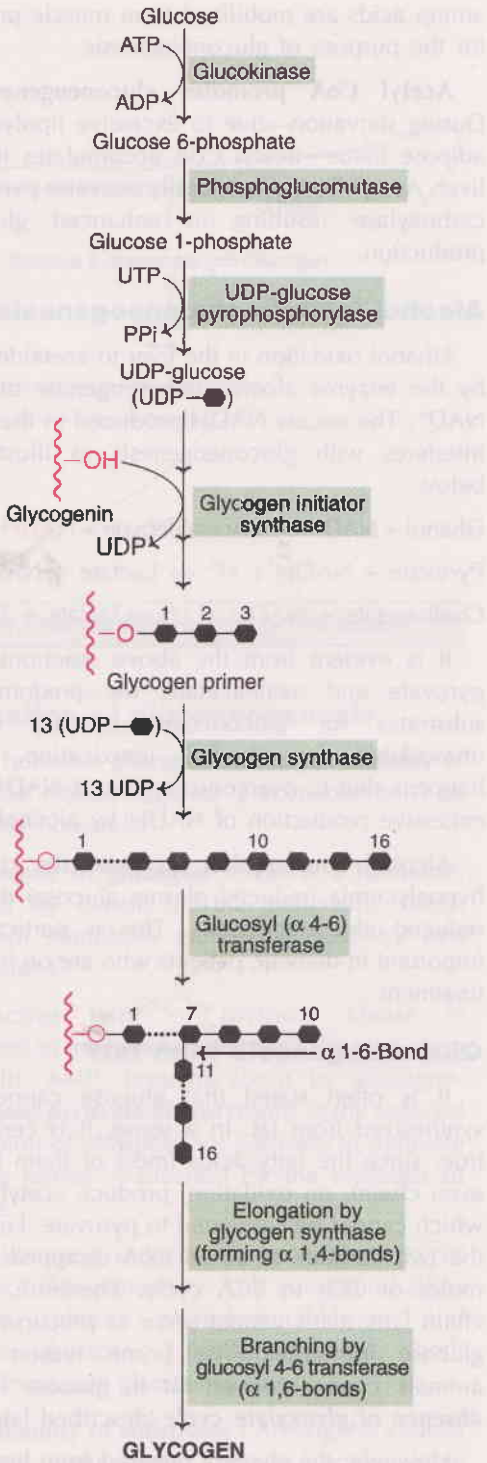


Fig. 13.14 : Glycogen synthesis from glucose (glycogenesis).

Of the two ATP utilized, one is required for the phosphorylation of glucose while the other is needed for conversion of UDP to UTP.

GLYCOGENOLYSIS

The ***degradation of stored glycogen*** in liver and muscle constitutes glycogenolysis. The pathways for the synthesis and degradation of glycogen are not reversible. An independent set of enzymes present in the cytosol carry out glycogenolysis. Glycogen is degraded by breaking α-1,4- and α-1,6-glycosidic bonds (***Fig.13.15***).

1. **Action of glycogen phosphorylase :** The α-1,4-glycosidic bonds (from the non-reducing ends) are cleaved sequentially by the enzyme glycogen phosphorylase to yield glucose 1-phosphate. This process—called ***phosphorolysis***—continues until four glucose residues remain on either side of branching point (α-1,6-glycosidic link). The glycogen so formed is known as ***limit dextrin*** which cannot be further degraded by phosphorylase. Glycogen phosphorylase possesses a molecule of pyridoxal phosphate, covalently bound to the enzyme.

2. **Action of debranching enzyme :** The branches of glycogen are cleaved by two enzyme activities present on a single polypeptide called ***debranching enzyme,*** hence it is a ***bifunctional enzyme.***

Glycosyl 4 : 4 transferase (oligo α-1,4 → 1,4 glucan transferase) activity removes a fragment of three or four glucose residues attached at a branch and transfers them to another chain. Here, one α-1,4-bond is cleaved and the same α-1,4 bond is made, but the places are different.

Amylo α-1,6-glucosidase breaks the α-1,6 bond at the branch with a single glucose residue and ***releases a free glucose.***

The remaining molecule of glycogen is again available for the action of phosphorylase and debranching enzyme to repeat the reactions stated in 1 and 2.

3. **Formation of glucose 6-phosphate and glucose :** Through the combined action of glycogen phosphorylase and debranching

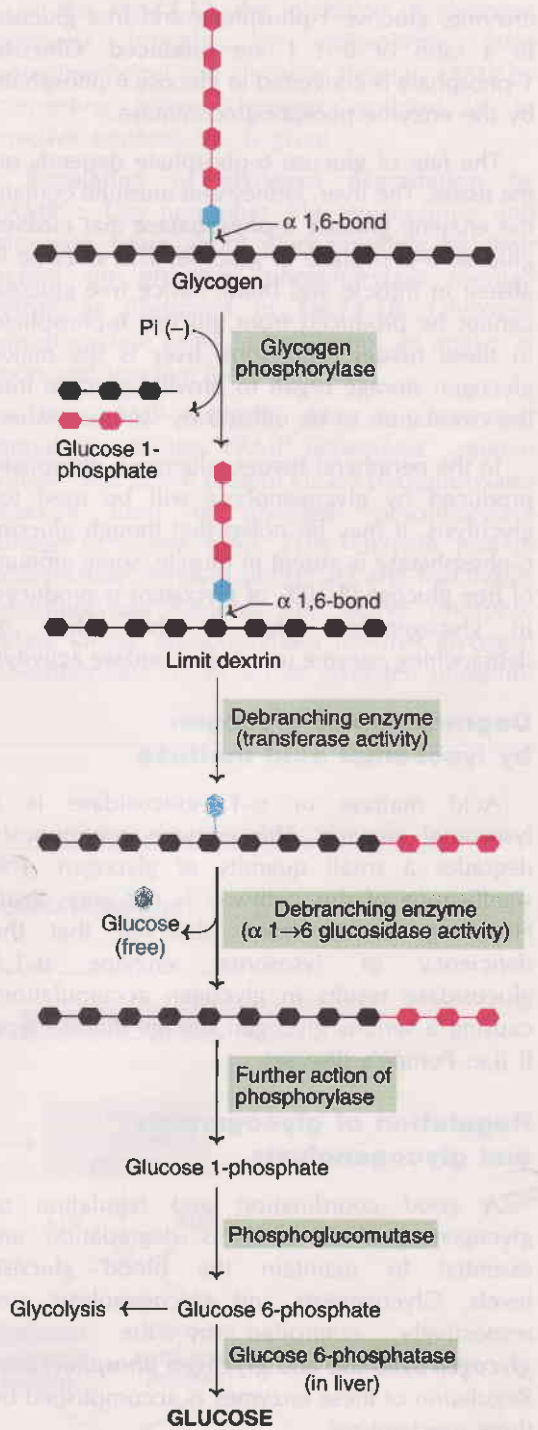


Fig. 13.15 : Glycogen degradation to glucose-glycogenolysis. (The ratio of glucose 1-phosphate to glucose is 8 : 1).

enzyme, glucose 1-phosphate and free glucose in a ratio of 8 : 1 are produced. Glucose 1-phosphate is converted to glucose 6-phosphate by the enzyme phosphoglucomutase.

The fate of glucose 6-phosphate depends on the tissue. The liver, kidney and intestine contain the enzyme ***glucose 6-phosphatase*** that cleaves glucose 6-phosphate to glucose. This enzyme is absent in muscle and brain, hence free glucose cannot be produced from glucose 6-phosphate in these tissues. Therefore, liver is the major glycogen storage organ to provide glucose into the circulation to be utilised by various tissues.

In the peripheral tissues, glucose 6-phosphate produced by glycogenolysis will be used for glycolysis. It may be noted that though glucose 6-phosphatase is absent in muscle, some amount of free glucose (8-10% of glycogen) is produced in glycogenolysis due to the action of debranching enzyme (α-1,6-glucosidase activity).

Degradation of glycogen by lysosomal acid maltase

Acid maltase or α-1,4-glucosidase is a lysosomal enzyme. This enzyme continuously degrades a small quantity of glycogen. The significance of this pathway is not very clear. However, it has been observed that the deficiency of lysosomal enzyme α-1,4 glucosidase results in glycogen accumulation, causing a serious glycogen storage disease type II (i.e. Pompe's disease).

Regulation of glycogenesis and glycogenolysis

A good coordination and regulation of glycogen synthesis and its degradation are essential to maintain the blood glucose levels. Glycogenesis and glycogenolysis are, respectively, controlled by the enzymes ***glycogen synthase*** and ***glycogen phosphorylase***. Regulation of these enzymes is accomplished by three mechanisms

1. Allosteric regulation
2. Hormonal regulation
3. Influence of calcium.

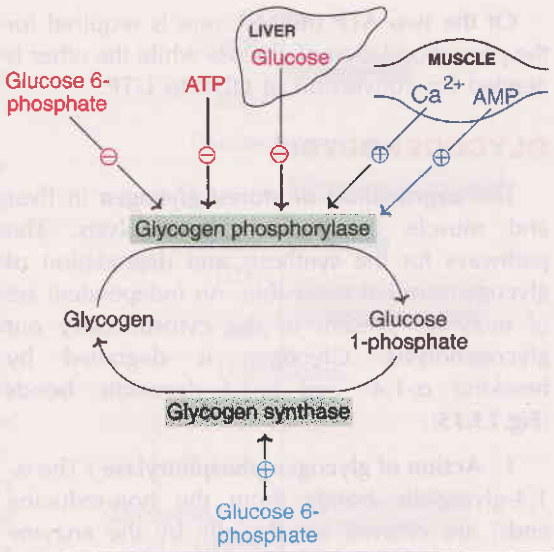


Fig. 13.16 : Allosteric regulation of glycogenolysis and glycogenesis (⊖ : Inhibition; ⊕ : Activation).

1. **Allosteric regulation of glycogen metabolism :** There are certain metabolites that allosterically regulate the activities of glycogen synthase and glycogen phosphorylase. The control is carried out in such a way that glycogen synthesis is increased when substrate availability and energy levels are high. On the other hand, glycogen breakdown is enhanced when glucose concentration and energy levels are low. The allosteric regulation of glycogen metabolism is depicted in ***Fig.13.16***. In a well-fed state, the availability of ***glucose 6-phosphate*** is high which allosterically ***activates glycogen synthase*** for more glycogen synthesis. On the other hand, glucose 6-phosphate and ATP allosterically inhibit glycogen phosphorylase. Free ***glucose*** in liver also acts as an allosteric ***inhibitor of glycogen phosphorylase.***

2. **Hormonal regulation of glycogen metabolism :** The hormones, through a complex series of reactions, bring about covalent modification, namely phosphorylation and dephosphorylation of enzyme proteins which, ultimately control glycogen synthesis or its degradation.

cAMP as second messenger for hormones : The hormones like epinephrine and norepinephrine, and glucagon (in liver) activate

adenylate cyclase to increase the production of cAMP. The enzyme phosphodiesterase breaks down cAMP. The hormone insulin increases the phosphodiesterase activity in liver and lowers the cAMP levels.

Regulation of glycogen synthesis by cAMP : The glycogenesis is regulated by glycogen synthase. This enzyme exists in two forms—***glycogen synthase 'a'***—which is not phosphorylated and most active, and secondly, ***glycogen synthase 'b'*** as phosphorylated inactive form. Glycogen synthase '*a*' can be converted to '*b*' form (inactive) by phsophorylation. The degree of phosphorylation is proportional to the inactive state of enzyme. The process of phosphorylation is catalysed by a cAMP-dependent protein kinase. The protein kinase phosphorylates and inactivates glycogen synthase by converting '*a*' form to '*b*' form. The glycogen synthase '*b*' can be converted back to synthase '*a*' by protein phosphatase I.

In the ***Fig.13.17***, the inhibition of glycogen synthesis brought by epinephrine (also norepinephrine) and glucagon through cAMP by converting active glycogen synthase '*a*' to inactive synthase '*b*', is given.

Regulation of glycogen degradation by cAMP : The hormones like epinephrine and glucagon bring about glycogenolysis by their action on ***glycogen phosphorylase*** through cAMP as illustrated in ***Fig.13.18***. Glycogen phosphorylase exists in two forms, an active '*a*' form and inactive form '*b*'.

The cAMP—formed due to hormonal stimulus—activates cAMP dependent protein kinase. This active protein kinase phosphorylates inactive form of glycogen phsophorylase kinase to active form. (The enzyme protein phosphatase removes phosphate and inactivates phosphorylase kinase). The active phosphorylase kinase phosphorylates inactive glycogen phosphorylase '*b*' to active glycogen phospho-

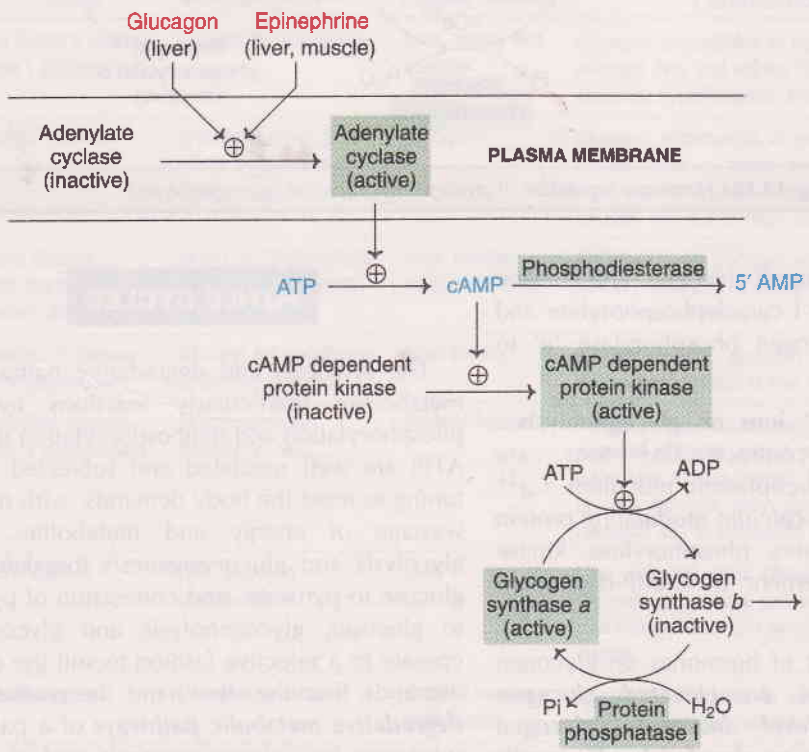


Fig. 13.17 : Hormonal regulation of glycogen synthesis (glycogenesis).

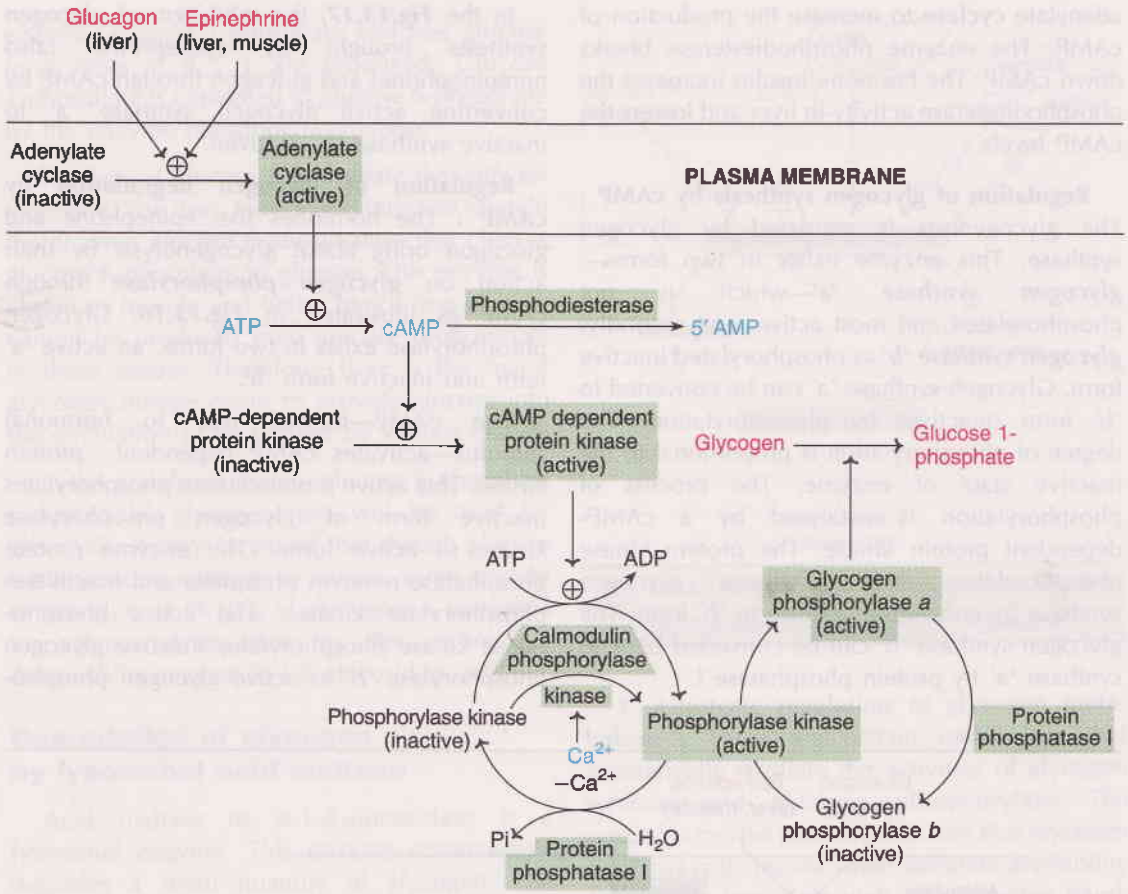


Fig. 13.18 : Hormonal regulation of glycogen degradation (glycogenolysis).

rylase '*a*' which degrades glycogen. The enzyme protein phosphatase I can dephosphorylate and convert active glycogen phosphorylase '*a*' to inactive '*b*' form.

3. **Effect of Ca^{2+} ions on glycogenolysis :** When the muscle contracts, Ca^{2+} ions are released from the sarcoplasmic reticulum. Ca^{2+} binds to ***calmodulin-calcium modulating protein*** and directly activates phosphorylase kinase without the involvement of cAMP-dependent protein kinase.

The overall effect of hormones on glycogen metabolism is that ***an elevated glucagon or epinephrine level increases glycogen degradation whereas an elevated insulin results in increased glycogen synthesis.***

FUTILE CYCLES

The synthesis and degradative pathways of metabolism (particularly reactions involving phosphorylation and dephosphorylation utilizing ATP) are well regulated and subjected to fine tuning to meet the body demands, with minimal wastage of energy and metabolites. Thus, glycolysis and gluconeogenesis (breakdown of glucose to pyruvate, and conversion of pyruvate to glucose), glycogenolysis and glycogenesis operate in a selective fashion to suit the cellular demands. If on the other hand, the ***synthesis and degradative metabolic pathways*** of a particular substance (say gluconeogenesis and glycolysis related to glucose) ***operate to the same extent***

simultaneously, this would result in futile cycles. However, futile cycles, consuming energy (ATP) are ***wasteful metabolic exercises***. They are minimally operative due to a well coordinated metabolic machinery.

GLYCOGEN STORAGE DISEASES

The ***metabolic defects*** concerned with the ***glycogen synthesis*** and ***degradation*** are collectively referred to as glycogen storage diseases. These disorders are due to defects in the enzymes which may be either generalized (affecting all tissues) or tissue-specific. The inherited disorders are characterized by deposition of normal or abnormal type of glycogen in one or more tissues. A summary of glycogen metabolism along with the defective enzymes in the glycogen storage disorders is depicted in ***Fig.13.19***. The biochemical lesions and the characteristic features of the disorders are given in ***Table 13.2***.

von Gierke's disease (type I)

The incidence of type I glycogen storage disease is 1 per 200,000 persons. It is transmitted by autosomal recessive trait. This disorder results in various biochemical manifestations.

1. **Fasting hypoglycemia :** Due to the defect in the enzyme ***glucose 6-phosphatase***, enough free glucose is not released from the liver into blood.

2. **Lactic acidemia :** Glucose is not synthesized from lactate produced in muscle and liver. Lactate level in blood increases and the pH is lowered (acidosis).

Table 13.2 Glycogen storage diseases – biochemical lesions and characteristic features

Type	*Name*	*Enzyme defect*	*Organ(s) involved*	*Characteristic features*
I	von Gierke's disease (type I glycogenosis)	Glucose 6-phosphatase	Liver, kidney and intestine	Glycogen accumulates in hepatocytes and renal cells, enlarged liver and kidney, fasting hypoglycemia, lactic acidemia; hyperlipidemia; ketosis; gouty arthritis.
II	Pompe's disease	Lysosomal α-1,4 glucosidase (acid maltase)	All organs	Glycogen accumulates in lysosomes in almost all the tissues; heart is mostly involved; enlarged liver and heart, nervous system is also affected; death occurs at an early age due to heart failure.
III	Cori's disease (limit dextrinosis, Forbe's disease)	Amylo α-1,6-glucosidase (debranching enzyme)	Liver, muscle, heart, leucocytes	Branched chain glycogen accumulates; liver enlarged; clinical manifestations are similar but milder compared to von Gierke's disease.
IV	Anderson's disease (amylopectinosis)	Glucosyl 4-6 transferase (branching enzyme)	Most tissues	A rare disease, glycogen with only few branches accumulate; cirrhosis of liver, impairment in liver function.
V	McArdle's disease (type V glycogenosis)	Muscle glycogen phosphorylase	Skeletal muscle	Muscle glycogen stores very high, not available during exercise; subjects cannot perform strenous exercise; suffer from muscle cramps; blood lactate and pyruvate do not increase after exercise; muscles may get damaged due to inadequate energy supply.
VI	Her's disease	Liver glycogen phosphorylase	Liver	Liver enlarged; liver glycogen cannot form glucose (pyruvate and lactate can be precursors for glucose); mild hypoglycemia and ketosis seen, not a very serious disease.
VII	Tarui's disease	Phosphofructokinase	Skeletal muscle, erythrocytes	Muscle cramps due to exercise; blood lactate not elevated; hemolysis occurs.

Rare glycogen disorders VIII, IX, X and XI have been identified. They are due to defects in the enzymes concerned with activating and deactivating liver phosphorylase.

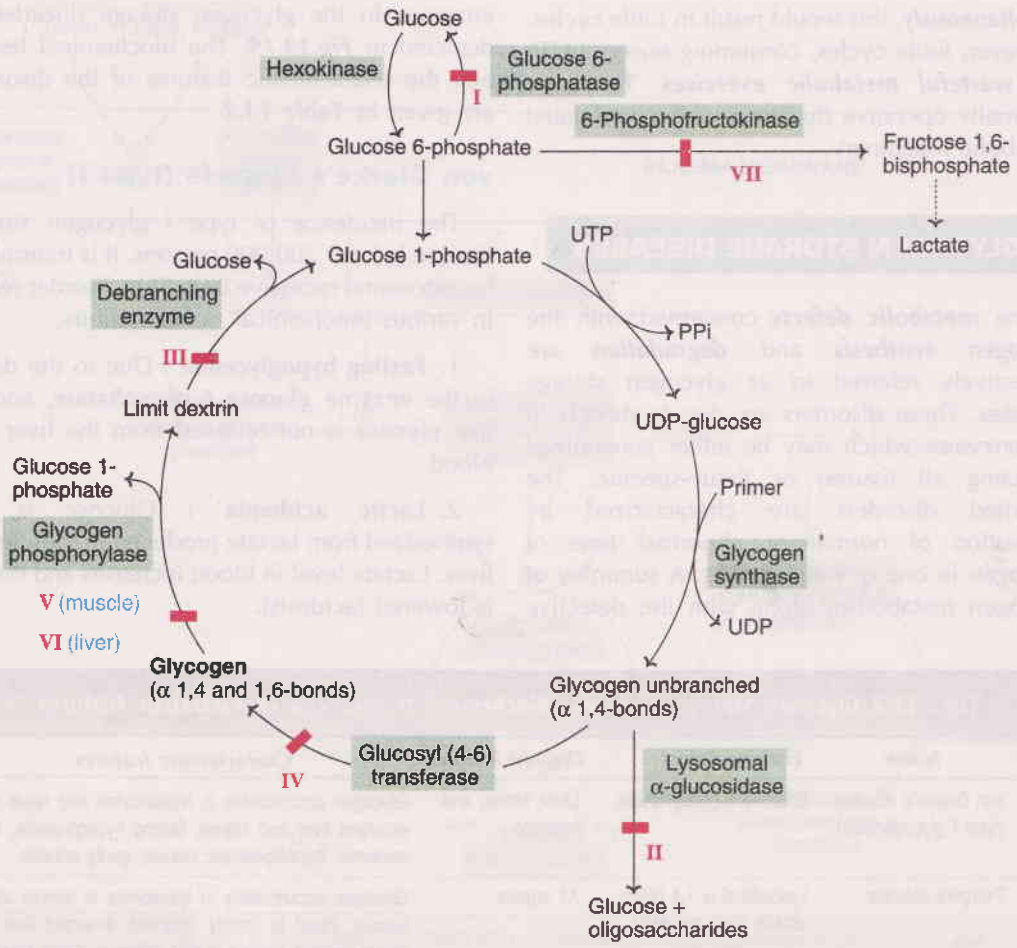


***Fig. 13.19** : Summary of glycogen metabolism with glycogen storage diseases (Red blocks indicate storage disease, I–von Gierke's disease; II–Pompe's disease; III–Cori's disease; IV–Anderson's disease; V–Mc Ardle's disease; VI–Her's disease; VII–Tarui's disease).*

3. **Hyperlipidemia** : There is a blockade in gluconeogenesis. Hence more fat is mobilized to meet energy requirements of the body. This results in increased plasma free fatty acids and ketone bodies.

4. **Hyperuricemia** : Glucose 6-phosphate that accumulates is diverted to pentose phosphate pathway, leading to ***increased synthesis of ribose phosphates*** which increase the cellular levels of phosphoribosyl pyrophosphate and enhance the metabolism of purine nucleotides to uric acid. ***Ele***vated plasma levels of uric acid (hyperuricemia) are often associated with ***gouty arthritis*** (painful joints).

The important features of the glycogen storage diseases are given in ***Table 13.2***.

HEXOSE MONOPHOSPHATE SHUNT

Hexose monophosphate pathway or ***HMP shunt*** is also called ***pentose phosphate pathway*** or phosphogluconate pathway. ***This is an alternative pathway to glycolysis and TCA cycle for the oxidation of glucose.*** However, HMP shunt is more anabolic in nature, since it is concerned with the biosynthesis of NADPH and pentoses.

HMP shunt—a unique multifunctional pathway

The pathway starts with glucose 6-phosphate. As such, no ATP is directly utilized or produced in HMP pathway. It is a unique multifunctional pathway, since there are several interconvertible substances produced which may proceed in different directions in the metabolic reactions.

Location of the pathway

The enzymes of HMP shunt are located in the ***cytosol***. The tissues such as ***liver, adipose tissue, adrenal gland, erythrocytes, testes*** and ***lactating mammary gland***, are highly active in HMP shunt. Most of these tissues are involved in the biosynthesis of fatty acids and steroids which are dependent on the supply of NADPH.

Reactions of the pathway

The sequence of reactions of HMP shunt (***Fig.13.20***) is divided into two phases—***oxidative*** and ***non-oxidative.***

1. **Oxidative phase :** Glucose 6-phosphate dehydrogenase (G6PD) is an NADP-dependent enzyme that converts glucose 6-phosphate to 6-phosphogluconolactone. The latter is then hydrolysed by the gluconolactone hydrolase to 6-phosphogluconate. The next reaction involving the synthesis of NADPH is catalysed by 6-phosphogluconate dehydrogenase to produce 3 keto 6-phosphogluconate which then undergoes decarboxylation to give ribulose 5-phosphate.

G6PD regulates HMP shunt : The first reaction catalysed by G6PD is most regulatory in HMP shunt. This enzyme catalyses an irreversible reaction. NADPH competitively inhibits G6PD. It is the ratio of NADPH/NAD^+ that ultimately determines the flux of this cycle.

2. **Non-oxidative phase :** The non-oxidative reactions are concerned with the interconversion of three, four, five and seven carbon monosaccharides. Ribulose 5-phosphate is acted upon by an epimerase to produce xylulose 5-phosphate while ribose 5-phosphate ketoisomerase converts ribulose 5-phosphate to ribose 5-phosphate.

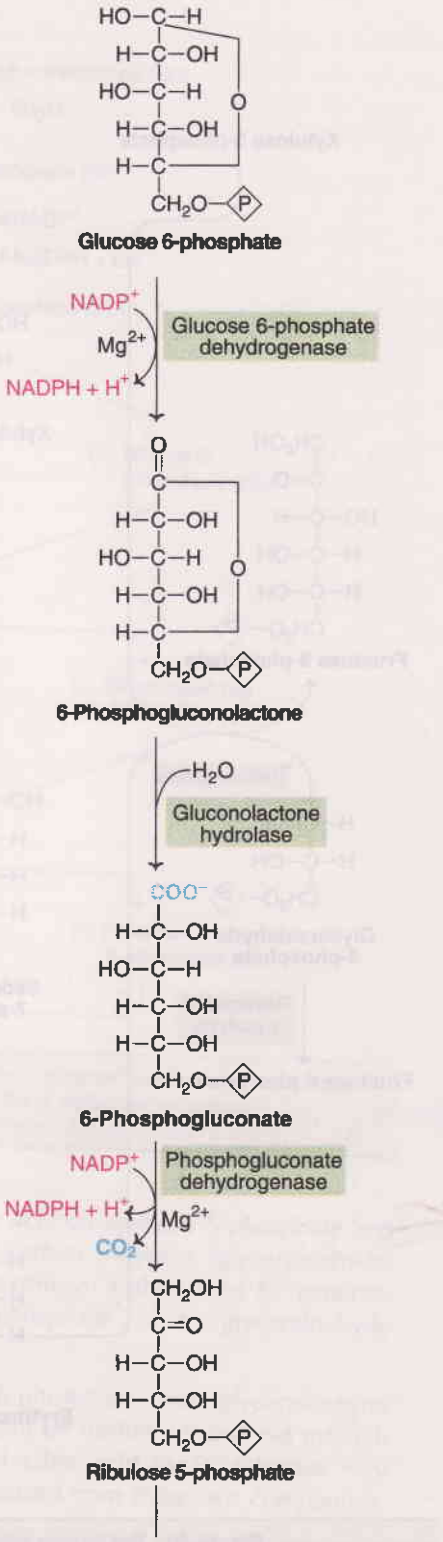


Fig. 13.20 contd. next page

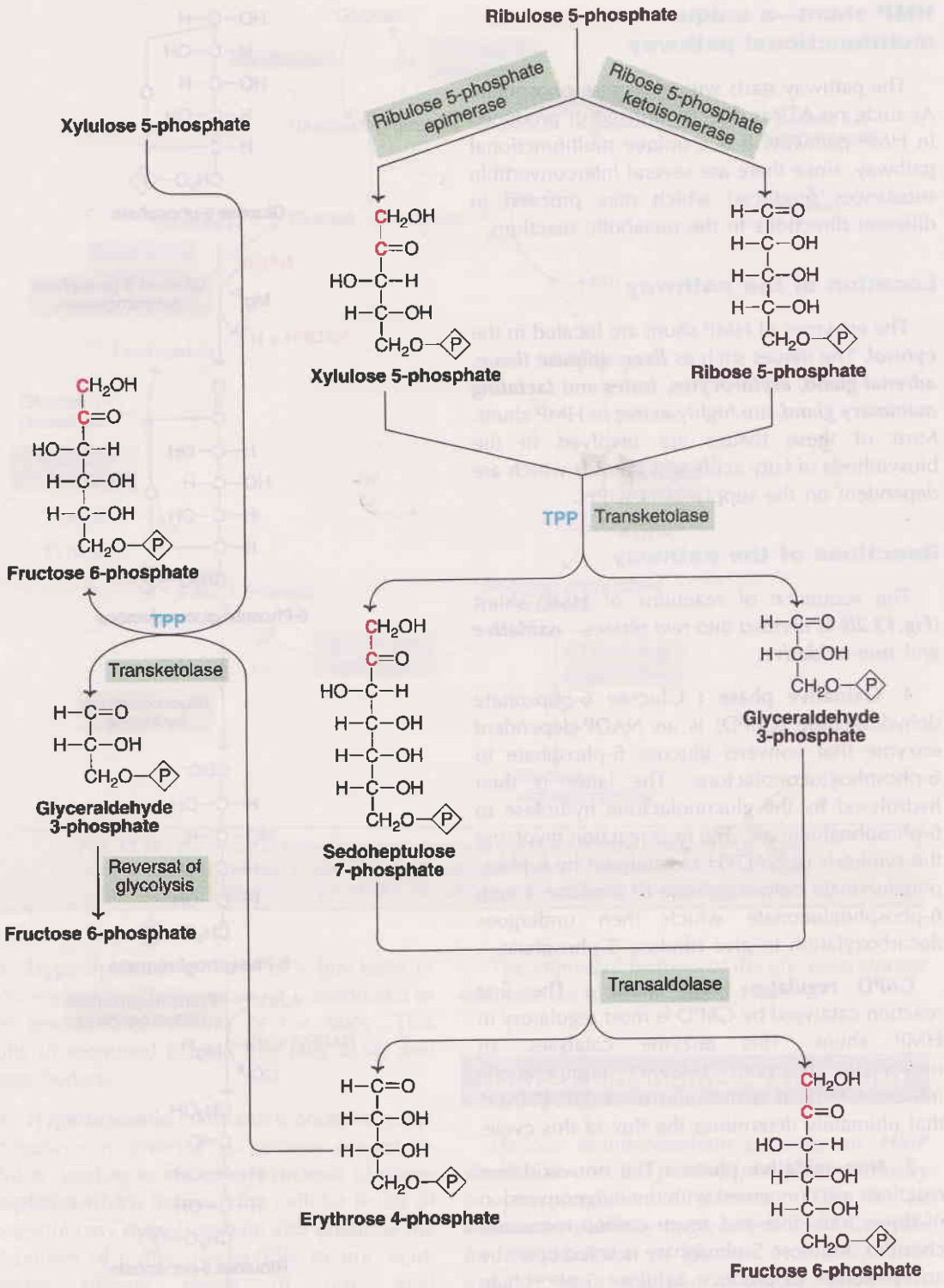


Fig. 13.20 : The hexose monophosphate shunt. (TPP – Thiamine pyrophosphate)

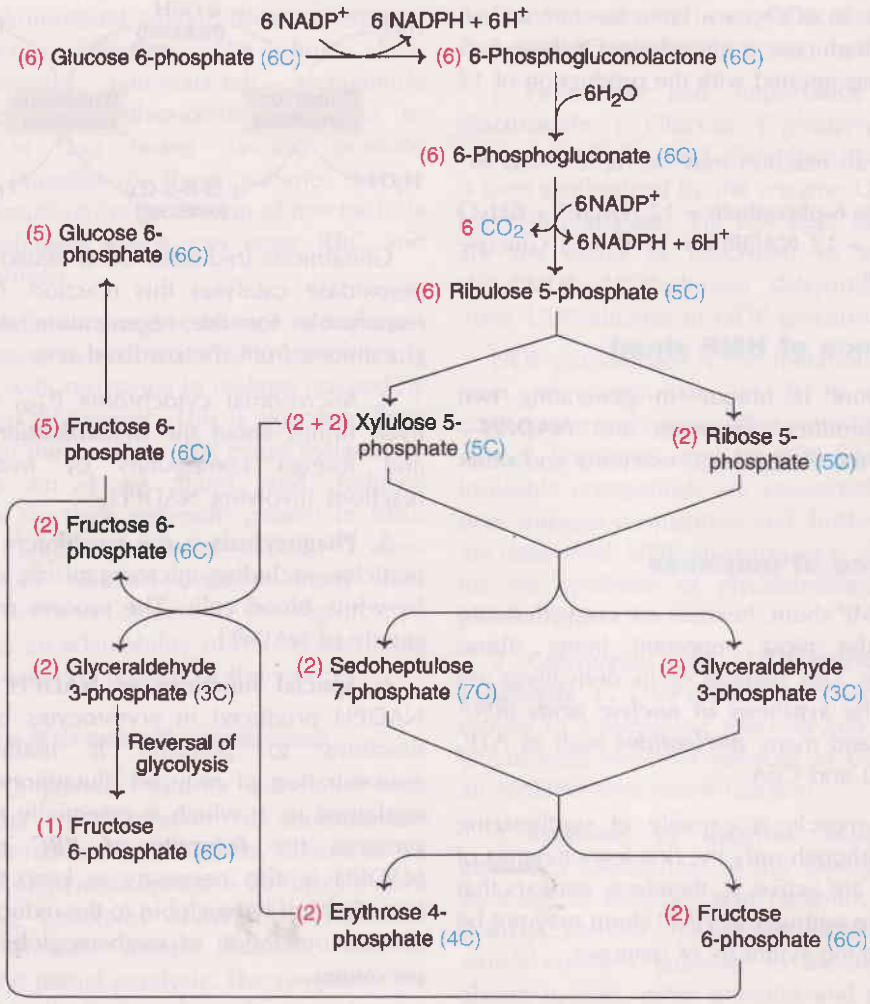


***Fig. 13.21** : Overview of hexose monophosphate shunt representing the number of molecules (prefix in red) and the number of carbon atoms (suffix in blue). Note that of the 6-molecules of glucose 6-phosphate that enter HMP shunt, one molecule is oxidized as 5-molecules are finally recovered.*

The enzyme ***transketolase*** catalyses the transfer of two carbon moiety from xylulose 5-phosphate to ribose 5-phosphate to give a 3-carbon glyceraldehyde 3-phosphate and a 7-carbon ***sedoheptulose 7-phosphate.*** Transketolase is dependent on the coenzyme ***thiamine pyrophosphate (TPP)*** and Mg^{2+} ions. Transaldolase brings about the transfer of a 3-carbon fragment (active dihydroxyacetone) from sedoheptulose 7-phosphate to glyceraldehyde 3-phosphate to give fructose 6-phosphate and four carbon erythrose 4-phosphate. Transketolase acts on xylulose 5-phosphate and transfers a 2-carbon fragment (glyceraldehyde) from it to erythrose 4-phosphate to generate fructose 6-phosphate and glyceraldehyde 3-phosphate.

Fructose 6-phosphate and glyceraldehyde 3-phosphate can be further catabolized through glycolysis and citric acid cycle. Glucose may also be synthesized from these two compounds.

An overview of HMP shunt is given in ***Fig.13.21***. For the complete oxidation of glucose

6-phosphate to $6CO_2$, we have to start with 6 molecules of glucose 6-phosphate. Of these 6, 5 moles are regenerated with the production of 12 NADPH.

The overall reaction may be represented as

6 Glucose 6-phosphate + 12 $NADP^+$ + $6H_2O$ $\longrightarrow$ $6CO_2$ + 12 NADPH + $12H^+$ + 5 Glucose 6-phosphate.

Significance of HMP shunt

HMP shunt is unique in generating two important products—***pentoses*** and ***NADPH***—needed for the biosynthetic reactions and other functions.

Importance of pentoses

In the HMP shunt, hexoses are converted into pentoses, the most important being ribose 5-phosphate. This pentose or its derivatives are useful for the ***synthesis of nucleic acids*** (RNA and DNA) and many ***nucleotides*** such as ATP, NAD^+, FAD and CoA.

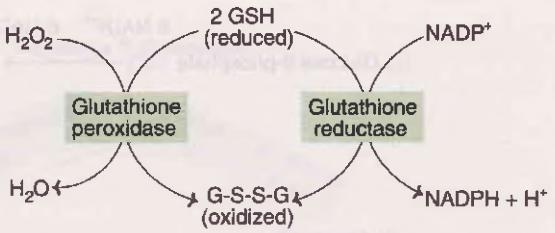
Skeletal muscle is capable of synthesizing pentoses, although only the first few enzymes of HMP shunt are active. It, therefore, appears that the complete pathway of HMP shunt may not be required for the synthesis of pentoses.

Importance of NADPH

1. NADPH is required for the reductive ***biosynthesis of fatty acids and steroids***, hence HMP shunt is more active in the tissues concerned with lipogenesis, e.g. adipose tissue, liver etc.

2. NADPH is used in the synthesis of certain amino acids involving the enzyme ***glutamate dehydrogenase.***

3. There is a continuous production of H_2O_2 in the living cells which can chemically damage unsaturated lipids, proteins and DNA. This is, however, prevented to a large extent through ***antioxidant reactions*** involving NADPH. Glutathione mediated reduction of H_2O_2 is given in the next column.



Glutathione (reduced, GSH) detoxifies H_2O_2, peroxidase catalyses this reaction. NADPH is responsible for the regeneration of reduced glutathione from the oxidized one.

4. Microsomal cytochrome P_{450} system (in liver) brings about the ***detoxification of drugs*** and foreign compounds by hydroxylation reactions involving NADPH.

5. **Phagocytosis** is the engulfment of foreign particles, including microorganisms, carried out by white blood cells. The process requires the supply of NADPH.

6. **Special functions of NADPH in RBC :** NADPH produced in erythrocytes has special functions to perform. It maintains the concentration of reduced glutathione (reaction explained in 3) which is essentially required to preserve the ***integrity of RBC membrane.*** NADPH is also necessary to keep the ferrous iron (Fe^{2+}) of hemoglobin in the reduced state so that accumulation of methemoglobin (Fe^{3+}) is prevented.

Glucose 6-phosphate dehydrogenase deficiency

G6PD deficiency is an inherited sex-linked trait. Although the deficiency occurs in all the cells of the affected individuals, it is more severe in RBC.

HMP shunt is the only means of providing NADPH in the erythrocytes. Decreased activity of G6PD impairs the synthesis of NADPH in RBC. This results in the accumulation of methemoglobin and peroxides in erythrocytes leading to ***hemolysis.***

Clinical manifestations in G6PD deficiency : Most of the patients with G6PD deficiency do not usually exhibit clinical symptoms. Some of them, however, develop ***hemolytic anemia*** if

they are administered oxidant drugs or exposed to a severe infection. The drugs such as primaquine (antimalarial), acetanilide (antipyretic), sulfamethoxazole (antibiotic) or ingestion of fava beans (favism) produce hemolytic jaundice in these patients. Severe infection results in the generation of free radicals (in macrophages) which can enter RBC and cause hemolysis.

G6PD deficiency and resistance to malaria : It is interesting to note that G6PD deficiency is associated with resistance to malaria (caused by *Plasmodium falciparum*). This is explained from the fact that the parasites that cause malaria are dependent on HMP shunt and reduced glutathione for their optimum growth in RBC. Therefore, G6PD deficiency—which is seen frequently in Africans—protects them from malaria, a common disease in this region. It is regarded as an adaptability of the people living in malaria-infected regions of the world.

Wernicke-Korsakoff syndrome

This is a genetic disorder associated with HMP shunt. An alteration in ***transketolase*** activity that reduces its affinity (by tenfold or so) with thiamine pyrophosphate is the biochemical lesion. The symptoms of Wernicke-Korsakoff syndrome include mental disorder, loss of memory and partial paralysis. The symptoms are manifested in alcoholics whose diets are vitamin-deficient.

In pernicious anemia, erythrocyte transketolase activity is found to increase.

URONIC ACID PATHWAY

This is an alternative oxidative pathway for glucose and is also known as ***glucuronic acid pathway*** (***Fig.13.22***). It is concerned with the synthesis of glucuronic acid, pentoses and vitamin, ascorbic acid (except in primates and guinea pigs). Dietary xylulose enters uronic acid pathway through which it can participate in other metabolisms. In most of the pathways of carbohydrate metabolism, phosphate esters participate, whereas, in uronic acid pathway, the free sugars or sugar acids are involved.

1. **Formation and importance of UDP-glucuronate :** Glucose 6-phosphate is first converted to glucose 1-phosphate. UDP-glucose is then synthesized by the enzyme UDP-glucose pyrophosphorylase. Till this step, the reactions are the same as described in glycogenesis (***Fig.13.14***). UDP-glucose dehydrogenase oxidizes UDP-glucose to UDP-glucuronate.

UDP-glucuronate is the metabolically active form of glucuronate which is utilized for conjugation with many substances like bilirubin, steroid hormones and certain drugs. Several insoluble compounds are converted to soluble ones through conjugation and, further, the drugs are detoxified. UDP-glucuronate is also required for the synthesis of glycosaminoglycans and proteoglycans.

2. **Conversion of UDP-glucuronate to L-gulonate :** UDP-glucuronate loses its UDP moiety in a hydrolytic reaction and releases D-glucuronate which is reduced to L-gulonate by an NADPH-dependent reaction.

3. **Synthesis of ascorbic acid in some animals :** L-Gulonate is the precursor for the synthesis of ascorbic acid (vitamin C) in many animals. The enzyme ***L-gulonolactone oxidase***—which converts gulonate to ascorbic acid—is absent in man, other primates and guinea pigs. Therefore, vitamin C has to be supplemented in the diet for these animals.

4. **Oxidation of L-gulonate :** L-Gulonate is oxidized to 3-ketogulonate and then decarboxylated to a pentose, L-xylulose. L-Xylulose is converted to D-xylulose via xylitol by a reduction (NADPH-dependent) followed by an oxidation (NAD^+-dependent) reaction. This is necessary since the D-xylulose (and not L-form)—after getting phosphorylated—can enter the hexose monophosphate shunt, for further metabolism.

Effect of drugs on uronic acid pathway

Administration of drugs (barbital, chlorobutanol etc.) significantly increases the uronic

acid pathway to achieve more synthesis of glucuronate from glucose. Certain drugs (aminopyrine, antipyrine) were found to enhance the synthesis of ascorbic acid in rats.

Essential pentosuria

This is a rare genetic disorder related to the deficiency of an NADP-dependent enzyme ***xylitol dehydrogenase.*** Due to this enzyme defect, L-xylulose cannot be converted to xylitol. The affected individuals excrete large amounts of L-xylulose in urine. Essential pentosuria is asymptomatic and the individuals suffer from no ill-effects. It has been reported that the administration of drugs aminopyrine and antipyrine increases the excretion of L-xylulose in pentosuric patients.

METABOLISM OF GALACTOSE

The disaccharide lactose, present in milk and milk products, is the principal dietary source of galactose. Lactase (β-galactosidase) of intestinal mucosal cells hydrolyses lactose to galactose and glucose. Galactose is also produced within the cells from the lysosomal degradation of glycoproteins and glycolipids. As is the case for fructose, galactose entry into the cells is not dependent on insulin.

The specific enzyme, namely galactokinase, phosphorylates galactose to galactose 1-phosphate. This reacts with UDP-glucose in an exchange reaction to form UDP-galactose in presence of the enzyme galactose 1-phosphate uridyltransferase (***Fig.13.23***). UDP-galactose is an active donor of galactose for many synthetic reactions involving the formation of compounds like lactose, glycosaminoglycans, glycoproteins, cerebrosides and

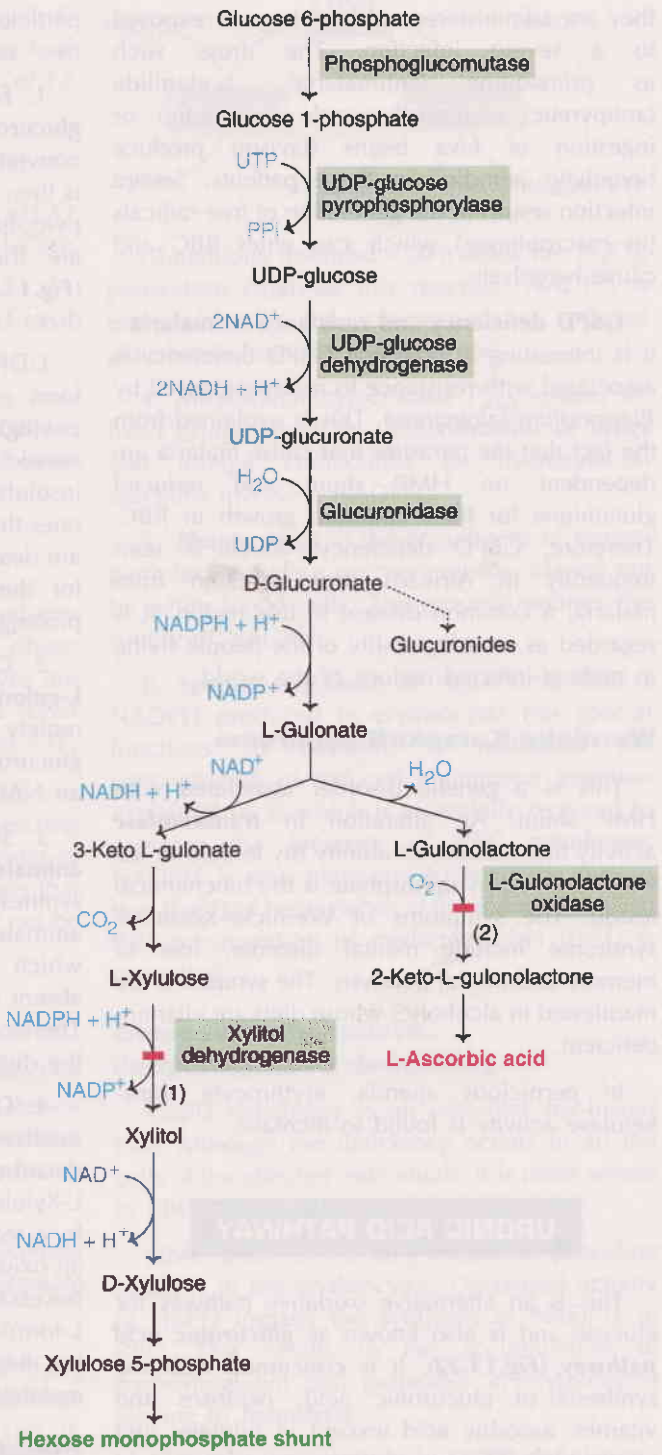


***Fig. 13.22** : Uronic acid pathway [UDP—uridine diphosphate];*
(1) Block in essential pentosuria;
(2) Enzyme absent in primates (including man) and guinea pigs].

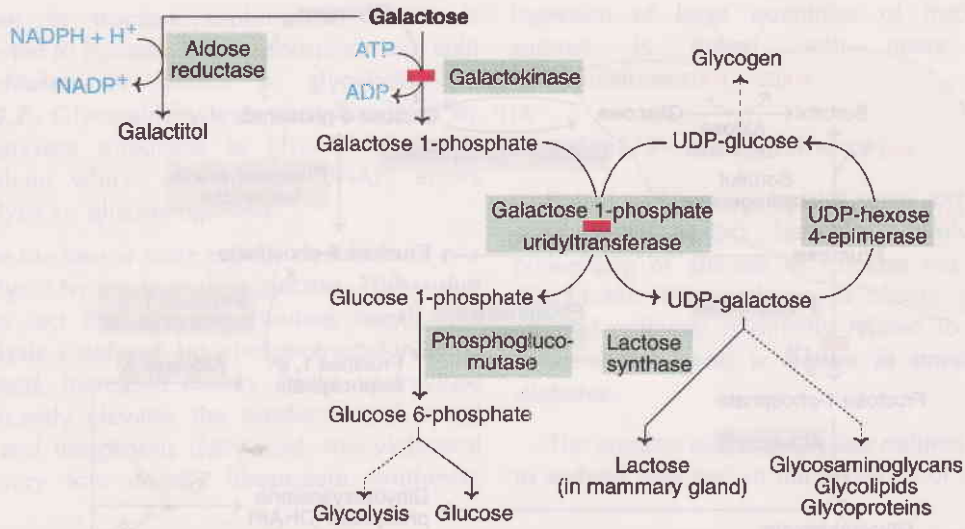


Fig. 13.23 : Metabolism of galactose (defects in galactosemia are indicated by red blocks).

glycolipids. UDP-galactose can be converted to UDP-glucose by UDP hexose 4-epimerase. In this way, galactose can enter the metabolic pathways of glucose. It may be noted that ***galactose is not an essential nutrient*** since UDP-glucose can be converted to UDP-galactose by the enzyme UDP-hexose 4-epimerase.

DISORDERS OF GALACTOSE METABOLISM

Classical galactosemia

Galactosemia is due to the deficiency of the enzyme ***galactose 1-phosphate uridyltransferase.*** It is a rare congenital disease in infants, inherited as an autosomal recessive disorder. The salient features of galactosemia are listed.

1. Galactose metabolism is impaired leading to ***increased galactose levels*** in circulation (***galactosemia***) and ***urine*** (***galactosuria***).

2. The accumulated galactose is diverted for the production of ***galactitol*** (***dulcitol***) by the enzyme aldose reductase (the same enzyme that converts glucose to sorbitol). Aldose reductase is present in lens, nervous tissue, seminal vesicles etc. The conversion of galactose to galactitol is insignificant in routine galactose metabolism. However, with increased levels of galactose (galactosemia), this pathway assumes significance. Galactitol (like sorbitol, discussed later) has been implicated in the development of ***cataract.***

3. The accumulation of galactose 1-phosphate and galactitol in various tissues like liver, nervous tissue, lens and kidney leads to impairment in their function.

4. The accumulation of galactose 1-phosphate in liver results in the depletion of inorganic phosphate (sequestering of phosphate) for other metabolic functions.

5. The clinical symptoms of galactosemia are—loss of weight (in infants) hepato-splenomegaly, jaundice, mental retardation etc. In severe cases, cataract, amino aciduria and albuminuria are also observed.

Diagnosis : Early detection of galactosemia is possible (biochemical diagnosis) by measuring the activity of galactose 1-phosphate uridyl-transferase in erythrocytes.

Treatment : The therapy includes the supply of diet deprived of galactose and lactose.

Galactokinase deficiency : The defect in the enzyme ***galactokinase,*** responsible for

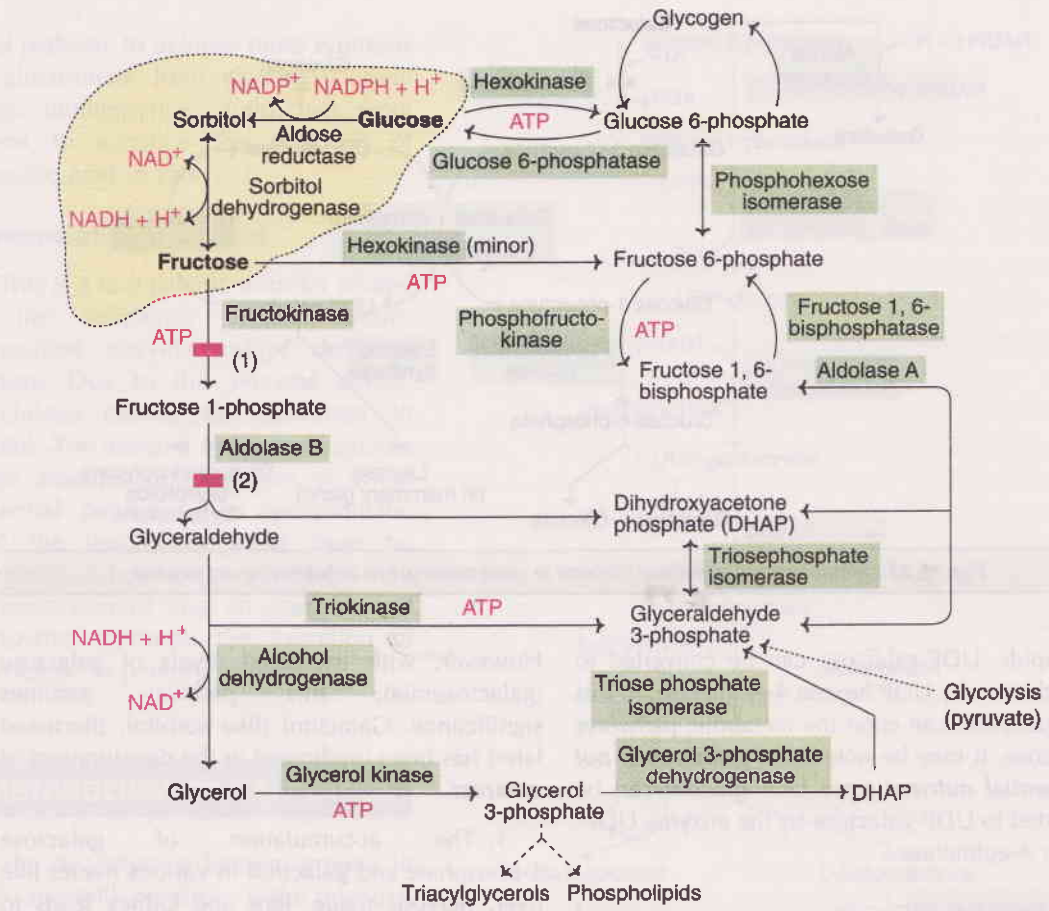


***Fig. 13.24** : Metabolism of fructose (Metabolic defects 1–Fructosuria; 2–Fructose intolerance).*
***(Note** : The shaded part represents the polyol pathway)*

phosphorylation of galactose, will also result in galactosemia and galactosuria. Here again galactose is shunted to the formation of galactitol. Generally, galactokinase-deficient individuals do not develop hepatic and renal complications. Development of cataract occurs at a very early age, sometimes within an year after birth. The treatment is the removal of galactose and lactose from the diet.

METABOLISM OF FRUCTOSE

The major dietary source of fructose is the disaccharide sucrose (cane sugar), containing equimolar quantities of fructose and glucose. It is also found in free form in honey and many fruits. In the body, entry of fructose into the cells is not controlled by the hormone insulin. This is in contrast to glucose which is regulated for its entry into majority of the tissues.

Fructose is mostly phosphorylated by fructokinase to fructose 1-phosphate. Fructokinase has been identified in liver, kidney and intestine. Hexokinase, which phosphorylates various monosaccharides, can also act on fructose to produce fructose 6-phosphate. However, hexokinase has low affinity (high K_m) for fructose, hence this is a minor pathway.

Fructose 1-phosphate is cleaved to glyceraldehyde and dihydroxyacetone phosphate (DHAP) by ***aldolase B (Fig.13.24)***. This is in

contrast to fructose 6-phosphate which is converted to fructose 1, 6-bisphosphate and split by ***aldolase A*** (details in glycolysis—***See Fig.13.2***). Glyceraldehyde is phosphorylated by the enzyme triokinase to glyceraldehyde 3-phosphate which, along with DHAP, enters glycolysis or gluconeogenesis.

The fructose is more rapidly metabolized (via glycolysis) by the liver than glucose. This is due to the fact that the rate limiting reaction in glycolysis catalysed by phosphofructokinase is bypassed. Increased dietary intake of fructose significantly elevates the production of acetyl CoA and lipogenesis (fatty acid, triacylglycerol and very low density lipoprotein synthesis). Ingestion of large quantities of fructose or sucrose is linked with many health complications.

Sorbitol / Polyol pathway

Polyol pathway (so termed since sorbitol is a polyhydroxy sugar) basically involves the conversion of glucose to fructose via sorbitol (***Fig.13.24***). This pathway is absent in liver. Sorbitol pathway is directly related to glucose concentration, and is ***higher in uncontrolled diabetes.***

The enzyme aldose reductase reduces glucose to sorbitol (glucitol) in the presence of NADPH.

BIOMEDICAL / CLINICAL CONCEPTS

- *A continuous presence of glucose—supplied through diet or synthesized in the body (gluconeogenesis)—is essential for the survival of the organism. Alcohol intoxication reduces gluconeogenesis.*
- *Human brain consumes about 120 g of glucose per day out of the 160 g needed by the body. Insufficient supply of glucose to brain may lead to coma and death.*
- *Liver glycogen serves as an immediate source for maintaining blood glucose levels, particularly between the meals. The glycogen stores in the liver get depleted after 12-18 hours of fasting.*
- *Muscle glycogen is primarily concerned with the supply of hexoses that undergo glycolysis to provide energy during muscle contraction.*
- *Glycogen storage diseases—characterized by deposition of normal or abnormal type of glycogen in one or more tissues—result in muscular weakness, or even death.*
- *The occurrence of HMP shunt (NADPH production) in the RBC is necessary to maintain the integrity of erythrocyte membrane and to prevent the accumulation of methemoglobin.*
- *Deficiency of glucose 6-phosphate dehydrogenase results in hemolysis of RBC, causing hemolytic anemia. The subjects of G6PD deficiency are, however, resistant to malaria.*
- *Uronic acid pathway is concerned with the production of glucuronic acid (involved in detoxification), pentoses and vitamin C. Man is incapable of synthesizing vitamin C due to the absence of a single enzyme—L-gulonolactone oxidase.*
- *The conversion of glucose to fructose is impaired in diabetes mellitus, causing accumulation of sorbitol. This compound has been implicated in the development of cataract, nephropathy, peripheral neuropathy etc.*
- *Severe cases of galactosemia are associated with the development of cataract, believed to be due to the accumulation of galactitol.*

Sorbitol is then oxidized to fructose by sorbitol dehydrogenase and NAD^+. Aldose reductase is absent in liver but found in many tissues like lens and retina of the eye, kidney, placenta, Schwann cells of peripheral nerves, erythrocytes and seminal vesicles. The enzyme sorbitol dehydrogenase is present in seminal vesicle, spleen and ovaries. Fructose is a preferred carbohydrate for energy needs of sperm cells due to the presence of sorbitol pathway.

Sorbitol pathway in diabetes mellitus

In uncontrolled diabetes (hyperglycemia), large amounts of glucose enter the cells which are not dependent on insulin. Significantly, the cells with increased intracellular glucose levels in diabetes (lens, retina, nerve cells, kidney etc.) possess high activity of aldose reductase and sufficient supply of NADPH. This results in a rapid and efficient conversion of glucose to sorbitol. The enzyme sorbitol dehydrogenase, however, is either low in activity or absent in these cells, hence sorbitol is not converted to fructose. Sorbitol cannot freely pass through the cell membrane, and accumulate in the cells where it is produced. Sorbitol—due to its hydrophilic nature—causes strong osmotic effects leading to swelling of the cells. Some of the ***pathological changes associated with diabetes*** (like cataract formation, peripheral neuropathy, nephropathy etc.) are ***believed to be due to the accumulation of sorbitol***, as explained above.

It is clearly known that in diabetic animals sorbitol content of lens, nerve, and glomerulus is elevated. This causes damage to tissues. It thus appears that majority of the complications associated with diabetes share a common pathogenesis as a consequence of polyol pathway. Certain inhibitors of aldose reductase can prevent the accumulation of sorbitol, and thus the associated complications. However, this approach is still at the experimental stage.

Defects in fructose metabolism

1. **Essential fructosuria** : Due to the deficiency of the enzyme hepatic ***fructokinase***, fructose is not converted to fructose 1-phosphate. This is an asymptomatic condition with excretion of fructose in urine. Treatment involves the restriction of dietary fructose.

2. **Hereditary fructose intolerance** : This is due to the absence of the enzyme ***aldolase B.*** Hereditary fructose intolerance causes intracellular accumulation of fructose 1-phosphate, severe hypoglycemia, vomiting, hepatic failure and jaundice. Fructose 1-phosphate allosterically inhibits liver phosphorylase and blocks glycogenolysis leading to hypoglycemia. Early detection and intake of diet free from fructose and sucrose, are advised to overcome fructose intolerance.

3. **Consumption of high fructose** : Fructose is rapidly converted to fructose 1-phosphate by fructokinase. The activity of the enzyme aldolase B is relatively less, and, due to this, fructose 1-phosphate accumulates in the cell. This leads to the depletion of intracellular inorganic phosphate (Pi) levels. The phenomenon of binding of Pi to the organic molecules (like fructose here)—that leads to the less availability of Pi for the essential metabolic functions—is known as ***sequestering of phosphate.*** Due to the decreased availability of Pi, which happens in overconsumption of fructose, the liver metabolism is adversely affected. This includes the lowered synthesis of ATP from ADP and Pi. High consumption of fructose over a long period is associated with increased uric acid in blood leading to gout. This is due to the excessive breakdown of ADP and AMP (accumulated due to lack of Pi) to uric acid.

METABOLISM OF AMINO SUGARS

When a hydroxyl group of a sugar is replaced by an amino group, the resultant compound is an amino sugar.

The important amino sugars are ***glucosamine***, ***galactosamine***, ***mannosamine***, ***sialic acid*** etc. They are essential components of glycosaminoglycans, glycolipids (gangliosides) and glycoproteins. They are also found in some

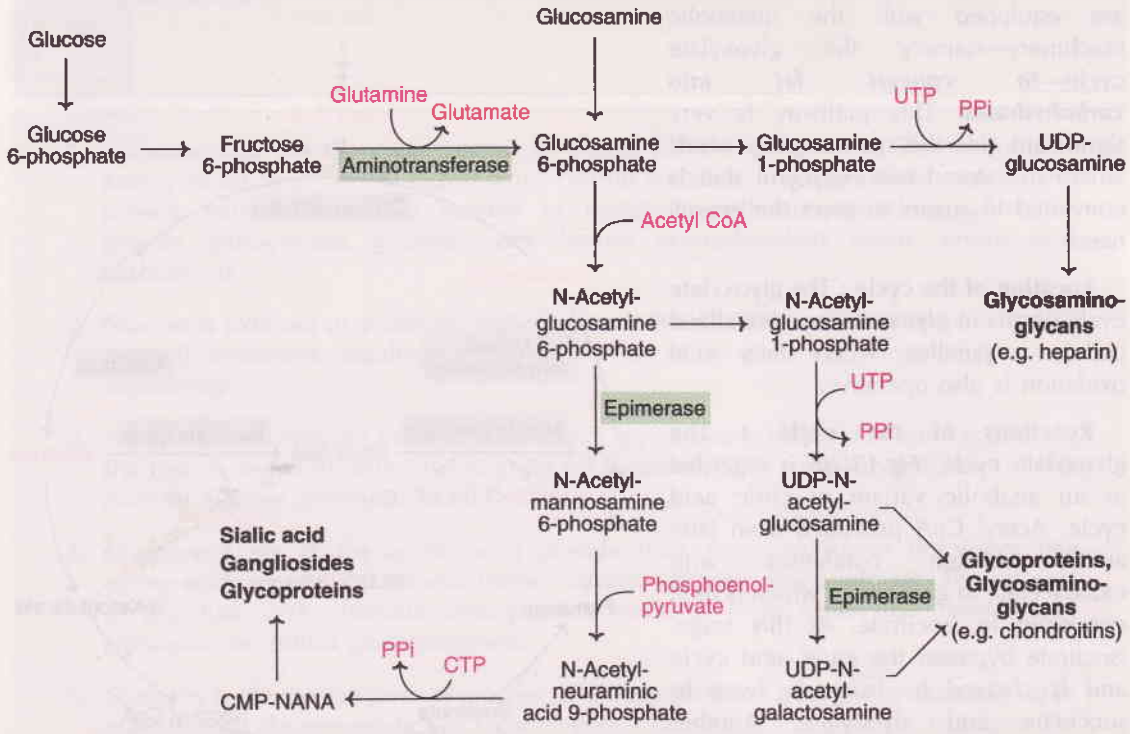


Fig. 13.25 : Summary of the synthesis of amino sugars.

oligosaccharides and certain antibiotics. It is estimated that about 20% of the glucose is utilized for the synthesis of amino sugars, which mostly occurs in the connective tissue.

The outline of the pathway for the synthesis of amino sugars is given in ***Fig.13.25***. Fructose 6-phosphate is the major precursor for glucosamine, N-acetylgalactosamine and N-acetylneuraminic acid (NANA). The utilization of the amino sugars for the formation of glycosaminolgycans, glycoproteins and gangliosides is also indicated in this figure.

Mucopolysaccharidoses

The ***lysosomal storage diseases*** caused by defects in the glycosaminoglycans (GAGs) are known as mucopolysaccharidoses. The diseases name is so given since the original name for GAGs was mucopolysaccharides (MPS). These are more than a dozen rare genetic diseases. Mucopolysaccharidoses are characterized by the accumulation of GAGs in various tissues that may result in skeletal deformities, and mental retardation. Mucopolysaccharidoses are important for elucidating the role of lysosomes in health and disease.

GAGs are degraded by a sequential action of lysosomal acid hydrolases e.g. exoglycosidases, sulfatases. Some important mucopolysaccharidoses and the enzyme defects are listed.

Hurler's syndrome (MPS I)–L-Iduronidase

Hunter's syndrome (MPS II)–Iduronate sulfatase

Sanfilippo syndrome (MPS III)–Four differt enzymes (e.g. heparan sulfamidase)

Sly syndrome (MPS VII)–β-Glucuronidase.

GLYOXYLATE CYCLE

The animals, including man, cannot carry out the net synthesis of carbohydrate from fat. However, the ***plants*** and many ***microorganisms***

are equipped with the metabolic machinery—namely the glyoxylate cycle—***to convert fat into carbohydrates.*** This pathway is very significant in the germinating seeds where the stored triacylglycerol (fat) is converted to sugars to meet the energy needs.

Location of the cycle : The glyoxylate cycle occurs in ***glyoxysomes***, specialized cellular organelles, where fatty acid oxidation is also operative.

Reactions of the cycle : The glyoxylate cycle (***Fig.13.26***) is regarded as an anabolic variant of citric acid cycle. Acetyl CoA produced from fatty acid oxidation condenses with oxaloacetate to give citrate which is then converted to isocitrate. At this stage, isocitrate bypasses the citric acid cycle and is cleaved by isocitrate lyase to succinate and glyoxylate. Another molecule of acetyl CoA is now utilized to combine with glyoxylate to form malate. This reaction is catalysed by malate synthase and the malate so formed enters citric acid cycle.

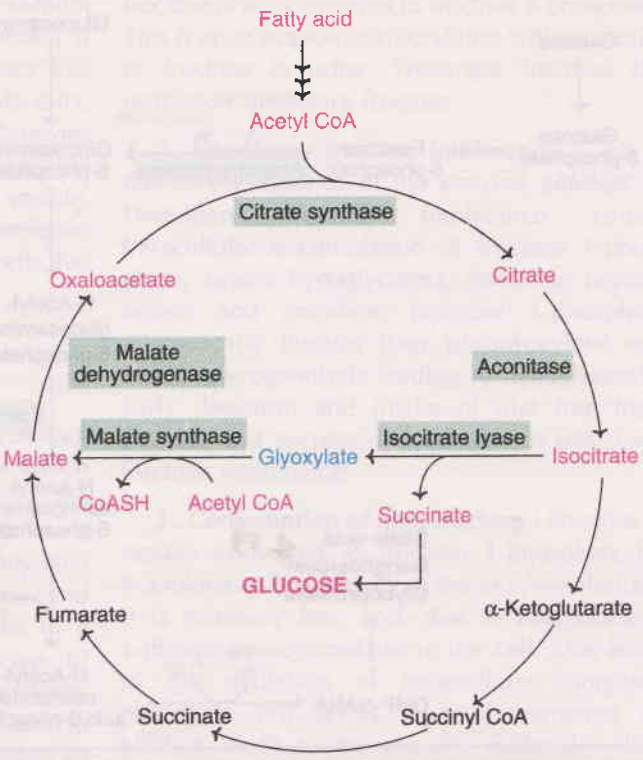


Fig. 13.26 : The glyoxylate cycle (enzymes represented in green shade).

The glyoxylate cycle is a cyclic pathway that results in the conversion of two 2-carbon fragments of acetyl CoA to 4-carbon compound, succinate. The succinate is converted to oxaloacetate and then to glucose involving the reactions of gluconeogenesis.

SUMMARY

1. *Carbohydrates are the major source of energy for the living cells. Glucose (normal fasting blood level 70-100 mg/dl) is the central molecule in carbohydrate metabolism, actively participating in a number of metabolic pathways—glycolysis, gluconeogenesis, glycogenesis, glycogenolysis, hexose monophosphate shunt, uronic acid pathway etc.*

2. *Glucose is oxidized in glycolysis, either in anaerobic (2 ATP formed) or aerobic (8 ATP formed) conditions, resulting in the formation of 2 moles of lactate or pyruvate, respectively.*

3. *Acetyl CoA is produced from pyruvate which is completely oxidized in citric acid cycle, the final common oxidative pathway for all foodstuffs. The complete oxidation of one mole of glucose generates 38 ATP.*

4. *Gluconeogenesis is the synthesis of glucose from noncarbohydrate precursors like amino acids (except leucine and lysine), lactate, glycerol, propionate etc. The reversal of glycolysis with alternate arrangements made at three irreversible reactions of glycolysis constitutes gluconeogenesis.*

5. *Glycogen is the storage form of glucose. The degradation of glycogen (glycogenolysis) in muscle meets the immediate fuel requirements, whereas the liver glycogen maintains the blood glucose level. Enzyme defects in synthesis or degradation of glycogen lead to storage disorders. von Gierke's disease (Type I) is due to the defect in the enzyme glucose 6-phosphatase.*

6. *Hexose monophosphate shunt (HMP shunt) is the direct oxidative pathway of glucose. HMP shunt assumes significance since it generates NADPH and pentoses, respectively required for the synthesis of lipids and nucleic acids.*

7. *Glucuronate—involved in the conjugation of bilirubin, steroid hormones and detoxification of drugs—is synthesized in uronic acid pathway. Due to a single enzyme defect (gulonolactone oxidase) in this pathway, man cannot synthesize ascorbic acid (vitamin C) whereas some animals can.*

8. *Galactosemia is mostly due to the defect in the enzyme galactose 1-phosphate uridyltransferase. This results in the diversion of galactose to produce galactitol which has been implicated in the development of cataract.*

9. *Glucose can be converted to fructose via sorbitol pathway. In prolonged hyperglycemia (uncontrolled diabetes), sorbitol accumulates in the tissues, resulting in cataract, nephropathy, peripheral neuropathy etc.*

10. *Amino sugars (glucosamine, galactosamine, mannosamine etc.), synthesized from fructose 6-phosphate are essential components of glycosaminoglycans, glycolipids and glycoproteins.*

SELF-ASSESSMENT EXERCISES

I. Essay questions

1. Describe briefly the metabolism of glucose 6-phosphate.
2. Give an account of glycogen metabolism.
3. Justify that citric acid cycle is the final common metabolic pathway for the oxidation of foodstuffs.
4. Discuss the synthesis of glucose from non-carbohydrate sources.
5. Describe the hexose monophosphate shunt and add a note on its significance.

II. Short notes

(a) Glycogenolysis, (b) UDPG, (c) Galactosemia, (d) Cori cycle, (e) 2, 3- BPG, (f) Glycogen storage diseases, (g) Essential fructosuria, (h) Conversion of pyruvate to acetyl CoA, (i) Energetics of TCA cycle, (j) TPP in carbohydrate metabolism.

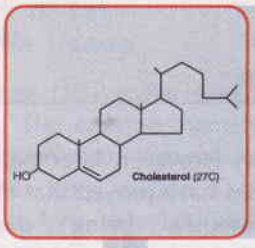
III. Fill in the blanks

1. Name the five vitamins required by pyruvate dehydrogenase or α-ketoglutarate dehydrogenase complex _____.
2. Muscle glycogen does not directly contribute to blood glucose due to absence of the enyme _____.
3. Ascorbic acid is not synthesized in man due to lack of the enzyme _____.
4. The compound implicated in the development of cataract in diabetic patients is _____.
5. Galactosemia is mostly due to the deficiency of the enzyme _____.
6. The two amino acids that are never glucogenic are _____ and _____.
7. Substrate level phosphorylation in citric acid cycle is catalysed by the enzyme _____.
8. The metabolic pathway concerned with the conversion of L-xylulose to D-xylulose is _____.
9. The name of the protein that has been identified to serve as a primer for glycogen synthesis is _____.
10. The metabolite among the citric acid cycle intermediates performing a catalytic role _____.

IV. Multiple choice questions

11. One of the following enzymes in glycolysis catalyses an irreversible reaction.
    (a) Hexokinase (b) Phosphofructokinase (c) Pyruvate kinase (d) All of them.
12. Synthesis of 2, 3-bisphosphoglycerate occurs in the tissue namely.
    (a) Liver (b) Kidney (c) Erythrocytes (d) Brain.
13. The hormone that lowers cAMP concentration in liver cells is
    (a) Glucagon (b) Insulin (c) Epinephrine (d) Thyroxine.
14. The number of ATP produced when a molecule of acetyl CoA is oxidized through citric acid cycle
    (a) 12 (b) 24 (c) 38 (d) 15.
15. The connecting link between HMP shunt and lipid synthesis is
    (a) Ribose (b) NADPH (c) Sedoheptulose 7-phosphate (d) NADH.

14 Metabolism of Lipids



Cholesterol, the most feared among lipids, speaks :

"Consumed through diet and produced in the body;
Participate in innumerable cellular functions;
Implicated in several health complications;
And blamed I am, for no fault of mine!"

Lipids are indispensable for cell structure and function. Due to their hydrophobic and non-polar nature, lipids differ from rest of the body compounds and are unique in their action.

Triacylglycerols —the body fuel reserve

Lipids constitute about 15-20% of the body weight in humans. Triacylglycerols ***(formerly triglycerides)*** are the most abundant lipids comprising 85-90% of body lipids. Most of the triacylglycerols (TG; also called ***neutral fat*** or depot fat) are stored in the ***adipose tissue*** and serve as energy reserve of the body. This is in contrast to carbohydrates and proteins which cannot be stored to a significant extent for energy purposes. Fat also acts as an insulating material for maintaining the body temperature of animals.

Why should fat be the fuel reserve of the body?

Triacylglycerols are the most predominant storage form of energy. There are two main reasons for fat being the fuel reserve of the body

1. Triacylglycerols (TG) are highly ***concentrated form of energy***, yielding ***9 Cal/g***, in contrast to carbohydrates and proteins that produce only 4 Cal/g. This is because fatty acids found in TG are in the reduced form.

2. The triacylglycerols are non-polar and hydrophobic in nature, hence ***stored in*** pure form without any association with water ***(anhydrous form)***. On the other hand, glycogen and proteins are polar. One gram of glycogen combines with 2 g of water for storage.

For the two reasons stated above, one gram of fat stored in the body yields nearly six times as much energy as one gram of (hydrated) glycogen. In a healthy adult individual (weighing 70 kg), about 10-11 kg of fat is stored (mostly in adipose tissue) which corresponds to a fuel reserve of 100,000 Cals. If this much of energy were to be stored as glycogen (instead of fat), then the weight of the person would increase by at least 55 kg! This explains why fat has been chosen as a fuel reserve during evolution.

Long chain fatty acids (of fat) are the ideal storage fuel reserves of the body. Fats can support the body's energy needs for long periods of food deprivation. In extreme cases, humans can fast and survive for 60–90 days, and the obese persons can survive even longer (6 months to one year!) without food.

Hibernating animals provide good example for utilizing fat reserve as fuel. For instance, bears go on hibernation for about 7 months and, during this entire period, the energy is derived from the degradation of fat stores. The ruby-throated humming birds fly non-stop between New England and West Indies (2,400 km!) at a speed of 40 km/hr for 60 hours! This is possible only due to the stored fat.

Other important body lipids

Phospholipids, glycolipids and cholesterol are major components of cell membranes. Cholesterol is also a precursor for bile acids and steroid hormones. Arachidonic acid—an unsaturated fatty acid—is the substrate for the synthesis of certain intercellular regulators—prostaglandins, thromboxanes, prostacyclins etc.

Transport of lipids

The insoluble lipids are solubilized in association with proteins to form ***lipoproteins*** in which form lipids are transported in the blood stream. Free lipids are undetectable in blood.

Chylomicrons, very low density lipoproteins (VLDL), low density lipoproteins (LDL), high density lipoproteins (HDL) and albumin-free fatty acids are the different lipoprotein complexes that transport lipids in the blood stream. Details of plasma lipoproteins and their metabolism are discussed later.

Plasma lipids

The various fractions of lipids in the plasma can be estimated by different methods after extracting them with lipid solvents. The plasma levels of lipids (***Table 14.1***) are often useful for assessing the health of the individuals.

Dynamic state of body lipids

It was earlier thought that the lipids are inert storage compounds and are less significant metabolically. However, later experiments with isotope studies have proved that the body lipids are continuously being degraded and resynthesized. As already stated, fat stored in the adipose tissue is the fuel reserve of the body. This is in a dynamic state.

Table 14.1 The plasma concentration of lipids (lipid profile) in humans

Lipid fraction	*Reference values (mg/dl)*
Total lipid	400-600
Total cholesterol	150–200
LDL-cholesterol	80–150
HDL-cholesterol	30–60
VLDL-cholesterol	20–40
Triglycerides	75–150
Phospholipids	150–200
Free fatty acids	5–15

The triacylglycerols transported from intestine (as chylomicrons) and liver (as VLDL) are stored in the adipose tissue. Besides, they are also utilized by muscle, liver, heart etc., as per the needs of the body. An overview of fat metabolism is depicted in ***Fig.14.1***.

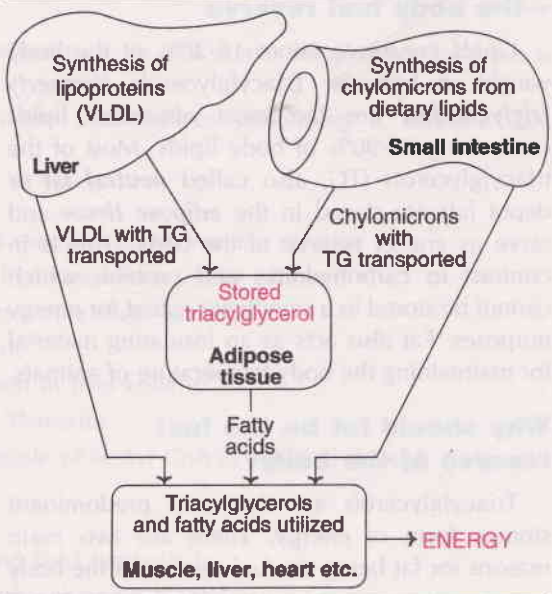


Fig. 14.1 : Overview of fat metabolism.

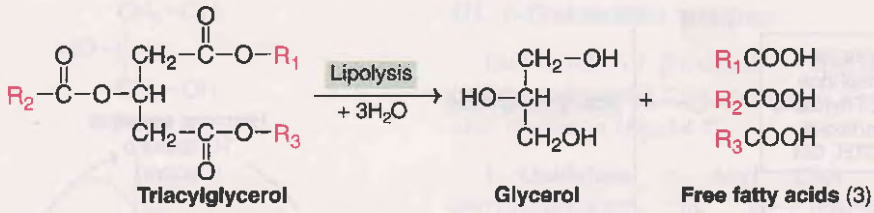


Fig. 14.2 : Complete hydrolysis (lipolysis) of triacylglycerol.

Mobilization of fat from adipose tissue

Triacylglycerol (TG) is the stored fat in the adipose tissue. The enzyme, namely ***hormone-sensitive triacylglycerol lipase,*** removes the fatty acid either from carbon 1 or 3 of the triacylglycerol to form diacylglycerol. The other two fatty acids of TG are cleaved by additional lipases specific for diacylglycerol and monoacylglycerol. The complete degradation of triacylglycerol to glycerol and free acids is known as ***lipolysis*** (***Fig.14.2***).

Regulation of hormone-sensitive TG-lipase

Hormone-sensitive TG-lipase is so named because its activity is mostly controlled by hormones. Lipase is present in an inactive form '*b*' and is activated (phosphorylated) by a cAMP dependent protein kinase to lipase '*a*'. Several hormones—such as ***epinephrine*** (most effective), norepinephrine, glucagon, thyroxine, ACTH etc.— enhance the activity of adenylate cyclase and, thus, increase lipolysis. On the other hand, insulin decreases cAMP levels and thereby inactivates lipase. Caffeine promotes lipolysis by increasing cAMP levels through its inhibition on phosphodiesterase activity. The control of cAMP mediated lipolysis is illustrated in ***Fig.14.3***.

As is evident from the foregoing discussion, increased levels of ***cAMP promote lipolysis***. In contrast, cAMP decreases fatty acid synthesis by inhibiting acetyl CoA carboxylase activity (discussed later). It should be therefore kept in mind that lipolysis and lipogenesis are not simultaneously operative (i.e. futile cycles are avoided. ***Refer p. 268***).

Fate of glycerol : The adipose tissue lacks the enzyme glycerol kinase, hence glycerol produced in lipolysis cannot be phosporylated here. It is transported to liver where it is activated to glycerol 3-phosphate. The latter may be used for the synthesis of triacylglycerols and phospholipids. Glycerol 3-phosphate may also enter glycolysis by getting converted to dihydroxyacetone phosphate (***Fig.14.4***).

Fate of free fatty acids : The fatty acids released by lipolysis in the adipocytes enter the circulation and are transported in a bound form to albumin. The free fatty acids enter various tissues and are utilized for the energy. About 95% of the energy obtained from fat comes from the oxidation of fatty acids. Certain tissues, however, cannot oxidize fatty acids, e.g. brain, erythrocytes.

FATTY ACID OXIDATION

The fatty acids in the body are mostly oxidized by ***β-oxidation***. β-Oxidation may be defined as the ***oxidation of fatty acids on the β-carbon atom***. This results in the sequential ***removal of*** a two carbon fragment, ***acetyl CoA***.

Fatty acid oxidation —stages and tissues

The β-oxidation of fatty acids involves three stages

I. Activation of fatty acids occurring in the cytosol;

II. Transport of fatty acids into mitochondria;

III. β-Oxidation proper in the mitochondrial matrix.

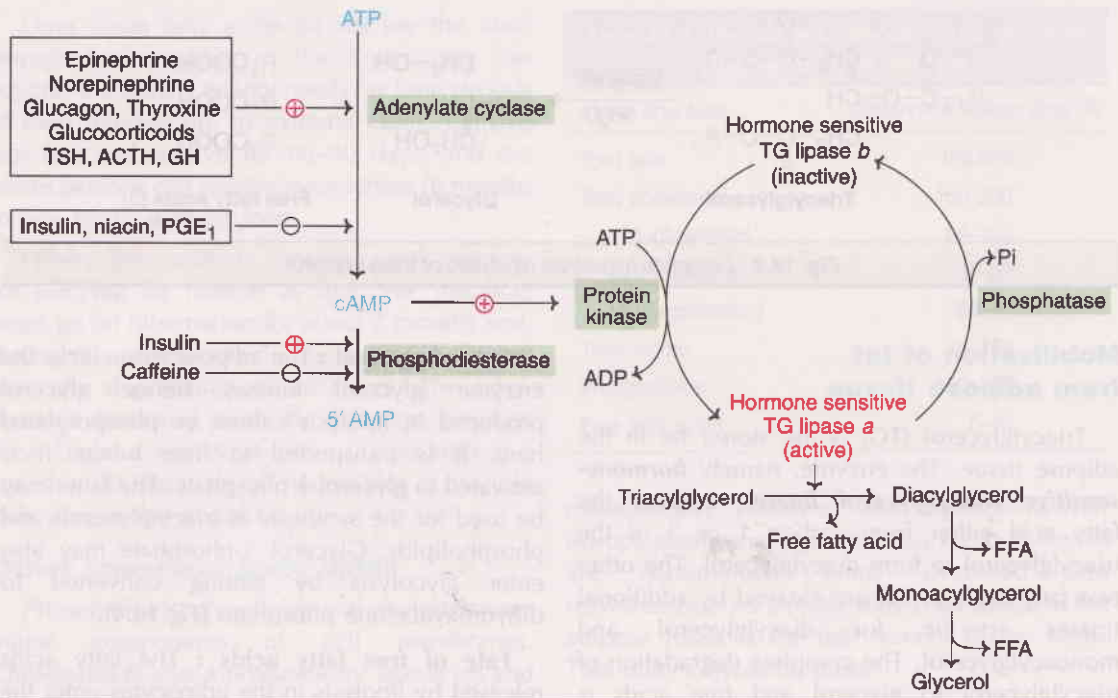


***Fig. 14.3** : Control of lipolysis in adipose tissue through cyclic AMP (⊕–Promoting and ⊖–Inhibiting effect; TSH–Thyroid stimulating hormone; ACTH–Adrenocorticotrophic hormone; GH–Growth hormone; PGE_1–Prostaglandin E_1; TG–Triacylglycerol; FFA–Free fatty acid).*

Fatty acids are oxidized by most of the tissues in the body. However, ***brain, erythrocytes and adrenal medulla cannot utilize fatty acids for energy requirement.***

I. Fatty acid activation

Fatty acids are activated to acyl CoA by ***thiokinases*** or ***acyl CoA synthetases.*** The reaction occurs in two steps and requires ATP, coenzyme A and Mg^{2+}. Fatty acid reacts with ATP to form acyladenylate which then combines with coenzyme A to produce acyl CoA (***Fig.14.5***). In the activation, ***two high energy phosphates are utilized,*** since ATP is converted to pyrophosphate (PPi). The enzyme ***inorganic pyrophosphatase*** hydrolyses PPi to phosphate (Pi). The immediate elimination of PPi makes this reaction totally irreversible.

Three different thiokinases, to activate long chain (10-20 carbon), medium chain (4-12 carbon) and short chain (< 4 carbon) fatty acids have been identified.

II. Transport of acyl CoA into mitochondria

The inner mitochondrial membrane is impermeable to fatty acids. A specialized ***carnitine carrier system*** (***carnitine shuttle***) operates to transport activated fatty acids from cytosol to the mitochondria. This occurs in four steps (***Fig.14.6***).

1. Acyl group of acyl CoA is transferred to ***carnitine*** (β-hydroxy γ-trimethyl aminobutyrate), catalysed by carnitine acyltransferase I (present on the outer surface of inner mitochondrial membrane).

2. The acyl-carnitine is transported across the membrane to mitochondrial matrix by a specific carrier protein.

3. Carnitine acyl transferase II (found on the inner surface of inner mitochondrial membrane) converts acyl-carnitine to acyl CoA.

4. The carnitine released returns to cytosol for reuse.

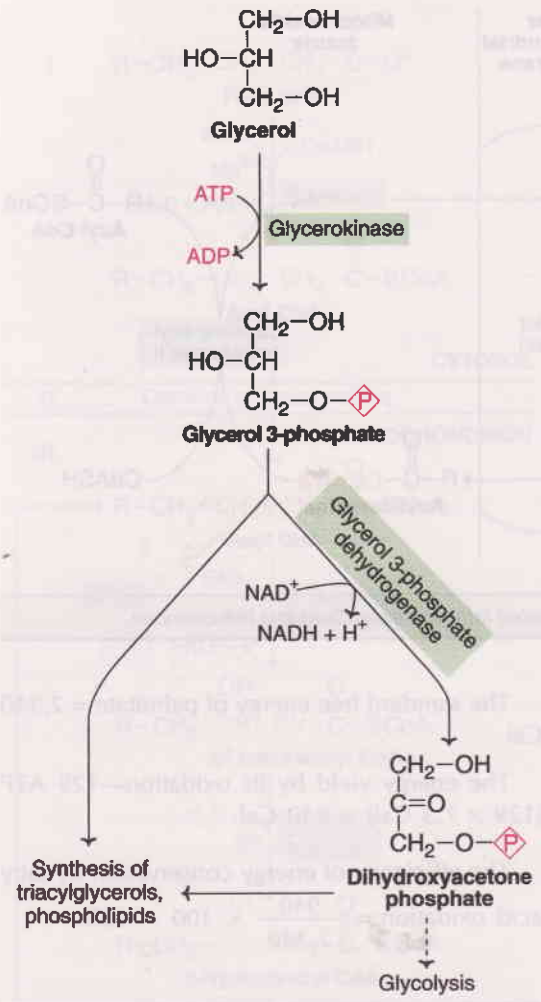


Fig. 14.4 : Fate of glycerol.

It should be noted that the coenzyme A used for activation is different from the one that finally combines with fatty acid in the mitochondria to form acyl CoA. Thus, the ***cell has two separate pools (cytosolic and mitochondrial) of coenzyme A.***

Inhibitor of carnitine shuttle : Carnitine acyl transferase I is inhibited by ***malonyl CoA***, a key metabolite involved in fatty acid synthesis that occurs in cytosol (details given later). In other words, while the fatty acid synthesis is in progress (reflected by high concentration of malonyl CoA), their oxidation does not occur, since carnitine shuttle is impaired.

III. β-Oxidation proper

Each cycle of β-oxidation, liberating a two carbon unit-acetyl CoA, occurs in a sequence of four reactions (***Fig.14.7***).

1. **Oxidation :** Acyl CoA undergoes dehydrogenation by an FAD-dependent flavoenzyme, acyl CoA dehydrogenase. A double bond is formed between α and β carbons (i.e., 2 and 3 carbons).

2. **Hydration :** Enoyl CoA hydratase brings about the hydration of the double bond to form β-hydroxyacyl CoA.

3. **Oxidation :** β-Hydroxyacyl CoA dehydrogenase catalyses the second oxidation and generates NADH. The product formed is β-ketoacyl CoA.

4. **Cleavage :** The final reaction in β-oxidation is the liberation of a 2 carbon fragment, acetyl CoA from acyl CoA. This occurs by a thiolytic cleavage catalysed by ***β-ketoacyl CoA thiolase*** (or simply thiolase).

The new acyl CoA, containing two carbons less than the original, reenters the β-oxidation cycle. The process continues till the fatty acid is completely oxidized.

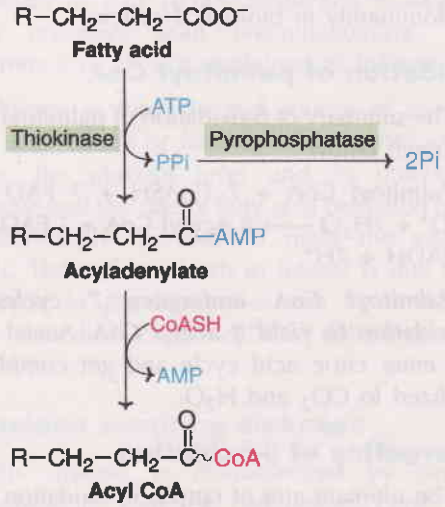


Fig. 14.5 : Activation of fatty acid to acyl CoA by the enzyme thiokinase.

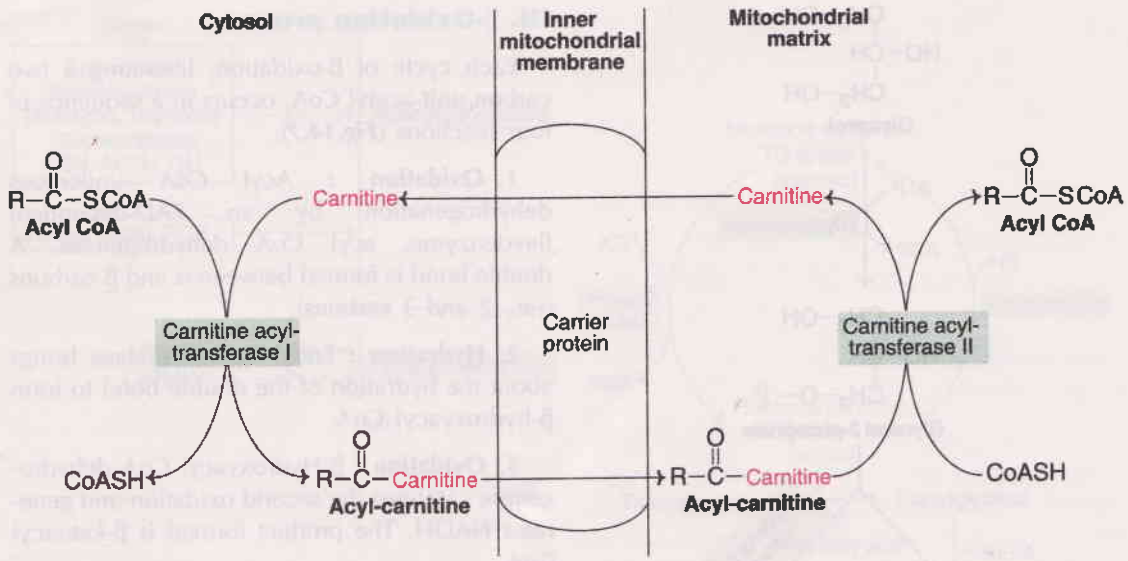


Fig. 14.6 : Carnitine shuttle for transport of activated fatty acid (acyl CoA) into mitochondria.

The overall reaction for each cycle of β-oxidation

C_n Acyl CoA + FAD + NAD^+ + H_2O + CoASH ⟶ $C_{(n-2)}$ Acyl CoA + Acetyl CoA + $FADH_2$ + NADH + H^+.

The scheme of fatty acid oxidation discussed above corresponds to saturated (no double bond) and even carbon fatty acids. This occurs most predominantly in biological system.

Oxidation of palmitoyl CoA

The summary of β-oxidation of palmitoyl CoA is shown below

Palmitoyl CoA + 7 CoASH + 7 FAD + 7 NAD^+ + $7H_2O$ ⟶ 8 Acetyl CoA + 7 $FADH_2$ + 7 NADH + $7H^+$

Palmitoyl CoA undergoes 7 cycles of β-oxidation to yield 8 acetyl CoA. Acetyl CoA can enter citric acid cycle and get completely oxidized to CO_2 and H_2O.

Energetics of β-oxidation

The ultimate aim of fatty acid oxidation is to generate energy. The energy obtained from the complete oxidation of palmitic acid (16 carbon) is given in ***Table 14.2*** and ***Fig.14.8.***

The standard free energy of palmitate = 2,340 Cal.

The energy yield by its oxidation—129 ATP (129 × 7.3 Cal) = 940 Cal.

The efficiency of energy conservation by fatty acid oxidation = $\frac{940}{2,340} \times 100 = 40\%$.

Table 14.2 Energetics of palmitic acid oxidation

Mechanism	*ATP yield*
I. β-Oxidation 7 cycles	
7 $FADH_2$ [oxidized by electron transport chain (ETC), each $FADH_2$ gives 2 ATP]	14
7 NADH (oxidized by ETC, each NADH liberates 3 ATP)	21
II. From 8 acetyl CoA	
Oxidized by citric acid cycle, each acetyl CoA provides 12 ATP	96
Total energy from one mole of palmitoyl CoA	131
Energy utilized for activation (formation of palmitoyl CoA)	–2
Net yield of oxidation of one molecule of palmitate	129

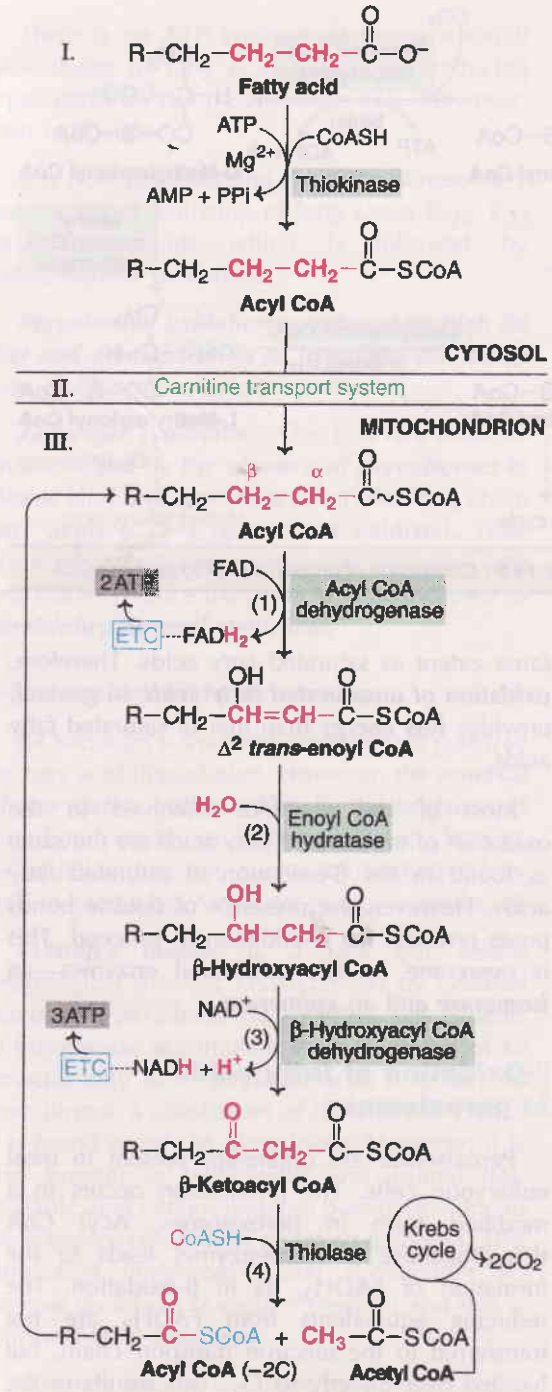


Fig. 14.7 : β-Oxidation of fatty acids : Palmitoyl CoA (16 carbon) undergoes seven cycles to yield 8 acetyl CoA [I–Activation; II–Transport; III–β Oxidation proper—(1) Oxidation, (2) Hydration, (3) Oxidation and (4) Cleavage].

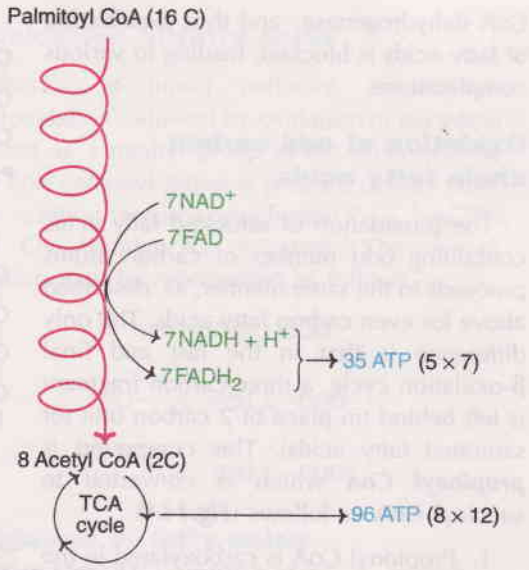


Fig. 14.8 : An overview of oxidation of palmitic acid.

SIDS—a disorder due to blockade in β-oxidation

The ***sudden infant death syndrome*** (SIDS) is an unexpected death of healthy infants, usually overnight. The real cause of SIDS is not known. It is now estimated that at least 10% of SIDS is due to ***deficiency of medium chain acyl CoA dehydrogenase***. The enzyme defect has a frequency of 1 in 10,000 births and is, in fact, more prevalent than phenylketonuria. The occurrence of SIDS is explained as follows

Glucose is the principal source of energy, soon after eating or feeding babies. After a few hours, the glucose level and its utilization decrease and the rate of fatty acid oxidation must simultaneously increase to meet the energy needs. The sudden death in infants is due to a ***blockade in β-oxidation*** caused by a deficiency in medium chain acyl CoA dehydrogenase (MCAD).

Jamaican vomiting sickness

This disease is characterized by severe hypoglycemia, vomiting, convulsions, coma and death. It is caused by eating unripe ackee fruit which contains an unusual toxic amino acid, ***hypoglycin A***. This ***inhibits*** the enzyme acyl

CoA dehydrogenase and thus ***β-oxidation*** of fatty acids is blocked, leading to various complications.

Oxidation of odd carbon chain fatty acids

The β-oxidation of saturated fatty acids containing odd number of carbon atoms proceeds in the same manner, as described above for even carbon fatty acids. The only difference is that in the last and final β-oxidation cycle, a three-carbon fragment is left behind (in place of 2 carbon unit for saturated fatty acids). This compound is ***propionyl CoA*** which is converted to succinyl CoA as follows (***Fig.14.9***)

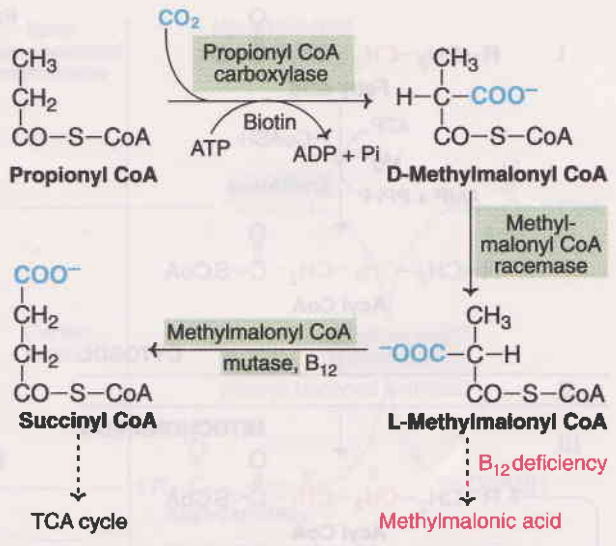


Fig. 14.9 : Conversion of propionyl CoA to succinyl CoA.

1. Propionyl CoA is carboxylated in the presence of ATP, CO_2 and vitamin ***biotin*** to D-methylmalonyl CoA.

2. Methylmalonyl CoA racemase converts the methylmalonyl CoA to L-form. This reaction (D → L) is essential for the entry of this compound into the metabolic reactions of the body.

3. The next enzyme, methylmalonyl CoA mutase, is dependent on ***vitamin B_{12}*** (deoxyadenosyl cobalamin). It catalyses the conversion of methylmalonyl CoA (a branched compound) to succinyl CoA (a straight chain compound), which can enter citric acid cycle.

Methylmalonic acidemia

Two types of methylmalonic acidemias are known

1. Due to ***deficiency of vitamin B_{12};***
2. Due to defect in the enzyme methylmalonyl CoA mutase.

In either case, there is an accumulation of methylmalonic acid in body, followed by its increased excretion in urine. This causes severe metabolic acidosis, damages the central nervous system and retards the growth. It is often fatal in the early years of life.

Oxidation of unsaturated fatty acids

Due to the presence of double bonds, the unsaturated fatty acids are not reduced to the same extent as saturated fatty acids. Therefore, ***oxidation of unsaturated fatty acids,*** in general, provides ***less energy*** than that of saturated fatty acids.

Most of the reactions involved in the oxidation of unsaturated fatty acids are the same as found in the β-oxidation of saturated fatty acids. However, the presence of double bonds poses problem for β-oxidation to proceed. This is overcome by two additional enzymes—an ***isomerase*** and an ***epimerase.***

β-Oxidation of fatty acids in peroxisomes

Peroxisomes are organelles present in most eukaryotic cells. The β-oxidation occurs in a modified form in peroxisomes. Acyl CoA dehydrogenase (a flavoenzyme) leads to the formation of $FADH_2$, as in β-oxidation. The reducing equivalents from $FADH_2$ are not transferred to the electron transport chain, but handed over directly to O_2. This results in the formation of H_2O_2, which is cleaved by catalase.

$$E\text{-}FADH_2 + O_2 \longrightarrow E\text{-}FAD + H_2O_2$$

$$H_2O_2 \xrightarrow{\text{Catalase}} H_2O + \frac{1}{2}O_2$$

There is no ATP synthesized in peroxisomal β-oxidation of fatty acids, since the reducing equivalents do not pass through ETC. However, heat is liberated.

It is now believed that the peroxisomes carry out the initial oxidation of long chain (C_{20}, C_{22} etc.) fatty acids which is followed by mitochondrial oxidation.

Peroxisomal oxidation is induced by high fat diet and administration of hypolipidemic drugs (e.g. clofibrate).

Zellweger syndrome : This is a rare disorder characterized by the ***absence of peroxisomes*** in almost all the tissues. As a result, the long chain fatty acids (C_{26}–C_{38}) are not oxidized. They accumulate in tissues, particularly in brain, liver and kidney. Hence the disorder is also known as ***cerebrohepatorenal syndrome.***

α-Oxidation of fatty acids

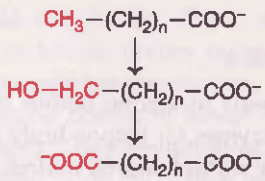
β-Oxidation is the most predominant pathway for fatty acid degradation. However, the ***removal of one carbon unit at a time*** by the oxidation of α-carbon atom of fatty acid is known. α-Oxidation does not involve the binding of fatty acid to coenzyme A and no energy is produced.

Refsum's disease is a rare but severe neurological disorder characterized by cerebral ataxia and peripheral neuropathy. The patients of this disease accumulate large quantities of an unusual fatty acid, ***phytanic acid.*** It is derived from phytol, a constituent of chlorophyll. Hence it is found mostly in plant foods. However, it is also present in milk lipids and animal fats. Phytanic acid cannot undergo β-oxidation due to the presence of a methyl group on carbon-3. This fatty acid undergoes initial α-oxidation (to remove α-carbon as carbon dioxide) and this is followed by β-oxidation.

Refsum's disease is caused by a defect in the α-oxidation due to the ***deficiency of the enzyme phytanic acid α-oxidase***. The result is that phytanic acid cannot be converted to a compound that can be degraded by β-oxidation. The patients should not consume diets containing chlorophyll (i.e., green leafy vegetables).

ω-Oxidation of fatty acids

This is a minor pathway. It involves hydroxylation followed by oxidation of ω-carbon present as a methyl group at the other end (at one end carboxyl group is present) of fatty acid. This reaction requires cytochrome P_{450}, NADPH and O_2, besides the enzymes. The overall reaction may be represented as follows.



Oxidation of fatty acids and metabolic water

Fatty acid oxidation (even other forms of aerobic respiration) is accompanied by the production of water, referred to metabolic water. For instance, when one molecule of palmitic acid is oxidized, it releases 16 molecules of water. This metabolic water has great significance in some animals. ***Camel*** can store ***lipids*** in its ***hump*** which is ***good source of water***, besides ***energy*** supply. For this reason, camel can travel in deserts for long periods even without food and water supply. Kangaroo rat is a small animal that is believed to live indefinitely without water. It consumes only oil rich seeds, and the metabolic water produced is adequate to meet its water needs. It may however, be noted that the use of metabolic water is an adaptation, and is accompanied by reduced output of urine.

KETONE BODIES

The compounds namely ***acetone, aceto-acetate*** and ***β-hydroxybutyrate*** (or 3-hydroxy-butyrate) are known as ketone bodies (***Fig.14.10***). Only the first two are true ketones while β-hydroxybutyrate does not possess a keto (C=O) group. ***Ketone bodies are water-soluble*** and ***energy yielding.*** Acetone, however, is an exception, since it cannot be metabolized.

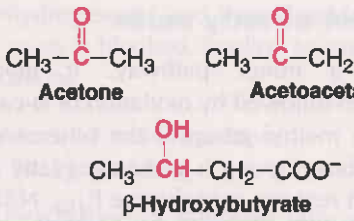


Fig. 14.10 : Structures of ketone bodies.

Ketogenesis

The synthesis of ketone bodies occurs in the ***liver***. The enzymes for ketone body synthesis are located in the ***mitochondrial matrix***. Acetyl CoA, formed by oxidation of fatty acids, pyruvate or some amino acids, is the precursor for ketone bodies. Ketogenesis occurs through the following reactions (***Fig.14.11***).

1. Two moles of acetyl CoA condense to form acetoacetyl CoA. This reaction is catalysed by thiolase, an enzyme involved in the final step of β-oxidation. Hence, acetoacetate synthesis is appropriately regarded as the reversal of thiolase reaction of fatty acid oxidation.

2. Acetoacetyl CoA combines with another molecule of acetyl CoA to produce β-hydroxy β-methyl glutaryl CoA (HMG CoA). ***HMG CoA synthase***, catalysing this reaction, ***regulates the synthesis of ketone bodies.***

3. HMG CoA lyase cleaves HMG CoA to produce acetoacetate and acetyl CoA.

4. Acetoacetate can undergo spontaneous decarboxylation to form acetone.

5. Acetoacetate can be reduced by a dehydrogenease to β-hydroxybutyrate.

The carbon skeleton of some amino acids (ketogenic) is degraded to acetoacetate or acetyl CoA and, therefore, to ketone bodies, e.g. leucine, lysine, phenylalanine etc.

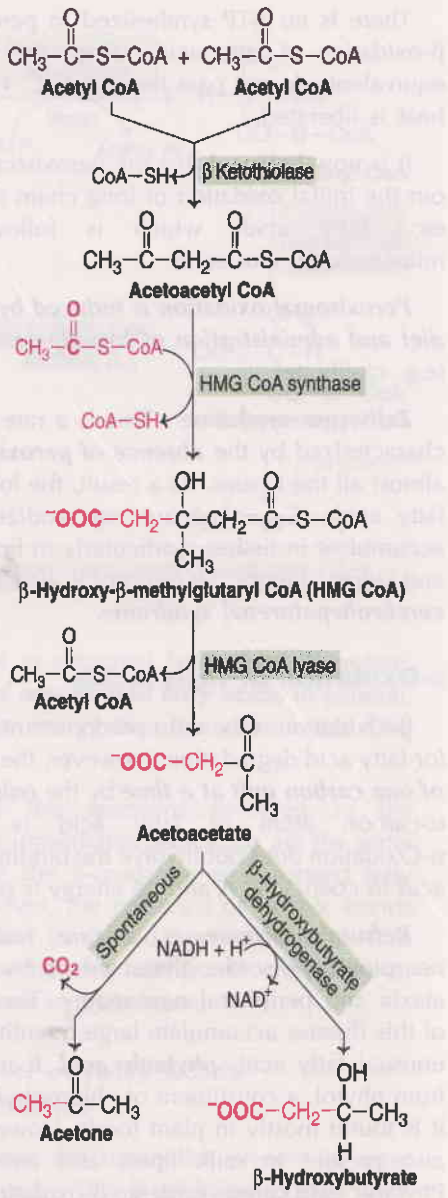


Fig. 14.11 : Synthesis of ketone bodies (ketogenesis).

Utilization of ketone bodies

The ketone bodies, being water-soluble, are easily transported from the liver to various tissues. The two ketone bodies—acetoacetate and β-hydroxybutyrate serve as important sources of energy for the ***peripheral tissues*** such as skeletal muscle, cardiac muscle, renal cortex etc. The tissues which lack mitochondria (e.g. erythrocytes) however, cannot utilize ketone bodies. The production of ketone bodies and their utilization become more significant when glucose is in short supply to the tissues, as observed in ***starvation***, and ***diabetes mellitus***.

During prolonged ***starvation***, ketone bodies are the major ***fuel source for the brain*** and other parts of central nervous system. It should be noted that the ability of the brain to utilize fatty acids for energy is very limited. The ketone bodies can meet 50-70% of the brain's energy needs. This is an adaptation for the survival of the organism during the periods of food deprivation.

Reactions of ketone bodies : β-Hydroxybutyrate is first converted to acetoacetate (reversal of synthesis) and metabolized. Acetoacetate is activated to acetoacetyl CoA by a mitochondrial enzyme ***thiophorase*** (succinyl CoA acetoacetate CoA transferase). The coenzyme A is donated by succinyl CoA, an intermediate in citric acid cycle. ***Thiophorase is absent in liver***, hence ketone bodies are not utilized by the liver. Thiolase cleaves acetoacetyl CoA to two moles of acetyl CoA (***Fig.14.12***).

The summary of ketone body synthesis, utilization and excretion is depicted in ***Fig.14.13***.

Overproduction of ketone bodies

In normal individuals, there is a constant production of ketone bodies by liver and their utilization by extrahepatic tissues. The concentration of ketone bodies in blood is maintained around 1 mg/dl. Their excretion in ***urine*** is very low and undetectable by routine tests (***Rothera's test***).

BIOMEDICAL / CLINICAL CONCEPTS

- *An adult human body contains about 10–11 kg of fat reserve corresponding to about 100,000 Cal. This can meet the energy requirements for several weeks of food deprivation in man.*
- *The sudden infant death syndrome (SIDS)—an unexpected overnight death of healthy infants—is attributed to a blockade in β-oxidation of fatty acids, caused by a deficiency of medium chain acyl CoA dehydrogenase (MCAD).*
- *Jamaican vomiting sickness is due to consumption of unripe ackee fruit containing hypoglycin A which blocks β-oxidation.*
- *Methylmalonic acidemia occurs either due to a deficiency of the vitamin B_{12} or a defect in an enzyme methyl malonyl CoA mutase. This disorder retards growth and damages central nervous system.*
- *Zellweger syndrome is caused by the absence of peroxisomes in tissues; as a result, the long chain fatty acids cannot be oxidized.*
- *Refsum's disease is due to a defect in α-oxidation of fatty acids. The patients are advised not to consume diets containing chlorophyll.*
- *Ketosis is commonly associated with uncontrolled diabetes mellitus and starvation. Diabetes ketoacidosis is dangerous—may result in coma or even death. Starvation, however, is not accompanied by ketoacidosis.*
- *Insulin promotes fatty acid synthesis by stimulating the conversion of pyruvate to acetyl CoA.*
- *The lack of the ability of the organisms to introduce double bonds in fatty acids beyond C_9 and C_{10} makes linoleic and linolenic acids essential to mammals.*

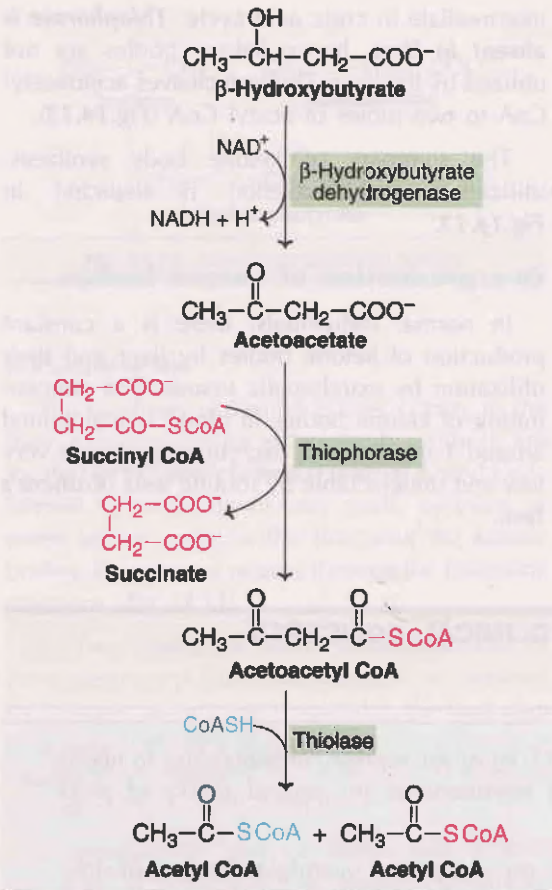


Fig. 14.12 : Metabolism (utilization) of ketone bodies to acetyl CoA.

When the rate of synthesis of ketone bodies exceeds the rate of utilization, their concentration in blood increases, this is known as ***ketonemia.*** Ketonemia is predominantly due to incresed production of ketone bodies rather than the deficiency in their utilization. The term ***ketonuria*** represents the excretion of ketone bodies in urine. The overall picture of ketonemia and ketonuria is commonly referred to as ***ketosis.*** Smell of acetone in breath is a common feature in ketosis. Ketosis is most commonly associated with starvation and severe uncontrolled diabetes mellitus.

Starvation : Starvation is accompanied by ***increased degradation of fatty acids*** (from the fuel reserve triacylglycerol) to meet the energy needs of the body. This causes an over-production of acetyl CoA which cannot be fully handled by citric acid cycle. Furthermore, TCA cycle is impaired due to deficiency of oxaloacetate, since most of it is diverted for glucose synthesis to meet the essential requirements (often unsuccessful) for tissues like brain. The result is an accumulation of acetyl CoA and its diversion for ***overproduction of ketone bodies.***

Diabetes mellitus : Diabetes mellitus is associated with insulin deficiency. This results in impaired carbohydrate metabolism and increased lipolysis, both of them ultimately leading to the accumulation of acetyl CoA and its conversion to ketone bodies. In severe diabetes, the ketone body concentration in blood plasma may reach 100 mg/dl and the urinary excretion may be as high as 500 mg/day.

Regulation of ketogenesis

The ketone body formation (particularly overproduction) occurs primarily due to non-availability of carbohydrates to the tissues. This is an outcome of excessive utilization of fatty acids to meet the energy requirements of the cells. The hormone ***glucagon stimulates ketogenesis*** whereas ***insulin inhibits.*** The increased ratio of glucagon/insulin in diabetes mellitus promotes ketone body formation. This is due to disturbances caused in carbohydrate and lipid metabolisms in diabetes, as discussed elsewhere (***Chapter 36***).

Ketogenic and antiketogenic substances

The dietary compounds are divided into two categories depending on whether they promote ketone body formation (ketogenic) or inhibit (antiketogenic).

The ketogenic substances include fatty acids and certain amino acids (leucine, lysine, tyrosine etc.). The antiketogenic substances are glucose, glycerol and glucogenic amino acids (e.g. glycine, alanine, serine, glutamate etc.)

Ketoacidosis

Both acetoacetate and β-hydroxybutyrate are strong acids. Increase in their concentration in blood would cause acidosis. The carboxyl group

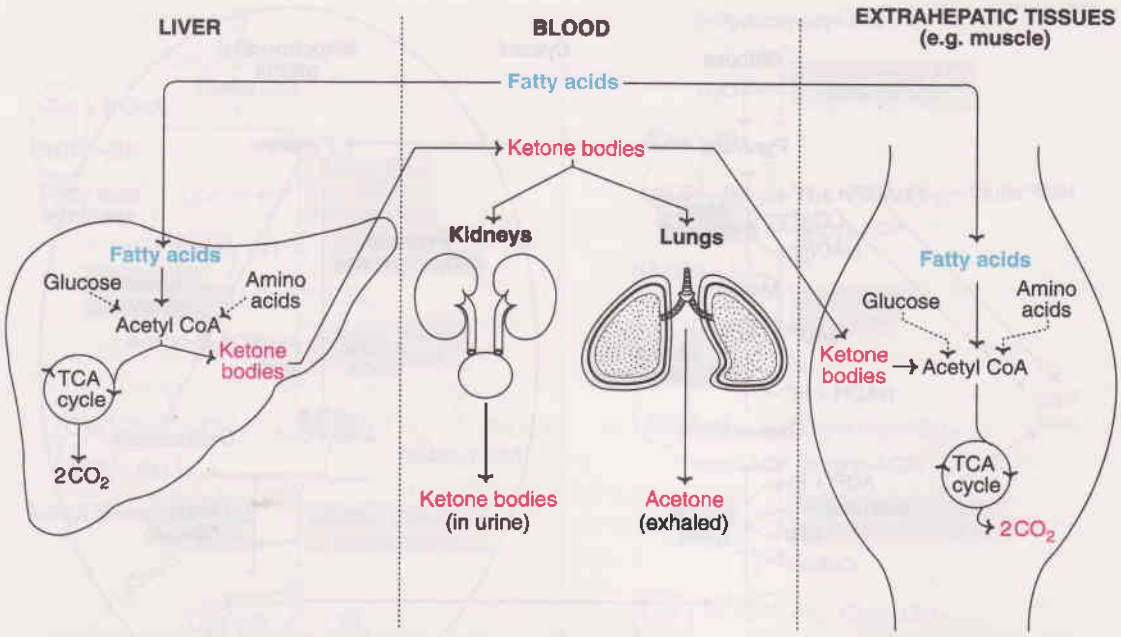


Fig. 14.13 : Summary of ketone body synthesis, utilization and excretion.

has a p^{Ka} around 4. Therefore, the ketone bodies in the blood dissociate and release H^+ ions which lower the pH. Further, the volume of plasma in the body is reduced due to dehydration caused by the excretion of glucose and ketone bodies. Diabetic ketoacidosis is dangerous—may result in coma, and even death, if not treated. Ketosis due to starvation is not usually accompanied by ketoacidosis.

Treatment of ketoacidosis : Rapid treatment of diabetic ketoacidosis is required to correct the metabolic abnormalities and the associated water and electrolyte imbalance. Administration of insulin is necessary to stimulate uptake of glucose by tissues and inhibition of ketogenesis.

BIOSYNTHESIS OF FATTY ACIDS

The dietary carbohydrates and amino acids, when consumed in excess, can be converted to fatty acids and stored as triacylglycerols. ***De novo*** (new) synthesis of fatty acids occurs predominantly in liver, kidney, adipose tissue and lactating mammary glands. The ***enzyme machinery*** for fatty acid production is located in the ***cytosomal fraction*** of the cell. Acetyl CoA is the source of carbon atoms while NADPH provides the reducing equivalents and ATP supplies energy for fatty acid formation. The fatty acid synthesis may be learnt in 3 stages

I. Production of acetyl CoA and NADPH

II. Conversion of acetyl CoA to malonyl CoA

III. Reactions of fatty acid synthase complex.

I. Production of acetyl CoA and NADPH

Acetyl CoA and NADPH are the prerequisites for fatty acid synthesis. Acetyl CoA is produced in the mitochondria by the oxidation of pyruvate and fatty acids, degradation of carbon skeleton of certain amino acids, and from ketone bodies. Mitochondria, however, are not permeable to acetyl CoA. An alternate or a bypass arrangement is made for the transfer of acetyl CoA to cytosol. Acetyl CoA condenses with oxaloacetate in mitochondria to form citrate. Citrate is freely transported to cytosol where it is cleaved by ***citrate lyase*** to liberate acetyl CoA and oxaloacetate. Oxaloacetate in the cytosol is converted to malate (***Fig.14.14***).

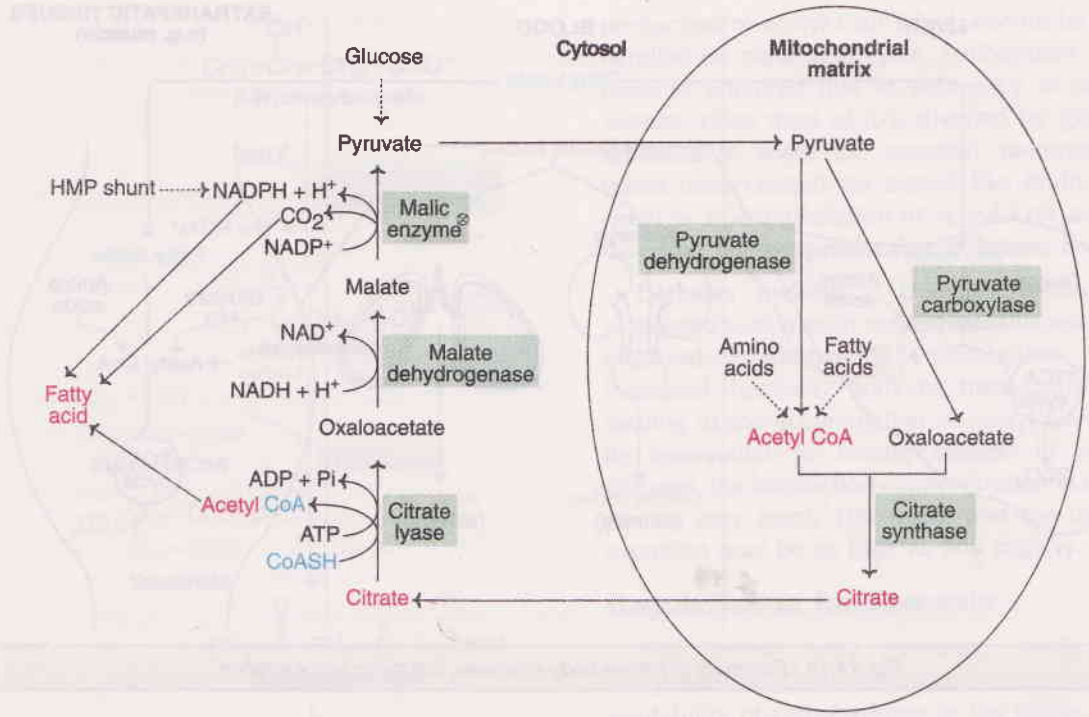


Fig. 14.14 : Transfer of acetyl CoA from mitochondria to cytosol (HMP shunt—Hexose monophosphate shunt; ⊗—also known as malate dehydrogenase).

Malic enzyme converts malate to pyruvate. NADPH and CO_2 are generated in this reaction. Both of them are utilized for fatty acid synthesis.

Advantages of coupled transport of acetyl CoA and NADPH : The transport of acetyl CoA from mitochondria to cytosol is coupled with the cytosomal production of NADPH and CO_2 which is highly advantageous to the cell for optimum synthesis of fatty acids.

II. Formation of malonyl CoA

Acetyl CoA is carboxylated to malonyl CoA by the enzyme ***acetyl CoA carboxylase*** (***Fig.14.15***). This is an ATP-dependent reaction and requires ***biotin*** for CO_2 fixation. The mechanism of action of acetyl CoA carboxylase is similar to that of pyruvate carboxylase (***Refer Chapter 7, Fig.7.29***). Acetyl CoA carboxylase is a regulatory enzyme in fatty acid synthesis (details given later).

III. Reactions of fatty acid synthase complex

The remaining reactions of fatty acid synthesis are catalysed by a multifunctional enzyme known as ***fatty acid synthase (FAS) complex.*** In eukaryotic cells, including man, the fatty acid synthase exists as a dimer with two identical units. Each monomer possesses the activities of seven different enzymes and an ***acyl carrier***

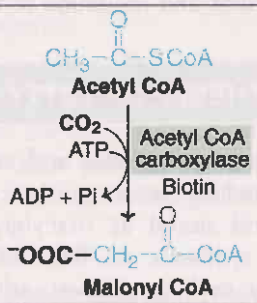


Fig. 14.15 : Conversion of acetyl CoA to malonyl CoA.

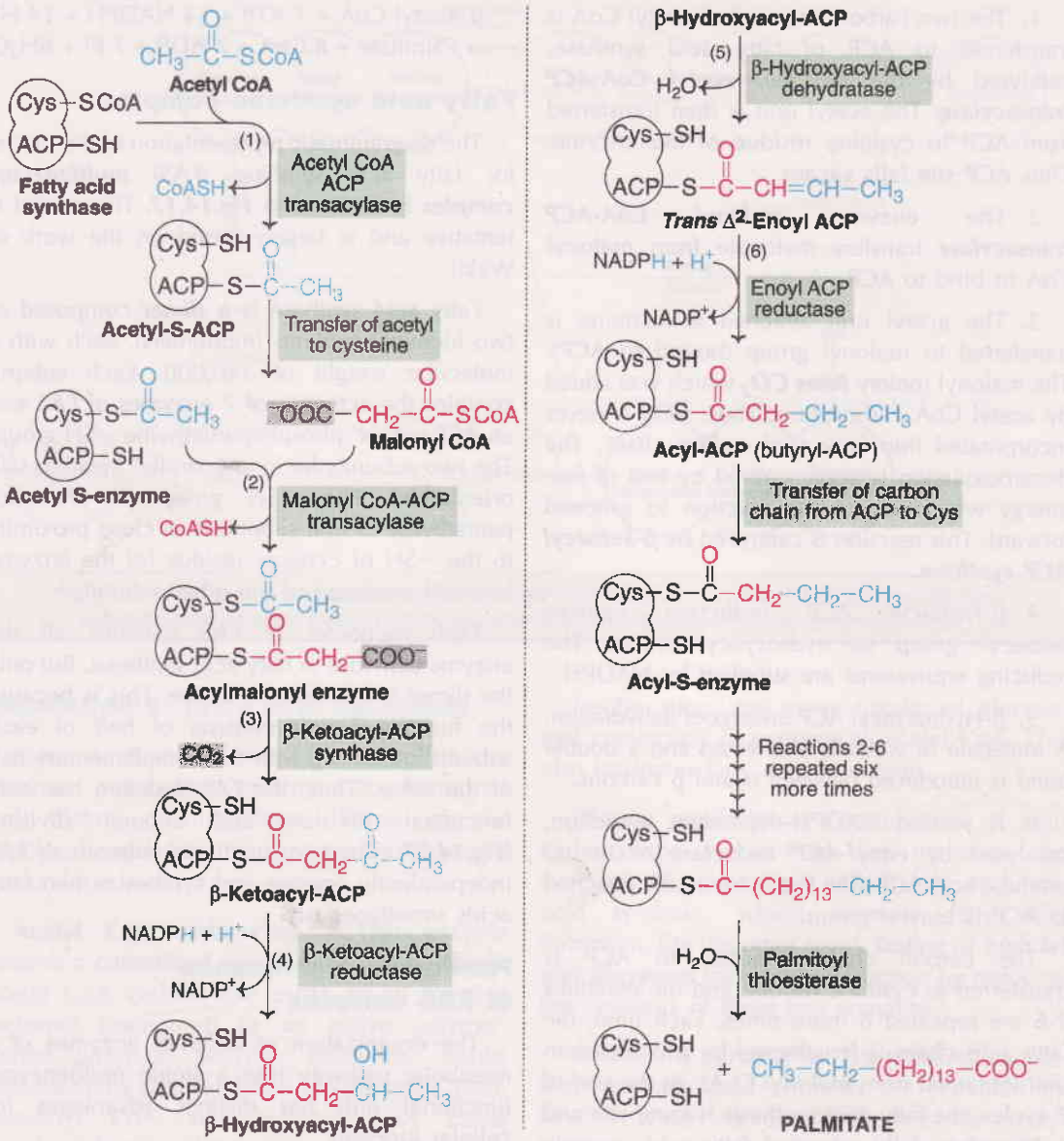


Fig. 14.16 contd. next column

***Fig. 14.16** : Biosynthesis of long chain fatty acid-palmitate. (Cys–Cysteine; ACP–Acyl carrier protein; The pathway repeats 7 times to produce palmitate; the first two carbons at the methyl end are directly from acetyl CoA, the rest of the carbons come from malonyl CoA).*

protein (ACP) bound to 4′-phosphopantetheine. Fatty acid synthase functions as a single unit catalysing all the seven reactions. Dissociation of the synthase complex results in loss of the enzyme activities. In the lower organisms (prokaryotes), the fatty acid synthesis is carried out by a multienzyme complex in association with a separate acyl carrier protein. This is in contrast to eukaryotes where ACP is a part of fatty acid synthase.

The sequence of reactions of the extra—mitochondrial synthesis of fatty acids (palmitate) is depicted in ***Fig.14.16***, and described in the next page.

1. The two carbon fragment of acetyl CoA is transferred to ACP of fatty acid synthase, catalysed by the enzyme, ***acetyl CoA-ACP transacylase***. The acetyl unit is then transferred from ACP to cysteine residue of the enzyme. Thus ACP site falls vacant.

2. The enzyme ***malonyl CoA-ACP transacylase*** transfers malonate from malonyl CoA to bind to ACP.

3. The acetyl unit attached to cysteine is transferred to malonyl group (bound to ACP). The malonyl moiety ***loses CO_2*** which was added by acetyl CoA carboxylase. Thus, CO_2 is never incorporated into fatty acid carbon chain. The decarboxylation is accompanied by loss of free energy which allows the reaction to proceed forward. This reaction is catalyzed by ***β-ketoacyl ACP synthase.***

4. β-Ketoacyl ACP reductase reduces ketoacyl group to hydroxyacyl group. The reducing equivalents are supplied by NADPH.

5. β-Hydroxyacyl ACP undergoes dehydration. A molecule of water is eliminated and a double bond is introduced between α and β carbons.

6. A second NADPH-dependent reduction, catalysed by ***enoyl-ACP reductase*** occurs to produce acyl-ACP. The four-carbon unit attached to ACP is butyryl group.

The carbon chain attached to ACP is transferred to cysteine residue and the reactions 2-6 are repeated 6 more times. Each time, the fatty acid chain is lengthened by a two-carbon unit (obtained from malonyl CoA). At the end of 7 cycles, the fatty acid synthesis is complete and a 16-carbon fully saturated fatty acid—namely palmitate—bound to ACP is produced.

7. The enzyme palmitoyl thioesterase separates palmitate from fatty acid synthase. This completes the synthesis of palmitate.

Summary of palmitate synthesis

Of the 16 carbons present in palmitate, only two come from acetyl CoA directly. The remaining 14 are from malonyl CoA which, in turn, is produced by acetyl CoA. The overall reaction of palmitate synthesis is summarized

$$8\ \text{Acetyl CoA} + 7\ \text{ATP} + 14\ \text{NADPH} + 14\ H^+ \longrightarrow \text{Palmitate} + 8\ \text{CoA} + 7\ \text{ADP} + 7\ \text{Pi} + 6H_2O$$

Fatty acid synthase complex

The diagrammatic representation of the model for fatty acid synthase (FAS) ***multienzyme complex*** is depicted in ***Fig.14.17***. This model is tentative and is largely based on the work of Wakil.

Fatty acid synthase is a ***dimer*** composed of two identical subunits (monomers), each with a molecular weight of 240,000. Each subunit contains the activities of 7 enzymes of FAS and an ACP with 4'-phosphopantetheine −SH group. The two subunits lie in antiparallel (head-to-tail) orientation. The −SH group of phosphopantetheine of one subunit is in close proximity to the −SH of cysteine residue (of the enzyme ketoacyl synthase) of the other subunit.

Each monomer of FAS contains all the enzyme activities of fatty acid synthesis. But only the dimer is functionally active. This is because the functional unit consists of half of each subunit interacting with the complementary half of the other. Thus, the FAS structure has both functional division and subunit division (***Fig.14.17***). The two functional subunits of FAS independently operate and synthesize two fatty acids simultaneously.

Functional significance of FAS complex

The organization of different enzymes of a metabolic pathway into a single multienzyme functional unit has distinct advantages for cellular function

1. The FAS complex offers ***great efficiency*** that is free from interference of other cellular reactions for the synthesis of fatty acids.

2. Since the entire process of the metabolic pathway is confined to the complex, there are no permeability barriers for the various intermediates.

3. The multienzyme polypeptide complex is coded by a single gene. Thus, there is a ***good coordination*** in the synthesis of all enzymes of the FAS complex.

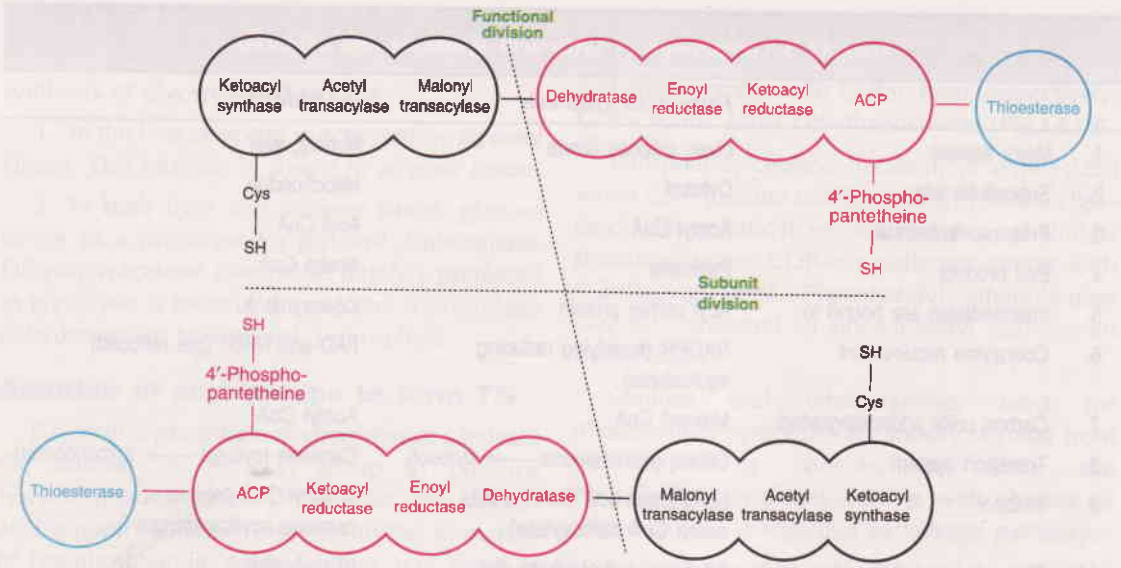


Fig. 14.17 : Fatty acid synthase multienzyme complex (ACP–Acyl carrier protein; FAS has two identical subunits which organize into two functional subunits to simultaneously synthesize two fatty acids).

Regulation of fatty acid synthesis

Fatty acid production is controlled by enzymes, metabolites, end products, hormones and dietary manipulations. Some of the important regulatory mechanisms are discussed hereunder.

Acetyl CoA carboxylase : This enzyme controls a ***committed step in fatty acid synthesis.*** Acetyl CoA carboxylase exists as an inactive protomer (monomer) or an active polymer. Citrate promotes polymer formation, hence increases fatty acid synthesis. On the other hand, palmitoyl CoA and malonyl CoA cause depolymerization of the enzyme and, therefore, inhibit fatty acid synthesis.

Hormonal influence : Hormones regulate acetyl CoA carboxylase by a separate mechanism—phosphorylation (inactive form) and dephosphorylation (active form) of the enzyme. Glucagon, epinephrine and norepinephrine inactivate the enzyme by cAMP-dependent phosphorylation. Insulin, on the other hand, dephosphorylates and activates the enzyme. Thus, ***insulin promotes fatty acid synthesis*** while ***glucagon inhibits.***

Insulin stimulates tissue uptake of glucose, and conversion of pyruvate to acetyl CoA. This also facilitates fatty acid formation.

Dietary regulation : Consumption of high carbohydrate or fat-free diet increases the synthesis of acetyl CoA carboxylase and fatty acid synthase, which promote fatty acid formation. On the other hand, ***fasting*** or ***high fat diet decreases*** fatty acid production by reducing the synthesis of these two enzymes.

Availability of NADPH : The reducing equivalents for fatty acid synthesis are provided by NADPH which come either from citrate (acetyl CoA) transport or hexose monophosphate shunt. About 50-60% of required NADPH is obtained from ***HMP shunt***, which significantly influences fatty acid synthesis.

Desaturation of fatty acid chains

A microsomal enzyme system called ***fatty acyl CoA desaturase*** is responsible for the formation of unsaturated fatty acids. This reaction also involves flavin-dependent cytochrome b_5 reductase, NADH and molecular O_2. The monounsaturated fatty acids—namely oleic

Table 14.3 Comparison of fatty acid synthesis and oxidation

		Fatty acid synthesis	*β-Oxidation*
1.	Major tissues	Liver, adipose tissue	Muscle, liver
2.	Subcellular site	Cytosol	Mitochondria
3.	Precursor/substrate	Acetyl CoA	Acyl CoA
4.	End product	Palmitate	Acetyl CoA
5.	Intermediates are bound to	Acyl carrier protein	Coenzyme A
6.	Coenzyme requirement	NADPH (supplying reducing equivalents)	FAD and NAD^+ (get reduced)
7.	Carbon units added/degraded	Malonyl CoA	Acetyl CoA
8.	Transport system	Citrate (mitochondria ⟶ cytosol)	Carnitine (cytosol ⟶ mitochondria)
9.	Inhibitor	Long chain acyl CoA (inhibits acetyl CoA carboxylase)	Malonyl CoA (inhibits carnitine acyltransferase I)
10.	The pathway increased	After rich carbohydrate diet	In starvation
11.	Hormonal status that promotes	High ratio of insulin/glucagon	Low ratio of insulin/glucagon
12.	Status of enzyme(s)	Multifunctional enzyme complex	Individual enzymes

acid and palmitoleic acid—are, respectively, synthesized from stearate and palmitate.

Mammals lack the ability to introduce double bonds in fatty acids beyond carbons 9 and 10. Hence, linoleic acid (18 : 2; 9, 12) and linolenic acid (18 : 3; 9, 12, 15) are essential for man in the diet. However, arachidonic acid (20 : 4; 5, 8, 11, 14) can be synthesized from linoleic acid by desaturation and chain elongation. Arachidonic acid is the precursor for eicosanoids (prostaglandins and thromboxanes), a group of compounds with diversified functions, discussed elsewhere (***Chapter 32***).

SYNTHESIS OF LONG CHAIN FATTY ACIDS FROM PALMITATE

Palmitate is the end product of the reactions of fatty acid synthase system that occurs in cytosol. Further, ***chain elongation*** can take place either in ***mitochondria*** or in ***endoplasmic reticulum*** (microsomes), by separate mechanisms. The microsomal chain elongation is more predominant and involves successive additions of malonyl CoA with the participation of NADPH. These reactions are similar to that catalysed by fatty acid synthase. A specific group of enzymes, namely ***elongases***, bring about fatty acid chain elongation.

The mitochondrial chain elongation is almost a reversal of β-oxidation of fatty acids. Acetyl CoA molecules are successively added to fatty acid to lengthen the chain. The reducing equivalents are derived from NADPH.

Comparison between fatty acid synthesis and oxidation

The synthesis of fatty acids and their oxidation are two distinct and independent pathways. A comparison between these two metabolic pathways in given in ***Table 14.3***.

SYNTHESIS OF TRIACYLGLYCEROLS

Triacylglycerol (TG) synthesis mostly occurs in ***liver*** and ***adipose tissue***, and to a lesser extent in other tissues. Fatty acids and glycerol must be activated prior to the synthesis of triacylglycerols. Conversion of fatty acids to acyl CoA by thiokinase is already described (***See Fig.14.5***).

Synthesis of glycerol 3-phosphate

Two mechanisms are involved for the synthesis of glycerol 3-phosphate

1. In the liver, glycerol is activated by glycerol kinase. This enzyme is absent in adipose tissue.

2. In both liver and adipose tissue, glucose serves as a precursor for glycerol 3-phosphate. Dihydroxyacetone phosphate (DHAP) produced in glycolysis is reduced by glycerol 3-phosphate dehydrogenase to glycerol 3-phosphate.

Addition of acyl groups to form TG

Glycerol 3-phosphate acyltransferase catalyses the transfer of an acyl group to produce lysophosphatidic acid. DHAP can also accept acyl group, ultimately resulting in the formation of lysophosphatidic acid. Another acyl group is added to lysophosphatidic acid to form phosphatidic acid (1,2-diacylglycerol phosphate). The enzyme phosphatase cleaves off phosphate of phosphatidic acid to produce diacylglycerol. Incorporation of another acyl group finally results in synthesis of triacylglycerol (***Fig.14.18***).

The three fatty acids found in triacylglycerol are not of the same type. A saturated fatty acid is usually present on carbon 1, an unsaturated fatty acid is found on carbon 2, and carbon 3 may have either.

The intermediates of TG synthesis phosphatidic acid and diacylglycerol are also utilized for phospholipid synthesis (described later).

METABOLISM OF PHOSPHOLIPIDS

Phospholipids are a specialized group of lipids performing a variety of functions. These include the membrane structure and functions, involvement in blood clotting, and supply of arachidonic acid for the synthesis of prostaglandins (for details ***Refer Chapter 32***).

Synthesis of phospholipids

Phospholipids are synthesized from phosphatidic acid and 1,2-diacylglycerol, intermediates in the production of triacylglycerols (***Fig.14.18***). Phospholipid synthesis occurs in the smooth endoplasmic reticulum.

1. **Formation of lecithin and cephalin :** Choline and ethanolamine first get phosphorylated and then combine with CTP to form, respectively, CDP-choline and CDP-ethanolamine (***Fig.14.19***).

Phosphatidylcholine (lecithin) is synthesized when CDP-choline combines with 1,2-diacylglycerol. Phosphatidyl ethanolamine (cephalin) is produced when CDP-ethanolamine reacts with 1,2-diacylglycerol. Phosphatidyl ethanolamine can be converted to phosphatidyl choline on methylation.

Choline and ethanolamine, used for phospholipid synthesis, are mostly derived from the preexisting phospholipids. Thus, the phospholipid synthesis starting with choline or ethanolamine is regarded as ***salvage pathway***.

2. **Synthesis of phosphatidylserine :** Phosphatidyl ethanolamine can exchange its ethanolamine group with free serine to produce phosphatidylserine. The latter, on decarboxylation, gives the former.

3. **Formation of phosphatidylinositol :** CDP-diacylglycerol produced from phosphatidic acid combines with inositol to form phosphatidyl inositol (PI). This phospholipid contains ***arachidonic acid*** on carbon 2 of glycerol which serves as a substrate for ***prostaglandin synthesis***. Further, PI is important for signal transmission across membranes.

4. **Synthesis of phosphatidyl glycerol and cardiolipin :** CDP-diacylglycerol combines with glycerol 3-phosphate to form phosphatidyl glycerol 3-phosphate, which then forms phosphatidylglycerol. The latter combines with another molecule of phosphatidylglycerol to finally produce cardiolipin (***Fig.14.19***). ***Cardiolipin*** is the only phospholipid possessing ***antigenic*** properties.

5. **Formation of plasmalogens :** These are phospholipids with fatty acid at carbon 1 bound by an ether linkage instead of ester linkage. An important plasmalogen, 1-alkenyl 2-acetyl glycerol 3-phosphocholine, causes blood platelet aggregation and is referred to as ***platelet-activating factor*** (PAF). The outline of the pathway for the synthesis of plasmalogens is depicted in ***Fig.14.20***.

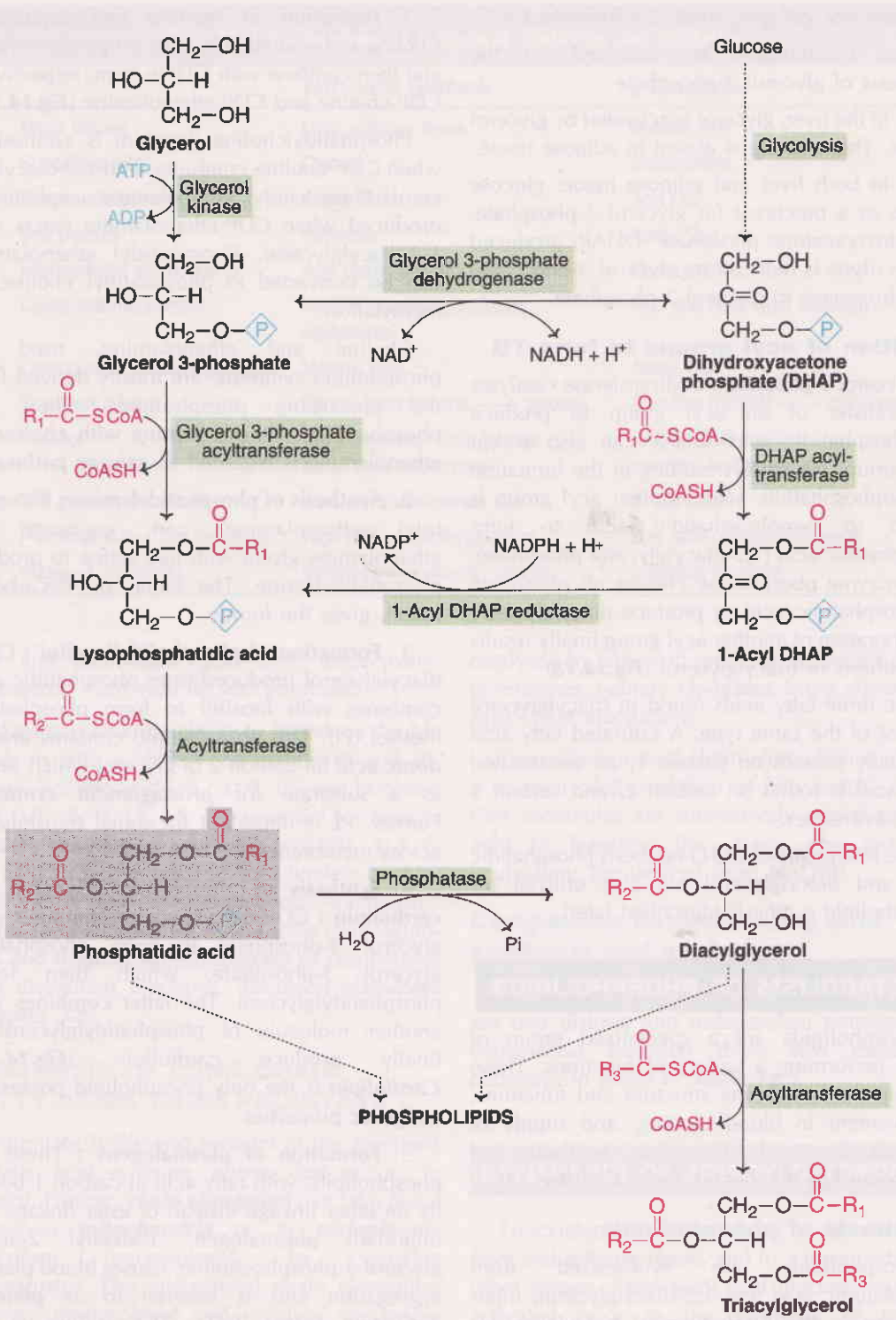


Fig. 14.18 : Synthesis of triacylglycerol.

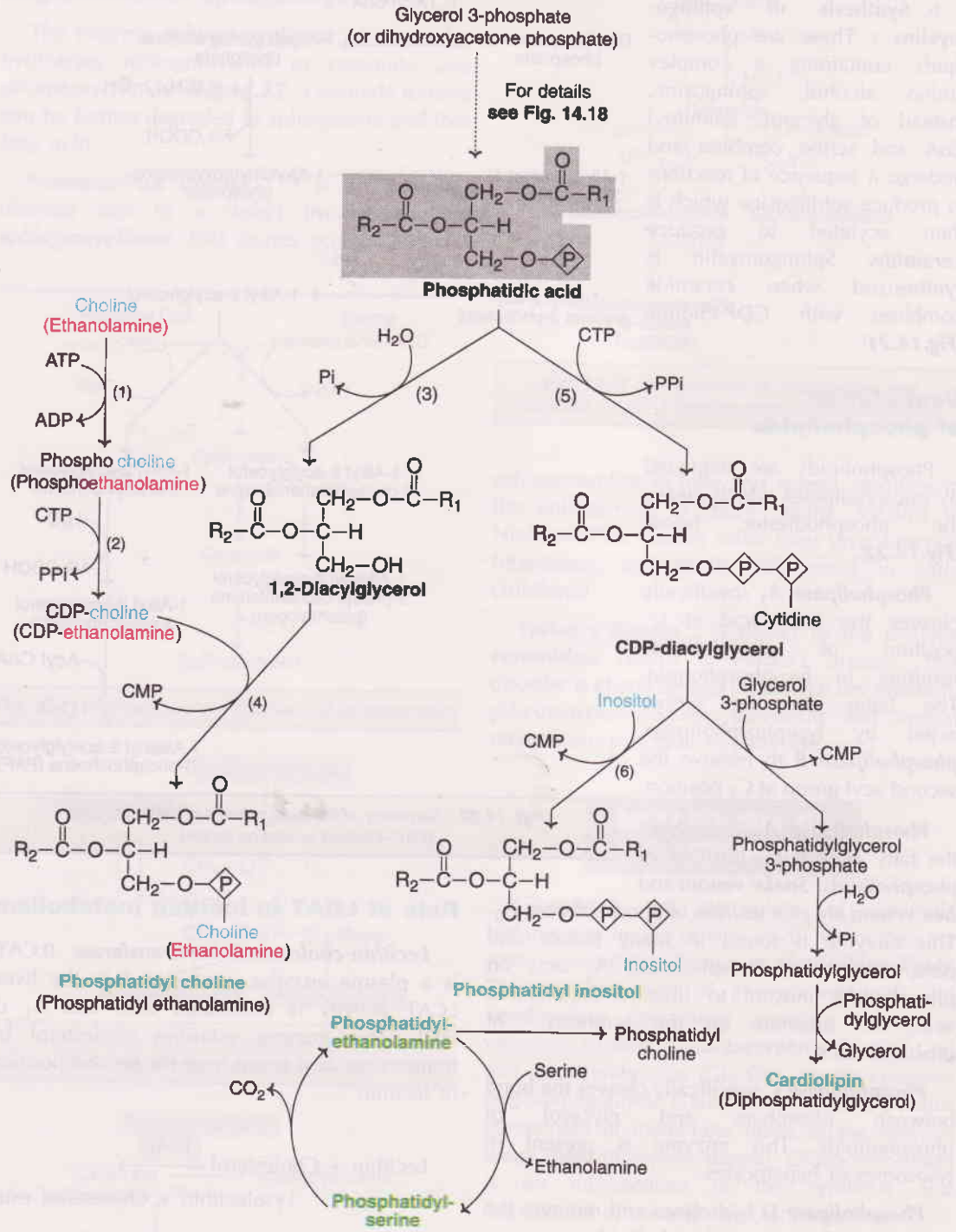


Fig. 14.19 : *Biosynthesis of phospholipids [The enzymes are numbered—(1) Choline kinase, (2) Phosphocholine cytidyltransferase, (3) Phosphatidate phosphohydrolase, (4) Phosphocholine diacylglycerol transferase, (5) CTP–Phosphatidate cytidyltransferase, (6) CDP–Diacylglycerol inositol transferase].*

6. **Synthesis of sphingomyelins :** These are phospholipids containing a complex amino alcohol, sphingosine, instead of glycerol. Palmitoyl CoA and serine combine and undergo a sequence of reactions to produce sphingosine which is then acylated to produce ***ceramide.*** Sphingomyelin is synthesized when ceramide combines with CDP-choline (***Fig.14.21***).

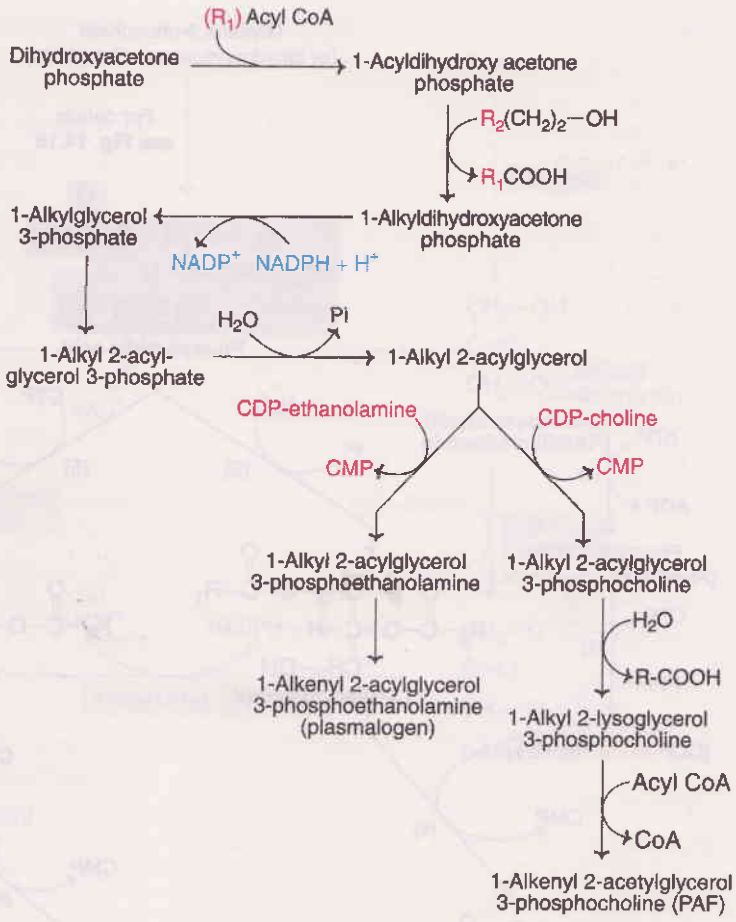


Fig. 14.20 : Summary of the biosynthesis of plasmalogens (PAF–Platelet activation factor).

Degradation of phospholipids

Phospholipids are degraded by phospholipases which cleave the phosphodiester bonds (***Fig.14.22***).

Phospholipase A_1 specifically cleaves the fatty acid at C_1 position of phospholipids resulting in lysophospholipid. The latter can be further acted by lysophospholipase, ***phospholipase B*** to remove the second acyl group at C_2 position.

Phospholipase A_2 hydrolyses the fatty acid at C_2 position of phospholipids. ***Snake venom*** and ***bee venom*** are rich sources of phospholipase A_2. This enzyme is found in many tissues and pancreatic juice. Phospholipase A_2 acts on phosphatidyl inositol to liberate arachidonic acid, the substrate for the synthesis of prostaglandins.

Phospholipase C specifically cleaves the bond between phosphate and glycerol of phospholipids. This enzyme is present in lysosomes of hepatocytes.

Phospholipase D hydrolyses and removes the nitrogenous base from phospholipids.

The degraded products of phospholipids enter the metabolic pool and are utilized for various purposes.

Role of LCAT in lecithin metabolism

Lecithin-cholesterol acyltransferase (LCAT) is a plasma enzyme, synthesized in the liver. LCAT activity is associated with apo A_1 of HDL. This enzyme esterifies cholesterol by transferring acyl group from the second position of lecithin

$$\text{Lecithin + Cholesterol} \xrightarrow{\text{LCAT}} \text{Lysolecithin + Cholesterol ester}$$

The above reaction is responsible for the reverse cholesterol transport mediated by HDL (more details given under lipoprotein metabolism).

Degradation of sphingomyelins

The enzyme ***sphingomyelinase*** of lysosomes hydrolyses sphingomyelins to ceramide and phosphorylcholine (***Fig.14.23***). Ceramide formed can be further degraded to sphingosine and free fatty acid.

Niemann-Pick disease : It is an inherited disorder due to a defect in the enzyme ***sphingomyelinase***. This causes accumulation of sphingomyelins in liver and spleen, resulting in the enlargement of these organs. Victims of Niemann-Pick disease suffer from severe mental retardation, and death may occur in early childhood.

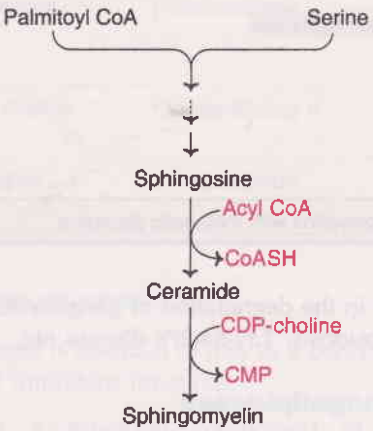


Fig. 14.21 : An outline of the synthesis of sphingomyelin.

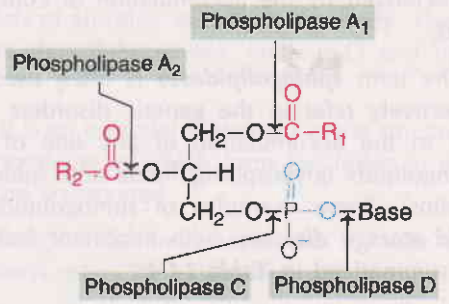


Fig. 14.22 : Degradation of phospholipids by phospholipases.

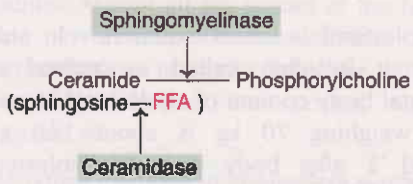


Fig. 14.23 : Site of action of sphingomyelinase and ceramidase on sphingomyelin.

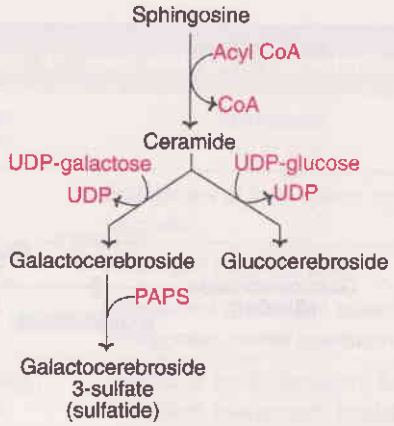


Fig. 14.24 : Biosynthesis of cerebrosides and sulfatides (PAPS–Phosphoadenosyl phosphosulfate).

Farber's disease : A defect in the enzyme ***ceramidase*** results in Farber's disease. This disorder is characterized by skeletal deformation, subcutaneous nodules, dermatitis and mental retardation. It is fatal in early life.

METABOLISM OF GLYCOLIPIDS

Glycolipids are derivatives of ceramide (sphingosine bound to fatty acid), hence they are more appropriately known as ***glycosphingolipids.*** The simplest form of glycosphingolipids are ***cerebrosides*** containing ceramide bound to monosaccharides. ***Galactocerebroside*** (Gal-Cer) and glucocerebroside (Glu-Cer) are the common glycosphingolipids. Galactocerebroside is a major component of membrane lipids in the nervous tissue (high in myelin sheath). Glucocerebroside is an intermediate in the synthesis and degradation of complex glycosphingolipids.

Synthesis of cerebrosides

The outline of the synthesis of cerebrosides and sulfatide is given in ***Fig.14.24.***

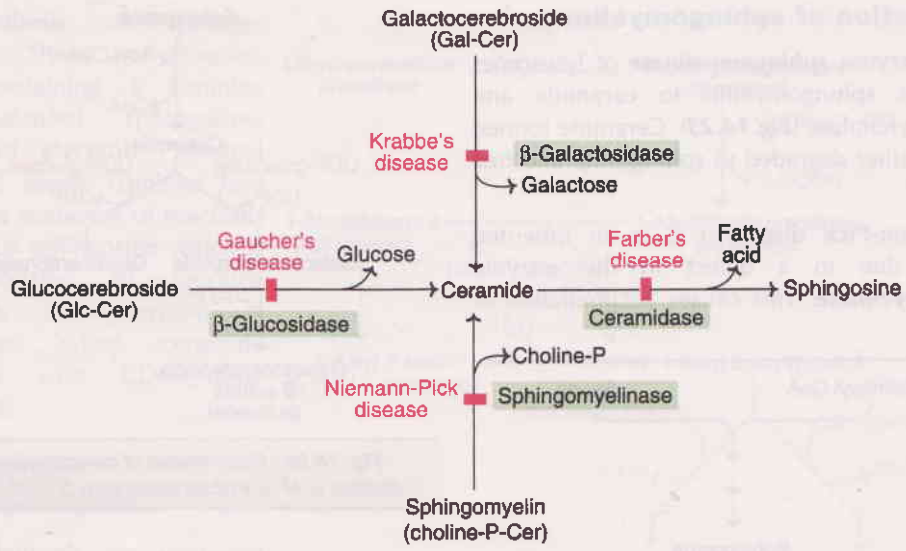


Fig. 14.25 : Degradation of cerebrosides and sphingomyelins with metabolic disorders.

Metabolic disorders of cerebrosides

The degradation of cerebrosides along with the associated inborn errors is depicted in ***Fig.14.25***.

Gaucher's disease : This is due to a defect in the enzyme ***β-glucosidase.*** As a result, tissue glucocerebroside levels increase. This disorder is commonly associated with enlargement of liver and spleen, osteoporosis, pigmentation of skin, anemia, mental retardation etc. Sometimes, Gaucher's disease is fatal.

Krabbe's disease : Defect in the enzyme ***β-galactosidase*** results in the accumulation of galactocerebrosides. A total absence of myelin in the nervous tissue is a common feature. Severe mental retardation, convulsions, blindness, deafness etc. are seen. Krabbe's disease is fatal in early life.

Niemann-Pick disease and Farber's disease connected with sphingomylein metabolism are already described. They are also depicted in ***Fig.14.25***.

Gangliosides are complex glycosphingolipids mostly found in ganglion cells. They contain one or more molecules of N-acetylneuraminic acid (NANA) bound ceramide oligosaccharides. Defect in the degradation of gangliosides causes gangliosidosis, Tay-Sach's disease etc.

Sphinogolipidoses

Lipid storage diseases, representing lysosomal storage defects, are inherited disorders. They are characterized by the accumulation of complex lipids.

The term ***sphingolipidoses*** is often used to collectively refer to the genetic disorders that lead to the accumulation of any one of the sphingolipids (glycosphingolipids and sphingomyelins). Some examples of sphiogolipidoses (***lipid storage diseases***) with important features are summarized in ***Table 14.4***.

METABOLISM OF CHOLESTEROL

Cholesterol is found exclusively in animals, hence it is often called as ***animal sterol.*** The total body content of cholesterol in an adult man weighing 70 kg is about 140 g i.e., around 2 g/kg body weight. Cholesterol is ***amphipathic*** in nature, since it possesses both hydrophilic and hydrophobic regions in the structure.

Table 14.4 Some examples of sphingolipidoses (lipid storage diseases) with their characteristics

Disease	*Missing/defective enzyme*	*Major storage compound*	*Symptoms*
Niemann-Pick disease	Sphingomyelinase	Sphingomyelins	Enlargement of liver, spleen, mental retardation.
Farber's disease	Ceramidase	Ceramide	Painful and deformed joints.
Gaucher's disease	β-Glucosidase	Glucocerebroside	Enlargement of liver and spleen, osteoporosis, mental retardation.
Krabbe's disease	β-Galactosidase	Galactocerebrosides	Absence of myelin formation, liver and spleen enlargement, mental retardation.
Tay-Sachs disease	Hexosaminidase A	Ganglioside GM_2	Blindness, mental retardation, death within 2-3 years.
Fabry's disease	α-Galactosidase	Ceramide trihexoside	Renal failure, skin rash, pain in lower extremities.

Functions of cholesterol

Cholesterol is essential to life, as it performs a number of important functions

1. It is a structural component of cell membrane.

2. Cholesterol is the precursor for the synthesis of all other steroids in the body. These include steroid hormones, vitamin D and bile acids.

3. It is an essential ingredient in the structure of lipoproteins in which form the lipids in the body are transported.

4. Fatty acids are transported to liver as cholesteryl esters for oxidation.

CHOLESTEROL BIOSYNTHESIS

About 1 g of cholesterol is synthesized per day in adults. Almost all the tissues of the body participate in cholesterol biosynthesis. The largest contribution is made by ***liver*** (50%), ***intestine*** (15%), skin, adrenal cortex, reproductive tissue etc.

The enzymes involved in cholesterol synthesis are found in the ***cytosol*** and ***microsomal fractions*** of the cell. Acetate of ***acetyl CoA*** provides all the carbon atoms in cholesterol. The reducing equivalents are supplied by ***NADPH*** while ***ATP*** provides energy. For the production of one mole of cholesterol, 18 moles of acetyl CoA, 36 moles of ATP and 16 moles of NADPH are required.

By administering acetate with ^{14}C isotope label either on the methyl ($-CH_3$) group or carboxyl ($-COO$) group, the origin of carbon atoms in the entire molecule of cholesterol has been established. The sources of carbon atoms and the key intermediates of cholesterol formation are depicted in ***Fig.14.26***, and the detailed reactions are given in ***Fig.14.27***.

The synthesis of cholesterol may be learnt in 5 stages

1. Synthesis of HMG CoA
2. Formation of mevalonate (6C)
3. Production of isoprenoid units (5C)
4. Synthesis of squalene (30C)
5. Conversion of squalene to cholesterol (27C).

1. **Synthesis of β-hydroxy β-methylglutaryl CoA (HMG CoA) :** Two moles of acetyl CoA condense to form acetoacetyl CoA. Another molecule of acetyl CoA is then added to produce HMG CoA. These reactions are similar to that of

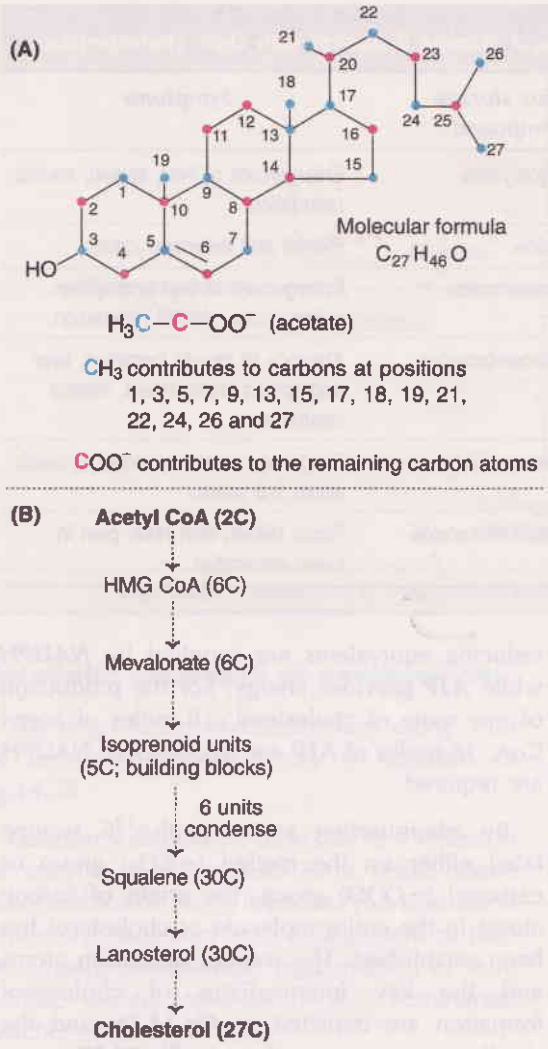


Fig. 14.26 : Outline of cholesterol biosynthesis— (A) Derivation of carbon atoms from acetate, (B) Key intermediates with the carbon atoms.

ketone body synthesis. However, the two pathways are distinct, since ketone bodies are produced in mitochondria while cholesterol synthesis occurs in cytosol. Thus, there exist ***two pools of HMG CoA*** in the cell. Further, two isoenzymes of HMG CoA synthase are known. The cytosomal enzyme is involved in cholesterol synthesis whereas the mitochondrial HMG CoA synthase participates in ketone body formation.

2. **Formation of mevalonate : *HMG CoA reductase*** is the ***rate limiting enzyme*** in cholesterol biosynthesis. This enzyme is present in endoplasmic reticulum and catalyses the reduction of HMG CoA to mevalonate. The reducing equivalents are supplied by NADPH.

3. **Production of isoprenoid units :** In a three-step reaction catalysed by kinases, mevalonate is converted to 3-phospho 5-pyrophospho-mevalonate which on decarboxylation forms isopentenyl pyrophosphate (IPP). The latter isomerizes to dimethylallyl pyrophosphate (DPP). Both IPP and DPP are 5-carbon isoprenoid units.

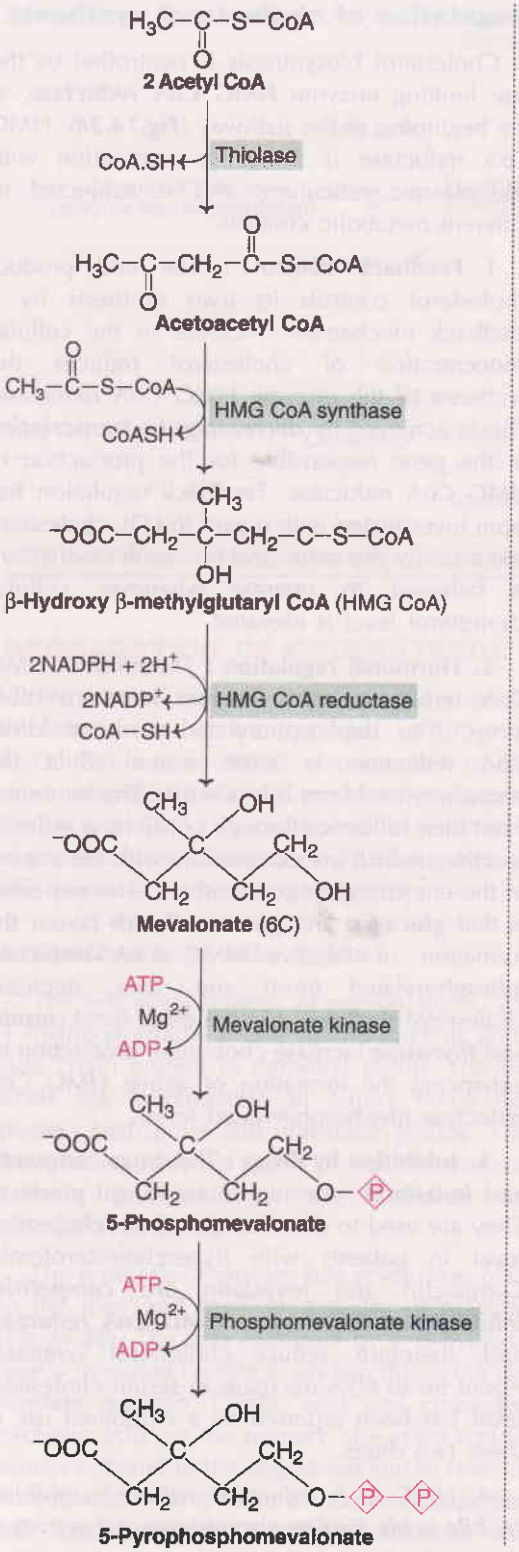
4. **Synthesis of squalene :** IPP and DPP condense to produce a 10-carbon geranyl pyrophosphate (GPP). Another molecule of IPP condenses with GPP to form a 15-carbon farnesyl pyrophosphate (FPP). Two units of farnesyl pyrophosphate unite and get reduced to produce a 30-carbon squalene.

5. **Conversion of squalene to cholesterol :** Squalene undergoes hydroxylation and cyclization utilizing O_2 and NADPH and gets converted to lanosterol. The formation of cholesterol from lanosterol is a multistep process with a series of about 19 enzymatic reactions. The following are the most important reactions

- Reducing the carbon atoms from 30 to 27.
- Removal of two methyl groups from C_4 and one methyl group from C_{14}.
- Shift of double bond from C_8 to C_5.
- Reduction in the double bond present between C_{24} and C_{25}.

The enzymes (about 19?) involved in the conversion of lanosterol to cholesterol are associated with endoplasmic reticulum. 14-Desmethyl lanosterol, zymosterol, cholestadienol and desmosterol are among the intermediates in the cholesterol biosynthesis. ***The penultimate product is 7-dehydrocholesterol which, on reduction, finally yields cholesterol.***

Cholesterol biosynthesis is now believed to be a part of a major metabolic pathway concerned with the synthesis of several other isoprenoid compounds. These include ubiquinone (coenzyme Q of electron transport chain) and dolichol (found in glycoprotein). Both of them are derived from farnesyl pyrophosphate.



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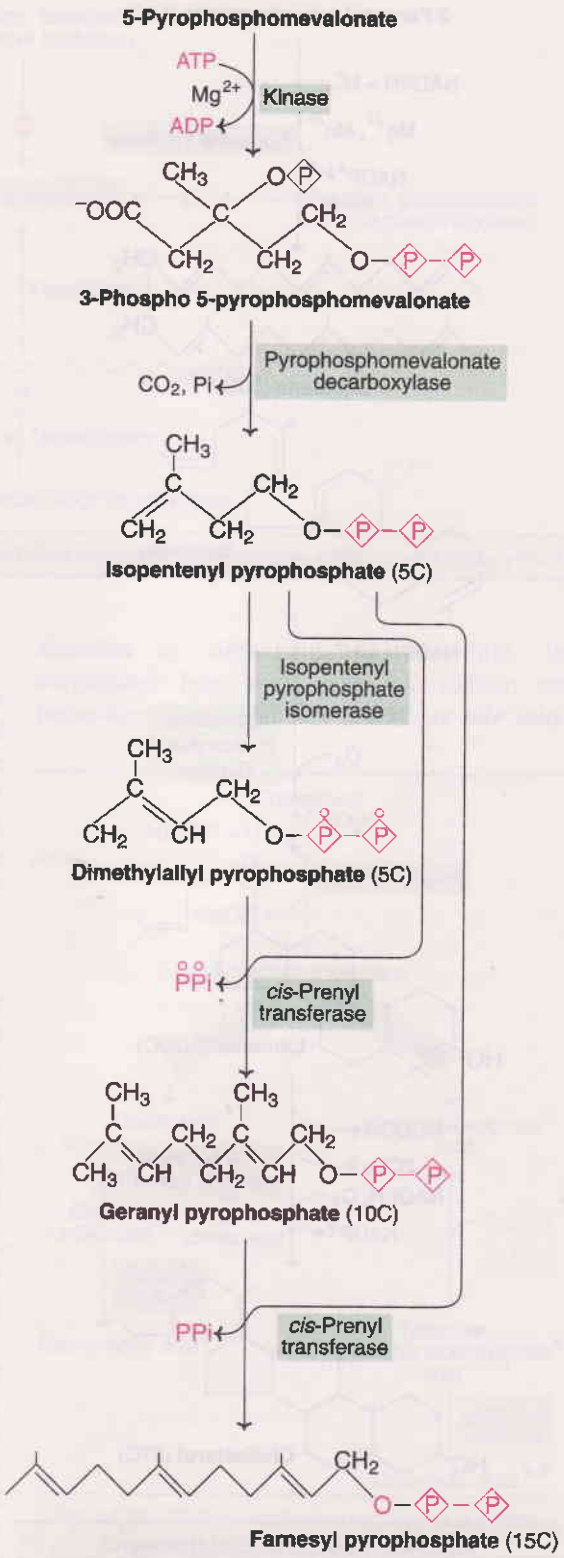

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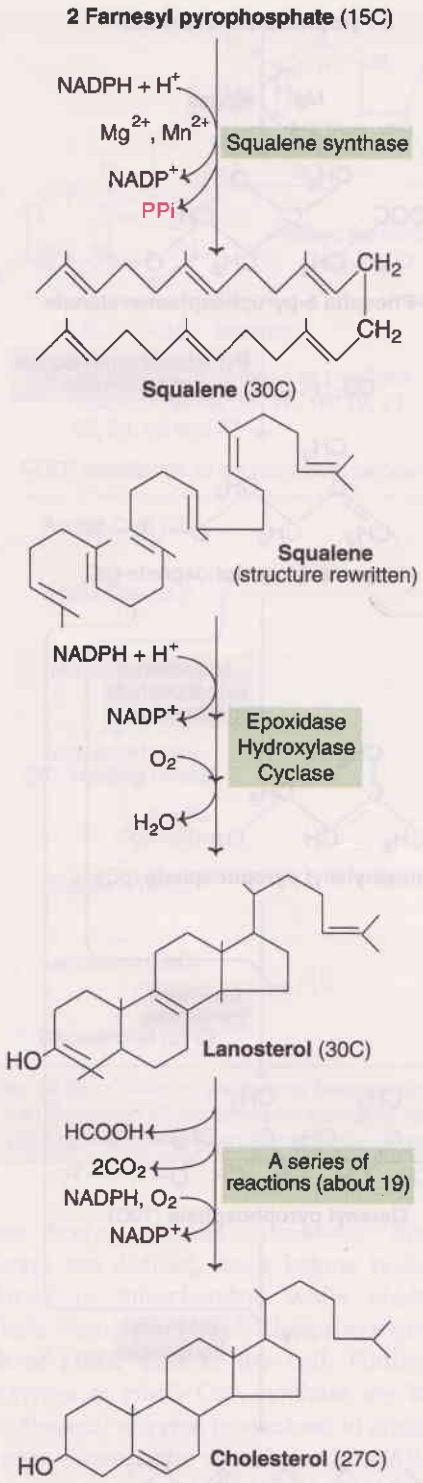

Fig. 14.27 : *Biosynthesis of cholesterol.*

Regulation of cholesterol synthesis

Cholesterol biosynthesis is controlled by the rate limiting enzyme ***HMG CoA reductase,*** at the beginning of the pathway (***Fig.14.28***). HMG CoA reductase is found in association with endoplasmic reticulum, and is subjected to different metabolic controls.

1. **Feedback control :** The end product cholesterol controls its own synthesis by a feedback mechanism. Increase in the cellular concentration of cholesterol reduces the synthesis of the enzyme HMG CoA reductase. This is achieved by ***decreasing the transcription*** of the gene responsible for the production of HMG CoA reductase. Feedback regulation has been investigated with regard to LDL-cholesterol taken up by the cells, and the same mechanism is believed to operate whenever cellular cholesterol level is elevated.

2. **Hormonal regulation :** The enzyme HMG CoA reductase exists in two interconvertible forms. The dephosphorylated form of HMG CoA reductase is more active while the phosphorylated form is less active. The hormones exert their influence through cAMP by a series of reactions which are comparable with the control of the enzyme glycogen synthase. The net effect is that ***glucagon*** and ***glucocorticoids*** favour the formation of inactive HMG CoA reductase (phosphorylated form) and, thus, decrease cholesterol synthesis. On the other hand, ***insulin*** and ***thyroxine*** increase cholesterol production by enhancing the formation of active HMG CoA reductase (dephosphorylated form).

3. **Inhibition by drugs :** The drugs ***compactin*** and ***lovastatin*** (mevinolin) are fungal products. They are used to decrease the serum cholesterol level in patients with hypercholesterolemia. Compactin and lovastatin are ***competitive inhibitors of*** the enzyme ***HMG CoA reductase*** and, therefore, reduce cholesterol synthesis. About 50 to 60% decrease in serum cholesterol level has been reported by a combined use of these two drugs.

4. HMG CoA reductase activity is inhibited by ***bile acids***. Fasting also reduces the activity of this enzyme.

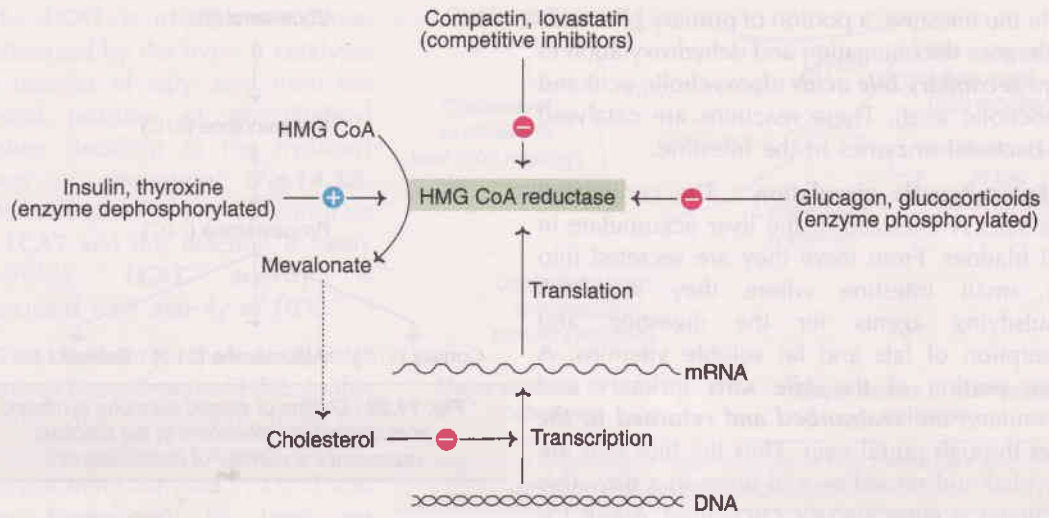

Fig. 14.28 : Regulation of cholesterol biosynthesis by HMG CoA reductase (⊕–Promoting effect; ⊖–Inhibitory effect).

DEGRADATION OF CHOLESTEROL

The steroid nucleus (ring structure) of the cholesterol cannot be degraded to CO_2 and H_2O. Cholesterol (50%) is converted to bile acids (excreted in feces), serves as a precursor for the synthesis of steroid hormones, vitamin D, coprostanol and cholestanol. The latter two are the fecal sterols, besides cholesterol.

I. Synthesis of bile acids

The bile acids possess 24 carbon atoms, 2 or 3 hydroxyl groups in the steroid nucleus and a side chain ending in carboxyl group. The bile acids are amphipathic in nature since they possess both polar and non-polar groups. They serve as emulsifying agents in the intestine and actively participate in the digestion and absorption of lipids.

The synthesis of primary bile acids takes place in the liver and involves a series of reactions (***Fig.14.29***). The step catalysed by 7 ***α-hydroxylase*** is inhibited by bile acids and this is the ***rate limiting*** reaction. Cholic acid and chenodeoxycholic acid are the primary bile acids and the former is found in the largest amount in bile. On conjugation with glycine or taurine, ***conjugated bile acids*** (glycocholic acid, taurocholic acid etc.) are formed which are more ***efficient in their function*** as surfactants. In the bile, the conjugated bile acids exist as sodium and potassium salts which are known as ***bile salts.***

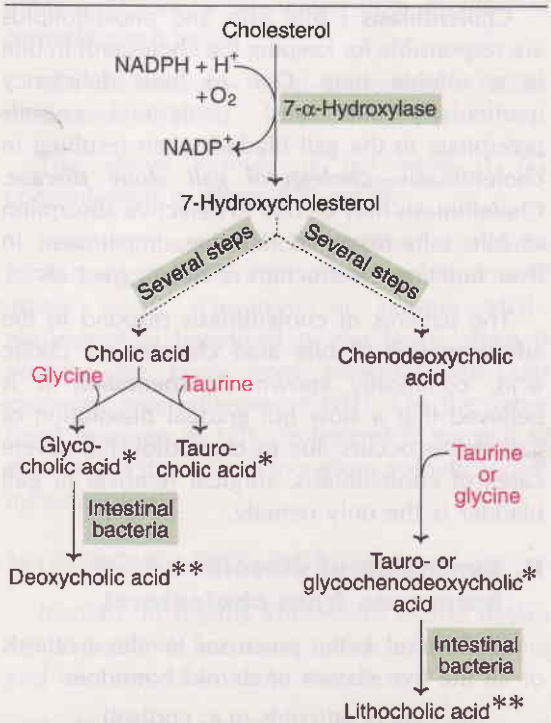

Fig. 14.29 : Outline of bile acid synthesis (–Primary bile acids, **–Secondary bile acids).*

In the intestine, a portion of primary bile acids undergoes deconjugation and dehydroxylation to form ***secondary bile acids*** (deoxycholic acid and lithocholic acid). These reactions are catalysed by bacterial enzymes in the intestine.

Enterohepatic circulation : The conjugated bile salts synthesized in the liver accumulate in gall bladder. From there they are secreted into the small intestine where they serve as emulsifying agents for the digestion and absorption of fats and fat soluble vitamins. A large portion of the ***bile salts*** (primary and secondary) are ***reabsorbed and returned to the liver*** through portal vein. Thus the bile salts are recycled and reused several times in a day. This is known as enterohepatic circulation. About 15-30 g of bile salts are secreted into the intestine each day and reabsorbed. However, a small portion of about 0.5 g/day is lost in the feces. An equal amount (0.5 g/day) is synthesized in liver to replace the lost bile salts. The ***fecal excretion of bile salts*** is the only ***route for the removal of cholesterol from the body.***

Cholelithiasis : Bile salts and phospholipids are responsible for keeping the cholesterol in bile in a soluble state. Due to their deficiency (particularly bile salts), cholesterol crystals precipitate in the gall bladder often resulting in cholelithiasis—***cholesterol gall stone disease.*** Cholelithiasis may be due to defective absorption of bile salts from the intestine, impairment in liver function, obstruction of biliary tract etc.

The patients of cholelithiasis respond to the administration of bile acid chenodeoxy cholic acid, commonly known as ***chenodiol.*** It is believed that a slow but gradual dissolution of gall stones occurs due to chenodiol. For severe cases of cholelithiasis, surgical removal of gall bladder is the only remedy.

II. Synthesis of steroid hormones from cholesterol

Cholesterol is the precursor for the synthesis of all the five classes of steroid hormones

(a) Glucocorticoids (e.g. cortisol)

(b) Mineralocorticoids (e.g. aldosterone)

(c) Progestins (e.g. progesterone)

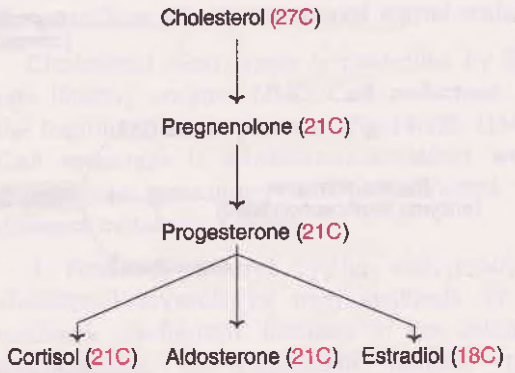

Fig. 14.30 : Outline of steroid hormone synthesis from cholesterol (Numbers in the brackets represent the number of carbon atoms).

(d) Androgens (e.g. testosterone)

(e) Estrogens (e.g. estradiol).

A brief outline of steroid hormonal synthesis is given in ***Fig.14.30*** and more details are discussed under 'Hormones' (***Chapter 19***).

III. Synthesis of vitamin D

7-Dehydrocholesterol, an intermediate in the synthesis of cholesterol, is converted to cholecalciferol (vitamin D_3) by ultraviolet rays in the skin.

A brief summary of prominent sources and the major pathways for utilization of cholesterol with the liver as the central metabolic organ is depicted in ***Fig.14.31.***

Transport of cholesterol

Cholesterol is present in the plasma lipoproteins in two forms

1. About 70-75% of it is in an esterified form with long chain fatty acids.

2. About 25-30% as free cholesterol. This form of cholesterol readily exchanges between different lipoproteins and also with the cell membranes.

Role of LCAT : High density lipoproteins (HDL) and the enzyme ***lecithin-cholesterol acyltransferase (LCAT)*** are responsible for the transport and elimination of cholesterol from the

body. LCAT is a plasma enzyme, synthesized by the liver. It catalyses the transfer of fatty acid from the second position of phosphatidyl choline (lecithin) to the hydroxyl group of cholesterol (***Fig.14.32***). HDL-cholesterol is the real substrate for LCAT and this reaction is freely reversible. ***LCAT activity*** is associated with ***apo-A_1 of HDL.***

The cholesterol (cholesteryl) ester forms an integral part of HDL. In this manner, the cholesterol from the peripheral tissues is trapped in HDL, by a reaction catalysed by LCAT and then transported to liver for degradation and excretion. This mechanism is commonly known as ***reverse cholesterol transport.***

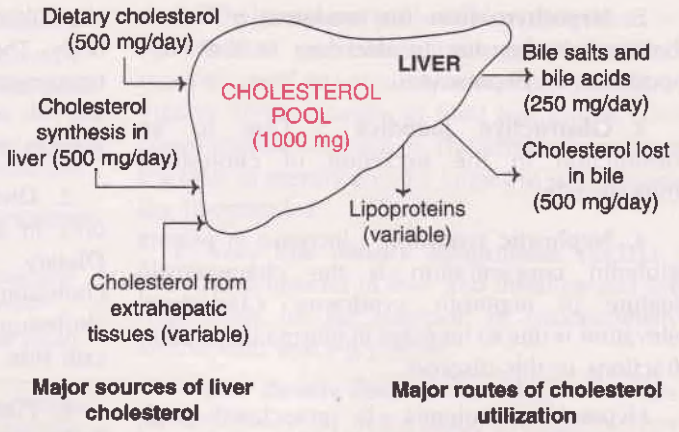

Fig. 14.31 *Summary of major sources of liver cholesterol and its utilization (values given in brackets are variable).*

Plasma cholesterol—biomedical importance

In healthy individuals, the total plasma cholesterol is in the range of 150-200 mg/dl. In the new born, it is less than 100 mg/dl and rises to about 150 mg/dl within an year. The ***women*** have relatively ***lower plasma cholesterol*** which is attributed to the hormones-***estrogens.*** Cholesterol level increases with increasing age (in women particularly after menopause), and also in pregnancy.

Plasma cholesterol is associated with different lipoprotein fractions (LDL, VLDL and HDL).

Total cholesterol can be estimated by many methods such as Libermann-Burchard reaction, Carr and Dructor method and, more recently, cholesterol oxidase method. HDL- cholesterol can be determined after precipitating LDL and VLDL by polyethylene glycol (PEG). VLDL cholesterol is equivalent to 1/5th of plasma triacylglycerol (TG) in a fasting state. LDL-cholesterol can be calculated from ***Friedewald formula*** given below.

LDL-cholesterol = Total cholesterol – (HDL-cholesterol + TG/5).

The above formula is not valid if TG concentration is above 400 mg/dl.

In adults, the normal LDL-cholesterol is about 80-150 mg/dl while HDL-cholesterol is around 30-60 mg/dl. Elevation in plasma HDL-cholesterol is beneficial to the body, since it protects the body from atherosclerosis and coronary heart diseases (CHD). On the other hand, increase in LDL-cholesterol is harmful to the body as it may lead to various complications, including CHD.

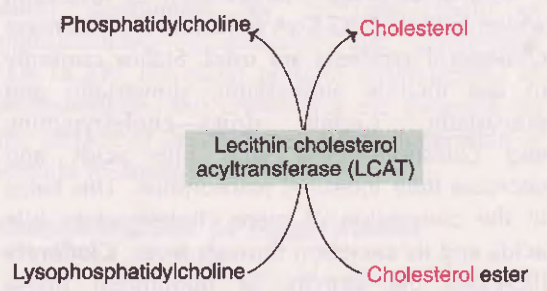

Fig. 14.32 : Reaction catalysed by LCAT.

HYPERCHOLESTEROLEMIA

Increase in plasma cholesterol (> 200 mg/dl) concentration is known as hypercholesterolemia and is observed in many disorders

1. **Diabetes mellitus :** Due to increased cholesterol synthesis since the availability of acetyl CoA is increased.

: This is in the HDL

2. **Hypothyroidism (m** : Due to an believed to be due t receptors on hepat retion of cholesterol

3. **Obstruc drome :** Increase in plasma obstructio tration is the characteristic through phrotic syndrome. Cholesterol ue to increase in plasma lipoprotein n this disorder.

ercholesterolemia is associated with ***rosclerosis*** and ***coronary heart disease.*** herosclerosis is characterized by deposition of cholesteryl esters and other lipids in the intima of the arterial walls often leading to hardening of coronary arteries and cerebral blood vessels. A positive correlation between raised plasma lipids with atherosclerosis on one hand and coronary heart disease on the other has been established. More specifically, LDL-cholesterol is positively correlated, whereas HDL-cholesterol is negatively correlated with cardiovascular diseases.

Bad cholesterol and good cholesterol : Cholesterol is a natural metabolite performing a wide range of functions (membrane structure, precursor for steroid hormones, bile acids). The usages good and bad to cholesterol, although inappropriate, are still in use. The cholesterol in high concentration, present in ***LDL***, is considered ***bad*** due to its involvement in altherosclerosis and related complications. Thus, LDL may be regarded as ***l***ethally ***d***angerous ***l***ipoprotein. On the other hand, ***HDL cholesterol*** is ***good*** since its high concentration counteracts atherogenesis. HDL may be considered as ***h***ighly ***d***esirable ***l***ipoprotein.

Control of hypercholesterolemia

Several measures are advocated to lower the plasma cholesterol level

1. **Consumption of PUFA :** Dietary intake of polyunsaturated fatty acids (PUFA) ***reduces the plasma cholesterol level***. PUFA will help in transport of cholesterol by LCAT mechanism (described earlier) and its excretion from the body. The oils with rich PUFA content include cottonseed oil, soya bean oil, sunflower oil, corn oil, fish oils etc. Ghee and coconut oil are poor sources of PUFA.

2. **Dietary cholesterol :** Cholesterol is found only in animal foods and not in plant foods. Dietary cholesterol ***influence*** on plasma cholesterol is ***minimal***. However, avoidance of cholesterol-rich foods is advocated to be on the safe side.

3. **Plant sterols :** Certain plant sterols and their esters (e.g. sitostanol esters) ***reduce*** plasma cholesterol levels. They inhibit the intestinal absorption of dietary cholesterol.

4. **Dietary fiber :** Fiber present in vegetables decreases the cholesterol absorption from the intestine.

5. **Avoiding high carbohydrate diet :** Diets rich in carbohydrates (particularly sucrose) should be avoided to control hypercholesterolemia.

6. **Impact of lifestyles :** Elevation in plasma cholesterol is obseved in people with smoking, abdominal obesity, lack of exercise, stress, high blood pressure, consumption of soft water etc. Therefore, adequate changes in the lifestyles will bring down plasma cholesterol.

7. **Moderate alcohol cosumption :** The beneficial effects of moderate alcohol intake are masked by the ill effects of chronic alcoholism. ***Red wine*** is particularly ***beneficial*** due to its antioxidants, besides low alcohol content.

8. **Use of drugs :** Drugs such as ***lovastatin*** which inhibit HMG CoA reductase and decrease cholesterol synthesis are used. Statins currently in use include atorvastatin, simvastatin and pravastatin. Certain drugs—cholestyramine and colestipol—bind with bile acids and decrease their intestinal reabsorption. This helps in the conversion of more cholesterol to bile acids and its excretion through feces. ***Clofibrate*** increases the activity of lipoprotein lipase and reduces the plasma cholesterol and triacylglycerols.

2. **Hypothyroidism (myxoedema) :** This is believed to be due to decrease in the HDL receptors on hepatocytes.

3. **Obstructive jaundice :** Due to an obstruction in the excretion of cholesterol through bile.

4. **Nephrotic syndrome :** Increase in plasma globulin concentration is the characteristic feature of nephrotic syndrome. Cholesterol elevation is due to increase in plasma lipoprotein fractions in this disorder.

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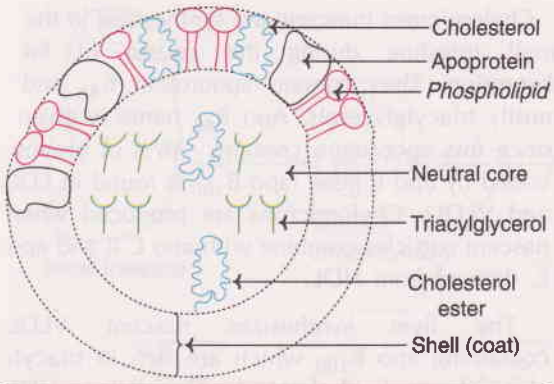


Fig. 14.33 : A general structure of lipoprotein complex. (Note : For the sake of clarity, only a part of the shell and core are filled with the constituents).

Hypocholesterolemia

A decrease in the plasma cholesterol, although less common, is also observed. Hyperthyroidism, pernicious anemia, malabsorption syndrome, hemolytic jaundice etc., are some of the disorders associated with hypocholesterolemia.

LIPOPROTEINS

Lipoproteins are ***molecular complexes*** that consist ***of lipids and proteins*** (conjugated proteins). They function as transport vehicles for lipids in blood plasma. Lipoproteins deliver the lipid components (cholesterol, triacylglycerol etc.) to various tissues for utilization.

Structure of lipoproteins

A lipoprotein basically consists of a neutral lipid core (with triacylglycerol and/or cholesteryl ester) surrounded by a coat shell of phospholipids, apoproteins and cholesterol (***Fig.14.33***). The polar portions (amphiphilic) of phospholipids and cholesterol are exposed on the surface of lipoproteins so that lipoprotein is soluble in aqueous solution.

Classification of lipoproteins

Five major classes of lipoproteins are identified in human plasma, based on their separation by electrophoresis (***Fig.14.34***).

1. **Chylomicrons :** They are synthesized in the intestine and transport exogenous (dietary) *triacylglycerol to various tissues. They consist of* highest (99%) quantity of lipid and lowest (1%) *concentration of protein. The chylomicrons are* the least in density and the largest in size, among the lipoproteins.

2. **Very low density lipoproteins (VLDL) :** They are produced in liver and intestine and are responsible for the transport of endogenously synthesized triacylglycerols.

3. **Low density lipoproteins (LDL) :** They are formed from VLDL in the blood circulation. They transport cholesterol from liver to other tissues.

4. **High density lipoproteins (HDL) :** They are mostly synthesized in liver. Three different fractions of HDL (1, 2 and 3) can be identified by ultracentrifugation. HDL particles transport cholesterol from peripheral tissues to liver (reverse cholesterol transport).

5. **Free fatty acids—albumin :** Free fatty acids in the circulation are in a bound form to albumin. Each molecule of albumin can hold

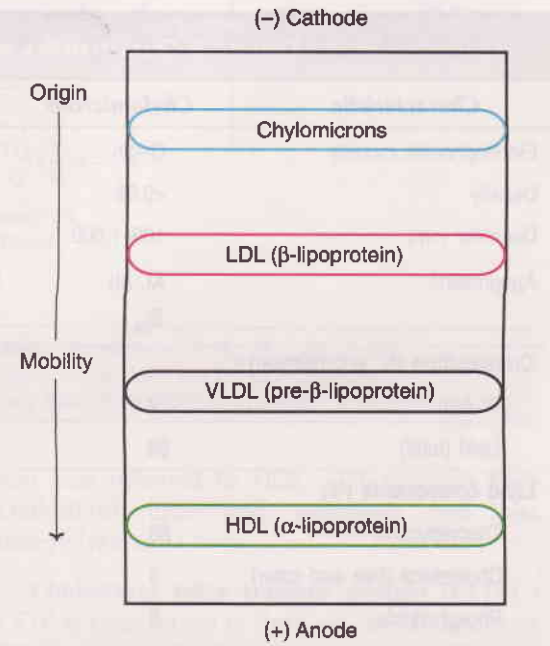


Fig. 14.34 : Electrophoresis of plasma (serum) lipoproteins.

about 20-30 molecules of free fatty acids. This lipoprotein cannot be separated by electrophoresis.

Apolipoproteins (apoproteins)

The protein components of lipoproteins are known as apolipoproteins or, simply, apoproteins. They perform the following functions

1. Act as structural components of lipoproteins.
2. Recognize the cell membrane surface receptors.
3. Activate enzymes involved in lipoprotein metabolism.

The comparative characteristic features of different lipoproteins with regard to electrophoretic patterns, size, composition etc. are given in ***Table 14.5***.

Metabolism of lipoproteins —a general view

A general picture of lipoprotein metabolism is depicted in ***Fig.14.35***.

Chylomicrons (nascent) are synthesized in the small intestine during the course of fat absorption. They contain apoprotein B_{48} and mostly triacylglycerols. Apo B_{48} name is given since this apoprotein contains 48% of protein coded by apo B gene (apo B_{100} is found in LDL and VLDL). Chylomicrons are produced when nascent particles combine with apo C II and apo E, derived from HDL.

The liver synthesizes nascent VLDL containing apo B_{100} which are rich in triacylglycerols and cholesterol. Circulating HDL donates apo C II and apo E to convert nascent VLDL to VLDL.

Role of lipoprotein lipase : The enzyme lipoprotein lipase is present in the capillary walls of adipose tissue, cardiac and skeletal muscle, besides other tissues. It hydrolyses a portion of triacylglycerols present in chylomicrons and VLDL to liberate free fatty acids and glycerol. Lipoprotein lipase is activated by apo C II.

Uptake of chylomicron remnants by liver : As the triacylglycerols of chylomicrons and VLDL are degraded, they lose the apo C II which

Table 14.5 Characteristics of human plasma lipoproteins

Characteristic	*Chylomicrons*	*VLDL*	*LDL*	*HDL*
Electrophoretic mobility	Origin	Pre-β	β	α
Density	<0.96	0.96–1.006	1.006–1.063	1.063–1.21
Diameter (nm)	100–1,000	30–90	20–25	10–20
Apoproteins	AI, AII B_{48}	B_{100}, CI, CII CIII, E	B_{100}	AI, AII, CI, CII, CIII, D, E
Composition (%, approximate)				
Protein	2	10	20	40
Lipid (total)	98	90	80	60
Lipid components (%)				
Triacylglycerol	88	55	12	12
Cholesterol (free and ester)	4	24	59	40
Phospholipids	8	20	28	47
Free fatty acids	—	1	1	1

(VLDL : Very low density lipoproteins; LDL : Low density lipoproteins; HDL : High density lipoproteins).

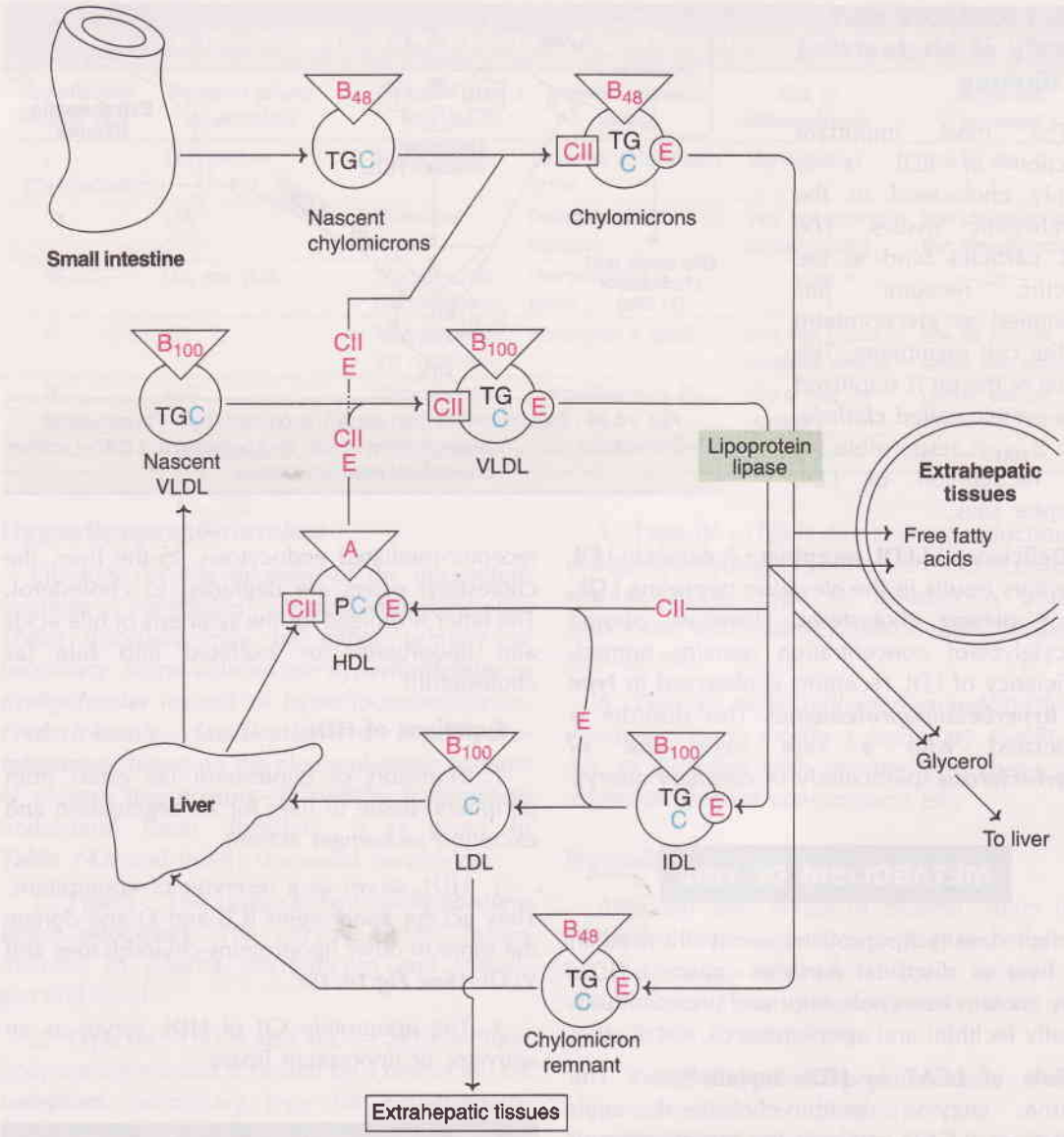


Fig. 14.35 : Summary of metabolism of lipoproteins (Apoproteins–A, B_{48}, B_{100}, CII and E; TG–Triacylglycerol; C–Cholesterol; P–Phospholipid; VLDL–Very low density lipoprotein; IDL–Intermediate density lipoprotein; LDL–Low density lipoprotein; HDL–High density lipoprotein).

is returned to HDL. The chylomicron remnants are taken up by receptors present on the hepatocytes of liver.

Conversion of VLDL to LDL

During the course of VLDL metabolism, intermediate density lipoprotein (IDL) is formed which lose apo-E and get converted to LDL. The apo E is returned to HDL. LDL contains high cholesterol (free and esterified) and less triacylglycerol.

Cholesterol ester transfer protein (CETP) : CETP is synthesized in the liver, and it facilitates the exchange of components between different lipoproteins. CETP can transfer cholesterol esters from HDL to VLDL or LDL, in exchange for TG.

LDL receptors and supply of cholesterol to tissues

The most important function of LDL is to supply cholesterol to the extrahepatic tissues. The LDL particles bind to the specific receptor pits (identified as glycoprotein) on the cell membrane. The shape of the pit is stabilized by a protein called ***clathrin***. Apo B_{100} is responsible for the recognition of LDL receptor sites.

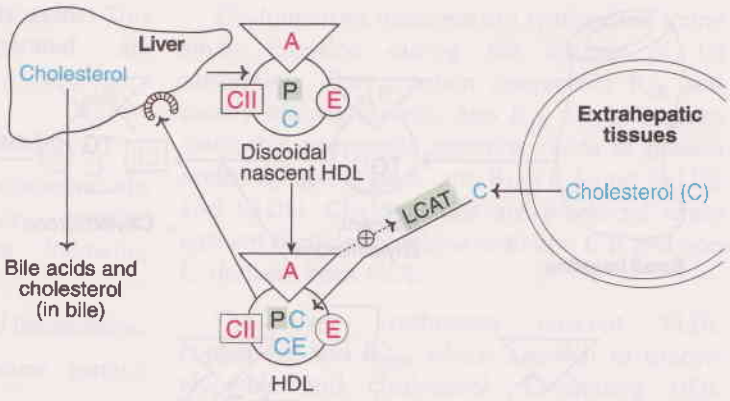


Fig. 14.36 : Metabolism of high density lipoproteins (P–Phospholipid; C–Cholesterol; CE–Cholesteryl ester; A, CII, E–Apoproteins; LCAT–Lecithin cholesterol acyltransferase).

Deficiency of LDL receptors : A defect in LDL receptors results in the elevation of plasma LDL, hence plasma cholesterol. However, plasma triacylglycerol concentration remains normal. Deficiency of LDL receptors is observed in ***type IIa hyperbetalipoproteinemia.*** This disorder is associated with a very high ***risk of atherosclerosis*** (particularly of coronary artery).

METABOLISM OF HDL

High density lipoproteins are synthesized in the liver as discoidal particles – nascent HDL. They contain free cholesterol and phospholipids (mostly lecithin) and apoproteins (A, CII, E etc.).

Role of LCAT in HDL metabolism : The plasma enzyme lecithin-cholesterol acyl-transferase (LCAT) catalyses the esterification of free cholesterol (by fatty acid of lecithin) present in the extrahepatic tissues and transfers to the HDL. Apoprotein A promotes the activity of LCAT. HDL also accepts free cholesterol from other lipoproteins in circulation and cell membrane of peripheral tissues (***Fig.14.36***). Any free cholesterol taken up by HDL undergoes LCAT-catalysed esterification. Due to the addition of cholesterol, HDL particles become spherical.

The HDL particles, with cholesteryl ester trapped inside, enter the hepatocytes by a receptor-mediated endocytosis. In the liver, the cholesteryl esters are degraded to cholesterol. The latter is utilized for the synthesis of bile acids and lipoproteins or excreted into bile (as cholesterol).

Functions of HDL

1. Transport of cholesterol (as ester) from peripheral tissue to liver for its degradation and excretion (scavenger action).

2. HDL serves as a reservoir of apoproteins. They accept apoproteins (CII and E) and donate the same to other lipoproteins-chylomicrons and VLDL (***See Fig.14.35***).

3. The apoprotein CII of HDL serves as an activator of lipoprotein lipase.

DISORDERS OF PLASMA LIPOPROTEINS

Inherited disorders of lipoproteins are encountered in some individuals resulting in ***primary*** hyper- or hypolipoproteinemias. These are due to genetic defects in lipoprotein metabolism and transport. The ***secondary*** acquired lipoprotein disorders are due to some other diseases (e.g. diabetes mellitus, nephrotic syndrome, atherosclerosis, hypothyrodism etc.), resulting in abnormal lipoprotein pattern which often resembles the primary inherited condition.

Table 14.6 Classification and characteristics of hyperlipoproteinemias (hyperlipidemias)

Hyperlipoproteinemia Type	*Increased plasma lipoprotein(s)*	*Increased plasma lipid (most)*	*Probable metabolic defect*	*Risk of atherosclerosis*	*Suggested treatment*
I	Chylomicrons	Triacylglycerols	Deficiency of lipoprotein lipase	May increase	Low fat diet
IIa	LDL	Cholesterol	Deficiency of LDL receptors	Very high (mostly in coronary artery)	Low cholesterol fat diet; cholestyramine
IIb	LDL and VLDL	Triacylglycerols and cholesterol	Overproduction of apo-B	— do —	— do —
III	IDL	Triacylglycerols and cholesterol	Abnormality in apo-E	Very high (mostly in peripheral vessels)	Low fat and low caloric diet; clofibrate
IV	VLDL	Triacylglycerols	Overproduction of TG	May or may not increase	Low fat and low caloric diet; niacin
V	Chylomicrons and VLDL	Triacylglycerols	—	— do —	— do —

Hyperlipoproteinemias

Elevation in one or more of the lipoprotein fractions constitutes hyperlipoproteinemias. These disorders may be either primary or secondary. Some authors use ***hyperlipidemias*** or ***dyslipidemias*** instead of hyperlipoproteinemias. ***Frederickson's classification of hyperlipoproteinemias***—based on the electrophoretic patterns of plasma lipoproteins—is widely accepted to understand these disorders. It is given in ***Table 14.6*** and briefly discussed hereunder.

1. **Type I :** This is due to familial ***lipoprotein lipase deficiency.*** The enzyme defect causes increase in plasma chylomicron and triacylglycerol levels.

2. **Type IIa :** This is also known as hyperbetalipoproteinemia and is caused by ***a defect in LDL receptors.*** Secondary type IIa hyperlipoproteinemia is observed in association with diabetes mellitus, hypothyroidism, nephrotic syndrome etc. This disorder is characterized by hypercholesterolemia.

3. **Type IIb :** Both LDL and VLDL increase along with elevation in plasma cholesterol and triacylglycerol. This is believed to be due to ***overproduction of apo B.***

4. **Type III :** This is commonly known as ***broad beta disease*** and characterized by the appearance of a broad β-band corresponding to ***intermediate density lipoprotein*** (IDL) on electrophoresis.

5. **Type IV :** This is due to overproduction of endogenous triacylglycerols with a concomitant ***rise in VLDL.*** Type IV disorder is usually associated with obesity, alcoholism, diabetes mellitus etc.

6. **Type V :** Both chylomicrons and VLDL are elevated. This is mostly a secondary condition, due to disorders such as obesity, diabetes and excessive alcohol consumption etc.

Hypolipoproteinemias

Although low levels of plasma lipids (not HDL!) within the normal range may be beneficial to the body, very low lipid levels are undesirable. These are commonly associated with certain abnormalities

1. **Familial hypobetalipoproteinemia :** It is an inherited disorder probably due to an impairment in the synthesis of apoprotein B. The plasma LDL concentration in the affected individuals is between 10 to 50% of normal values. This disorder is harmless, and the individuals have healthy and long life.

2. **Abetalipoproteinemia :** This is a rare disorder due to a defect in the synthesis of apoprotein B. It is characterized by a total absence of β-lipoprotein (LDL) in plasma. Triacylglycerols are not found in plasma, but they accumulate in liver and intestine. Serum cholesterol level is low. Abetalipoproteinemia is associated with decreased absorption of fat

and fat-soluble vitamins. Impairment in physical growth and mental retardation are commonly observed.

Familial alpha-lipoprotein deficiency (Tangier disease) : The plasma HDL particles are almost absent. Due to this, the reverse transport of cholesterol is severely affected leading to the accumulation of cholesteryl esters in tissues. An absence of apoprotein C II—which activates lipoprotein lipase—is also found. The plasma triacylglycerol levels are elevated. The affected individuals are at an increased risk for atherosclerosis.

FATTY LIVER

The normal concentration of lipid (mostly phospholipid) in liver is around 5%. Liver is not a storage organ for fat, unlike adipose tissue. However, in certain conditions, lipids—especially the ***triacylglycerols—accumulate*** excessively in liver, resulting in fatty liver (***Fig.14.37***). In the normal liver, Kupffer cells contain lipids in the form of droplets. In fatty liver, droplets of triacylglycerols are found in the entire cytoplasm of hepatic cells. This causes impairment in metabolic functions of liver. Fatty liver is associated with fibrotic changes and cirrhosis, Fatty liver may occur due to two main causes.

1. Increased synthesis of triacylglycerols
2. Impairment in lipoprotein synthesis.

1. **Increased triacylglycerol synthesis :** Mobilization of free fatty acids from adipose tissue and their influx into liver is much higher than their utilization. This leads to the overproduction of triacylglycerols and their accumulation in liver. ***Diabetes mellitus***, ***starvation***, ***alcoholism*** and ***high fat diet*** are associated with increased mobilization of fatty acids that often cause fatty liver. Alcohol also inhibits fatty acid oxidation and, thus, promotes fat synthesis and its deposition.

BIOMEDICAL / CLINICAL CONCEPTS

- *Niemann-Pick disease, caused by a defect in the enzyme sphingomyelinase, results in the accumulation of sphingomyelins in liver and spleen.*
- *About a dozen glycolipid storage diseases are known. These include Gaucher's disease and Krabbe's disease.*
- *Hypercholesterolemia is associated with atherosclerosis and coronary heart diseases. Consumption of polyunsaturated fatty acids and fiber decreases cholesterol in circulation. Drugs—such as lovastatin, cholestyramine, compactin and clofibrate—reduce plasma cholesterol.*
- *Cholelithiasis, a cholesterol gall stone disease, is caused by a defect in the absorption of bile salts from the intestine or biliary tract obstruction.*
- *High density lipoproteins—in association with lecithin-cholesterol acyltransferase (LCAT)—are responsible for the transport and elimination of cholesterol from the body.*
- *Hyperlipoproteinemias are a group of disorders caused by the elevation of one or more of plasma lipoprotein fractions.*
- *Excessive accumulation of triacylglycerols causes fatty liver which can often be prevented by the consumption of lipotropic factors (choline, betaine, methionine).*

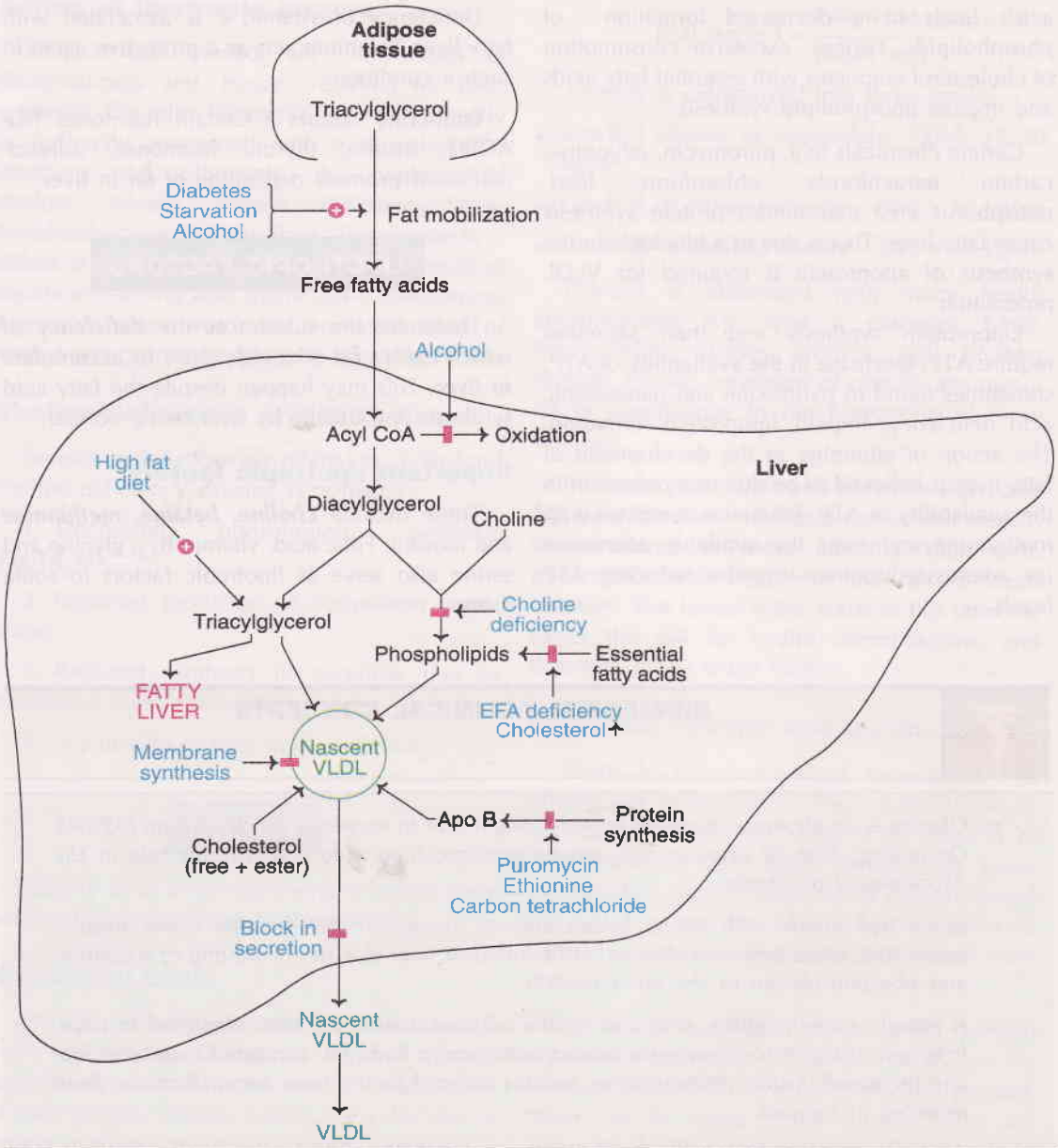


Fig. 14.37 : Development of fatty liver along with responsible factors.

2. **Impaired synthesis of lipoproteins :** The synthesis of very low density lipoproteins (VLDL) actively takes place in liver. VLDL formation requires phospholipids and apoprotein B. Fatty liver caused by impaired lipoprotein synthesis may be due to :

- a defect in phospholipid synthesis;
- a block in apoprotein formation;
- a failure in the formation/secretion of lipoprotein.

Among the three causes, fatty liver due to impairment in phospholipid synthesis has been studied in some detail. This is usually associated with the dietary ***deficiency of lipotropic factors*** such as choline, betaine, inositol etc. (more details given later). Deficiency of essential fatty

acids leads to a decreased formation of phospholipids. Further, excessive consumption of cholesterol competes with essential fatty acids and impairs phospholipid synthesis.

Certain chemicals (e.g. puromycin, ethionine, carbon tetrachloride, chloroform, lead, phosphorus etc.) that inhibit protein synthesis cause fatty liver. This is due to a blockade in the synthesis of apoprotein B required for VLDL production.

Lipoprotein synthesis and their secretion require ATP. Decrease in the availability of ATP, sometimes found in pyridoxine and pantothenic acid deficiency, impairs lipoprotein formation. The action of ethionine in the development of fatty liver is believed to be due to a reduction in the availability of ATP. Ethionine competes with methionine and traps the available adenosine (as adenosylethionine)—thereby reducing ATP levels.

Deficiency of vitamin E is associated with fatty liver. Selenium acts as a protective agent in such a condition.

Endocrine factors : Certain hormones like ACTH, insulin, thyroid hormones, adrenocorticoids promote deposition of fat in liver.

LIPOTROPIC FACTORS

These are the substances the ***deficiency of which causes fat*** (triacylglycerol) ***to accumulate in liver.*** This may happen despite the fatty acid synthesis and uptake by liver being normal.

Important lipotropic factors

These include ***choline, betaine, methionine*** and inositol. Folic acid, vitamin B_{12}, glycine and serine also serve as lipotropic factors to some extent.

BIOMEDICAL / CLINICAL CONCEPTS

- *Obesity is an abnormal increase in body weight due to excessive fat deposition (>25%). Overeating, lack of exercise and genetic predisposition play a significant role in the development of obesity.*
- *Some individuals with active brown adipose tissue do not become obese despite overeating, since whatever they eat is liberated as heat due to uncoupling of oxidation and phosphorylation in the mitochondria.*
- *A protein namely leptin, produced by the adipose tissue, has been identified in mice. Injection of leptin to obese mice caused reduction in body fat, increased metabolic rate and increased insulin concentration, besides reduced food intake. Leptin has also been detected in humans.*
- *Anorexia nervosa is a psychiatric disorder associated with total loss of appetite—mostly found in females in the age group 10–30 years.*
- *Atherosclerosis is characterized by hardening of arteries due to the accumulation of lipids and other compounds. The probable causes of atherosclerosis include hyperlipoproteinemias, diabetes mellitus, obesity, high consumption of saturated fat, lack of exercise and stress.*
- *Atherosclerosis and coronary heart disease are directly correlated with plasma cholesterol and LDL, inversely with HDL. Elevation of plasma lipoprotein a suggests increased risk of CHD.*
- *Alcoholism is associated with fatty liver, hyperlipidemia and atherosclerosis.*

Action of lipotropic factors

Choline and inositol are components of phospholipids and, hence, required for their synthesis. The other lipotropic factors are directly or indirectly concerned with transmethylation reactions and, ultimately, the synthesis of choline. Severe protein deficiency (e.g. kwashiorkor) causes fatty liver. This is due to a defect in the synthesis of choline as a result of insufficient amino acid (particularly methionine) supply. In other words the non-availability of methyl groups may lead to fatty liver (***Fig.14.37***).

Choline deficiency and fatty liver

Several explanations are offered to understand choline deficiency causing fatty liver :

1. Decreased phospholipid synthesis (***Fig.14.37***)
2. Impaired formation of lipoprotein membrane
3. Reduced synthesis of carnitine due to insufficient supply of methyl groups
4. Impairment in fatty acid oxidation.

OBESITY

Obesity is an abnormal increase in the body weight due to excessive fat deposition.

Nutritional basis

Men and women are considered as obese if their weight due to fat (in adipose tissue), respectively, exceeds more than 20% and 25% of body weight. Obesity is basically a disorder of excess calorie intake, in simple language—***overeating***. It has to be remembered that every 7 calories of excess consumption leads to 1 g fat deposit and increase in body weight. Overeating—coupled with ***lack of physical exercise***—contribute to obesity.

Body mass index (BMI)

Clinical obesity is represented by body mass index. BMI is calculated as the weight (in kilograms) divided by the height (in meters^2).

$$\text{BMI (kg/m}^2) = \frac{\text{Weight (kg)}}{[\text{height (m)}^2]}$$

Obesity is categorized into three grades

- Grade I obesity or overweight – BMI 25–30 kg/m^2
- Grade II or clinical obesity – BMI > 30 kg/m^2
- Grade III or morbid obesity – BMI > 40 kg/m^2

Obesity is associated with many health complications e.g. type II diabetes, CHD, hypertension, stroke, arthritis, gall bladder disease. Hence, treatment of obesity assumes a lot of significance in the prevention of these diseases.

In recent years, the ***ratio between waist and hip sizes*** (for men < 0.9 and for women < 0.85) is considered as ***more effective than BMI***, particularly with regard to the risk of heart diseases. The lower is the waist to hip ratio the lower the risk for health complications, and therefore better is the health.

Genetics, obesity and leptin

There is strong evidence to suggest that obesity has genetic basis. Thus, a child born to two obese people has about 75% chances of being obese. One gene namely ***ob gene***, expressed in adipocytes (of white adipose tissue) producing a protein called ***leptin*** (mol. wt. 16,000 daltons), is closely associated with obesity.

Leptin is regarded as a ***body weight regulatory hormone***. It binds to a specific receptor in the brain and functions as a ***lipostat***. When the fat stores in the adipose tissue are adequate, leptin levels are high. This signals to restrict the feeding behaviour and limit fat deposition. Further, leptin stimulates lipolysis and inhibits lipogenesis. Any genetic defect in leptin or its receptor will lead to extreme overeating and massive obesity. Treatment of such obese individuals with leptin has been shown to reverse obesity.

During starvation, leptin levels fall which promote feeding, and fat production and its deposition.

Obesity and adipose tissue

There are two types of adipose tissues

1. **White adipose tissue :** The fat is mostly stored and this tissue is metabolically less active.

2. **Brown adipose tissue :** The stored fat is relatively less but the tissue is metabolically very active.

Brown adipose tissue possesses high proportion of mitochondria and cytochromes but low activity of ATP synthase. This is an active centre for the oxidation of fatty acids and glucose and is responsible for the ***diet-induced thermogenesis***.

The peculiarity of mitochondria of brown adipose tissue is that the oxidation and phosphorylation are not coupled. Mitochondrial oxidation produces ***more heat*** and ***less ATP***. A specific protein—namely ***thermogenin***—has been isolated in the inner membrane of these mitochondria. Thermogenin functions like an uncoupler and dissipates the energy in the form of heat, and thus blocks the formation of ATP.

Brown adipose tissue is mostly found in hibernating animals, and the animals exposed to cold, besides the newborn. In adult humans, though not a prominent tissue, it is located in the thoracic region. It is significant to note that ***brown adipose tissue is almost absent in obese persons***. Some individuals are fortunate to have active brown adipose tissue. They eat and liberate it as heat with the result that they do not become obese.

CACHEXIA

This is exactly opposite of what is seen in obesity. Cachexia is characterized by a failure to maintain normal lipid stores in the body. It involves higher rate of fat mobilization than the deposition. In extreme cases, the adipose tissue may totally disappear.

Anorexia nervosa is a total loss of appetite. This is mostly seen in females in the age group 10-30 years. Surprisingly, majority of the affected individuals are from wealthy families where food is aplenty. And some members in these families may be even obese! Anorexia nervosa is more a psychiatric disease.

XANTHOMATOSIS

The deposition of yellow-orange colour lipids in liver, spleen and flat bones of the skull is known as xanthomatosis (*Greek* : xanthos—yellow). This is usually associated with severe hyperlipidemia and hypercholesterolemia.

ATHEROSCLEROSIS

Atherosclerosis (*Greek*: athere—mush) is a complex disease characterized by thickening or ***hardening of arteries due to the accumulation of lipids*** (particularly cholesterol, free, and esterified) collagen, fibrous tissue, proteoglycans, calcium deposits etc. in the inner arterial wall. Atherosclerosis is a progressive disorder that narrows and ultimately blocks the arteries. Infarction is the term used to indicate the stoppage of blood flow resulting in the death of affected tissue. ***Coronary arteries***—the arteries supplying blood to heart—are the most commonly affected leading to myocardial infarction or heart attacks.

The incidence of atherosclerosis and coronary heart diseases are higher in developed countries (e.g. USA, U.K.) than in the developing countries (India, Africa etc.).

Causes of atherosclerosis and CHD : The development of atherosclerosis and the risk for the coronary heart disease (CHD) is directly correlated with plasma cholesterol and LDL. On the other hand, plasma HDL is inversely correlated with CHD.

Disorders that may cause atherosclerosis

Certain diseases are associated with atherosclerosis. These include diabetes mellitus, hyperlipoproteinemias, nephrotic syndrome, hypothyroidism etc. Many other factors like obesity, high consumption of saturated fat, excessive smoking, lack of physical exercise,

hypertension, stress etc., are the probable causes of atherosclerosis.

Relation between HDL and CHD

The increased levels of plasma HDL (good cholesterol) are correlated with a low incidence of cardiovascular disorders. ***Women*** have higher HDL and are ***less prone to heart diseases*** compared to men. This is attributed to ***estrogens*** in women. Strenuous physical exercise, moderate alcohol intake, consumption of unsaturated fatty acids (vegetable and fish oils), reduction in body weight—all tend to increase HDL levels and reduce the risk of cardiovascular diseases. Some more details on cholesterol and atherosclerosis are given under hypercholesterolemia.

Lipoprotein a and CHD

Lipoprotein a (Lp-a) is almost identical in structure to LDL. Lp-a contains an additional apoprotein, apo-a. Lp-a inhibits fibrinolysis. Recent studies have shown that elevation of lipoprotein-a in the plasma (>30 mg/dl) suggests increased risk of CHD. It is hypothesized that elevated Lp-a reduces the breakdown of blood clots and triggers heart attacks.

Antioxidants and atherosclerosis

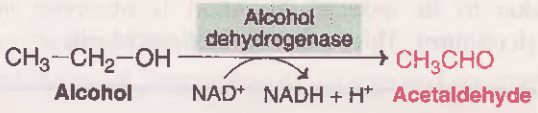
Antioxidants, in general, decrease the oxidation of LDL. There is some evidence, based on the epidemiological studies that taking of antioxidants (vitamins E and C or β-carotene) reduces the risk of atherosclerosis, and thereby CHD. However, more research is needed in this direction.

ALCOHOL METABOLISM

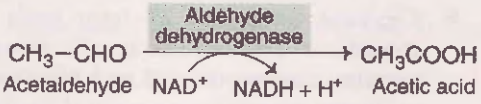
Walker has rightly said 'alcohol can be a food, a drug or a poison depending on the dose.' In small quantities, alcohol relieves tension and anxiety. Unfortunately, consumption of alcohol seldom ends with small doses, hence the beneficial effects are over-shadowed by the harmful effects.

Alcohol (ethanol or ethyl alcohol) is readily absorbed by the stomach and intestine. Consequently, less than 2% of the alcohol consumed is excreted through lungs, urine and sweat.

Alcohol gets oxidized in the liver by alcohol dehydrogenase to acetaldehyde.



Besides ADH, microsomal ethanol oxidizing system (MEOS) is also involved in the metabolism of alcohol. ***Aldehyde,*** produced by the action of either ADH or MEOS, is ***responsible for the manifestations of alcohol.*** The enzyme aldehyde dehydrogenase converts aldehyde to acetic acid which then enters Krebs cycle in the form of acetyl CoA.



Since the activity of aldehyde dehydrogenase is less than that of alcohol dehydrogenase, acetaldehyde accumulates leading to various complications. ***Disulfiram,*** a drug used for the treatment of alcoholism, ***inhibits aldehyde dehydrogenase.***

Biochemical changes in alcoholism

The metabolism of alcohol (by both dehydrogenases) involves the consumption of NAD^+, and consequently a high $NADH/NAD^+$ ratio. This is mostly responsible for the metabolic alterations observed in alcoholism. Some of them are listed.

1. High concentration of NADH favours the conversion of pyruvate to lactate which may lead to ***lactic acidosis.***

2. ***Hypoglycemia*** due to reduced gluconeogenesis is observed. This happens as a result of decreased availability of pyruvate and oxaloacetate (the latter gets converted to malate by high NADH).

3. Citric acid cycle is impaired since the availability of oxaloacetate and NAD^+ is reduced. As a result, acetyl CoA accumulates which gets diverted towards ketogenesis, cholesterologenesis, and fatty acid synthesis. Accumulation of fats leads to fatty liver and hyperlipidemia.

4. Increased concentration of serum uric acid due to its reduced excretion is observed in alcoholism. This is due to lactic acidosis.

5. Acetaldehyde interferes with the functioning of neurotransmitters, with an overall effect of neurological depression.

6. Acetaldehyde causes headache, nausea, tachycardia, reduced blood pressure etc.

Effects of chronic alcoholism

Chronic alcoholism is associated with cirrhosis of liver, neurodegenerative changes, cardiomyopathy, diuresis, impotence etc.

SUMMARY

1. *Triacylglycerols (TG) are the highly concentrated form of energy, stored in adipose tissue. Hormone-sensitive lipase hydrolyses TG to free fatty acids which are transported as albumin-FFA complexes.*
2. *Fatty acids are activated (acyl CoA) and transported by carnitine to mitchondria where they get oxidized (mostly by β-oxidation) to liberate energy. Complete oxidation of one mole palmitate liberates 129 ATP.*
3. *Excessive utilization of fatty acids occurs in uncontrolled diabetes mellitus and starvation. This results in the overproduction of ketone bodies (in liver), namely acetone, acetoacetic acid and β-hydroxy butyric acid. The last two ketone bodies serve as energy source for peripheral tissues.*
4. *Fatty acid biosynthesis occurs from acetyl CoA in the cytosol through the involvement of a multienzyme complex associated with acyl carrier protein (ACP). The reducing equivalents ($NADPH + H^+$) are supplied mostly by HMP shunt.*
5. *Synthesis of triacylglycerols and phospholipids (PL) occurs from glycerol 3-phosphate and dihydroxyacetone phosphate with the addition of acyl CoA, and activated nitrogenous bases (for PL).*
6. *Cholesterol is synthesized from acetyl CoA in a series of reactions involving HMG CoA, mevalonate, isoprenoid units and squalene as the intermediates. Cholesterol serves as a precursor for bile acids, steroid hormones and vitamin D.*
7. *Lipoproteins are the transport vehicles for lipids in the plasma. Lipoprotein disorders are associated with abnormalities in their plasma levels. Elevation in LDL and VLDL—in association with cholesterol and TG—poses a serious health problem with increased risk of atherosclerosis and CHD.*
8. *Excessive accumulation of triacylglycerols in liver causes fatty liver, which may be due to increased production of TG or impairment in lipoprotein (VLDL) synthesis. The latter is mostly associated with the deficiency of certain substances called lipotropic factors (e.g. choline, betaine, methionine etc.)*
9. *Obesity is an abnormal increase in body weight (with more than 25% due to fat). Among the many causative factors of obesity, lack of active brown adipose tissues (which burn fat and liberate heat) in these individuals is gaining importance.*
10. *Atherosclerosis is a complex disease characterized by thickening of arteries due to the accumulation of lipids. Atherosclerosis and CHD are directly correlated with LDL and inversely with HDL of plasma.*

SELF-ASSESSMENT EXERCISES

I. Essay questions

1. Describe the functions and metabolism of phospholipids.
2. Give an account of cholesterol biosynthesis. Add a note on the significance of plasma cholesterol estimation.
3. Describe in detail the extramitochondrial synthesis of fatty acids.
4. Write about the types, characteristics and metabolism of lipoproteins. Add a note on lipoprotein disorders.
5. Give an account of fatty acid oxidation.

II. Short notes

(a) Carnitine, (b) LCAT, (c) Fatty liver, (d) Ketone bodies, (e) Lipotropic factors, (f) Acyl carrier protein, (g) Degradation of cholesterol, (h) HDL, (i) Lipoprotein lipase, (j) Brown adipose tissue.

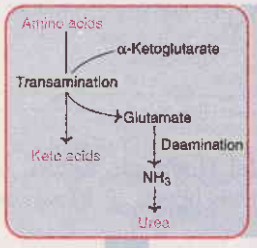
III. Fill in the blanks

1. The most predominant lipid component of chylomicrons _____.
2. Cholesterol synthesis is controlled by feedback inhibition of the enzyme _____.
3. A compound possessing hydrophobic and hydrophilic groups in its structure is known as _____.
4. Niemann-Pick disease is due to a defect in the enzyme _____.
5. The lipoprotein involved in the reverse cholesterol transport is _____.
6. The total number of ATP produced by the oxidation of a molecule of palmitic acid is _____.
7. The long chain fatty acids (C_{26}—C_{35}) are not oxidized due to the absence of peroxisomes. This disorder is known as _____.
8. Acetyl CoA from the mitochondria is transported into the cytosol after its conversion to _____.
9. Plasma lipoprotein that is inversely correlated with coronary heart disease is _____.
10. The fatty acid that is commonly found in the C_2 of triacylglycerols is _____.

IV. Multiple choice questions

11. The following substance(s) is (are) ketogenic
    (a) Fatty acids (b) Leucine (c) Lysine (d) All of them.
12. The lipoprotein possessing the highest quantity of phospholipid
    (a) HDL (b) LDL (c) VLDL (d) Chylomicrons.
13. Hypercholesterolemia is observed in the disorder(s)
    (a) Hypothyroidism (b) Diabetes mellitus (c) Nephrotic syndrome (d) All of them.
14. The two final products in the β-oxidation of odd chain fatty acids are
    (a) Acetyl CoA and malonyl CoA (b) Acetyl CoA and acetyl CoA (c) Acetyl CoA and propionyl CoA (d) Acetyl CoA and succinyl CoA.
15. Hormone sensitive lipase activity is inhibited by the hormone
    (a) Epinephrine (b) Insulin (c) Thyroxine (d) Glucocorticoids.

15 Metabolism of Amino Acids



The amino acids speak :

"We transaminate and deaminate to liberate ammonia;
That is detoxified in the liver to end product urea;
Greatly important to body are our nitrogen products;
Carbon skeleton is for glucose, fat or fuel."

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 wide variety of static (structural) and dynamic (enzymes, hormones, clotting factors, receptors etc.) functions. About half of the body protein (predominantly collagen) is present in the supportive tissue (skeleton and connective) while the other half is intracellular.

Proteins are nitrogen-containing macromolecules ***consisting of L-α-amino acids*** as the repeating units. Of the 20 amino acids found in proteins, half can be synthesized by the body (non-essential) while the rest have to be provided in the diet (essential amino acids).

The proteins on degradation (proteolysis) release individual amino acids. Amino acids are not just the structural components of proteins. Each one of the 20 naturally occurring amino acids undergoes its own metabolism and performs specific functions. Some of the amino acids also serve as precursors for the synthesis of many biologically important compounds (e.g. melanin, serotonin, creatine etc.). Certain amino acids may directly act as neurotransmitters (e.g. glycine aspartate, glutamate). ***Protein metabolism is more appropriately learnt as metabolism of amino acids.***

AMINO ACID POOL

An adult has about ***100 g of free amino acids*** which represent the amino acid pool of the body. The amino acid pool may be an oversimplification of the facts, since there is no single compartment—rather, several compartments exist.

Glutamate and ***glutamine*** together constitute ***about 50%***, and essential amino acids about 10% of the body pool (100 g). The concentration of intracellular amino acids is always higher than the extracellular amino acids. Amino acids enter the cells against a concentration gradient by active transport.

The amino acid pool of the body is maintained by the sources that contribute (input) and the metabolic pathways that utilize (output) the amino acids (***Fig.15.1***).

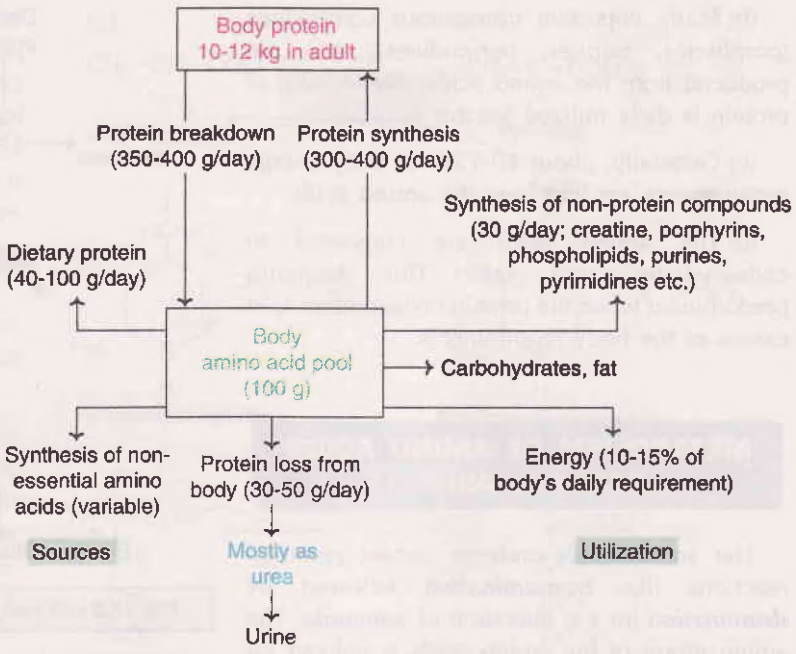


Fig. 15.1 : Overview of body's amino acid pool—sources and utilization.

I. Sources of amino acid pool

Turnover of body protein, intake of dietary protein and the synthesis of non-essential amino acids contribute to the body amino acid pool.

(a) **Protein turnover :** The protein present in the body is in a dynamic state. It is estimated that about ***300-400 g of protein per day*** is constantly degraded and synthesized which represents body protein turnover. There is a wide variation in the turnover of individual proteins. For instance, the plasma proteins and digestive enzymes are rapidly degraded, their half-lives being in hours or days. The structural proteins (e.g. collagen) have long half-lives, often in months and years.

Control of protein turnover : The turnover of the protein is influenced by many factors. A small polypeptide called ***ubiquitin*** (mol. wt. 8,500) tags with the proteins and facilitates degradation. Certain proteins with amino acid sequence proline, glutamine (one letter code E), serine and threonine (PEST sequence) are rapidly degraded.

(b) **Dietary protein :** There is a regular loss of nitrogen from the body due to degradation of amino acids. In healthy adults, it is estimated that about 30-50 g of protein is lost everyday from the body. This amount of protein (30-50 g/day) must, therefore, be supplied daily in the diet ***to maintain nitrogen balance***. The purpose of dietary protein is to supply amino acids (particularly the essential ones) for the synthesis of proteins and other nitrogen compounds.

There is ***no storage form of amino acids*** as is the case for carbohydrates (glycogen) and lipids (triacylglycerols). The excess intake of amino acids are metabolized—oxidized to provide energy, converted to glucose or fat. The amino groups are lost as urea and excreted. The protein consumption in developed countries is much higher than the recommended dietary allowance (i.e. 1g/kg body weight/day). The daily protein intake by an adult in most countries is 40-100 g. Protein is digested by proteolytic enzymes to amino acids which are absorbed in the intestine and enter the body pool of amino acids.

(c) **Synthesis of non-essential amino acids :** Ten out of the 20 naturally occurring amino acids can be synthesized by the body which contribute to the amino acid pool.

II. Utilization of amino acids from body pool

(a) Most of the body proteins (300-400 g/day) degraded are synthesized from the amino acid pool. These include enzymes, hormones, immunoproteins, contractile proteins etc.

(b) Many important nitrogenous compounds (porphyrins, purines, pyrimidines, etc.) are produced from the amino acids. About 30 g of protein is daily utilized for this purpose.

(c) Generally, about 10-15% of body energy requirements are met from the amino acids.

(d) The amino acids are converted to carbohydrates and fats. This becomes predominant when the protein consumption is in excess of the body requirements.

METABOLISM OF AMINO ACIDS —GENERAL ASPECTS

The amino acids undergo certain common reactions like ***transamination*** followed by ***deamination*** for the liberation of ***ammonia***. The amino group of the amino acids is utilized for the formation of ***urea*** which is an excretory ***end product*** of protein metabolism. The carbon skeleton of the amino acids is first converted to keto acids (by transamination) which meet one or more of the following fates.

1. Utilized to generate energy.
2. Used for the synthesis of glucose.
3. Diverted for the formation of fat or ketone bodies.
4. Involved in the production of non-essential amino acids.

A general picture of amino acid metabolism is depicted in ***Fig.15.2***.

The details of general and specific metabolic reactions of amino acids are described in the following pages.

TRANSAMINATION

The ***transfer of an amino ($-NH_2$) group from an amino acid to a keto acid*** is known as transamination. This process involves the interconversion of a pair of amino acids and a pair of keto acids, catalysed by a group of enzymes called ***transaminases*** (recently, ***aminotransferases***).

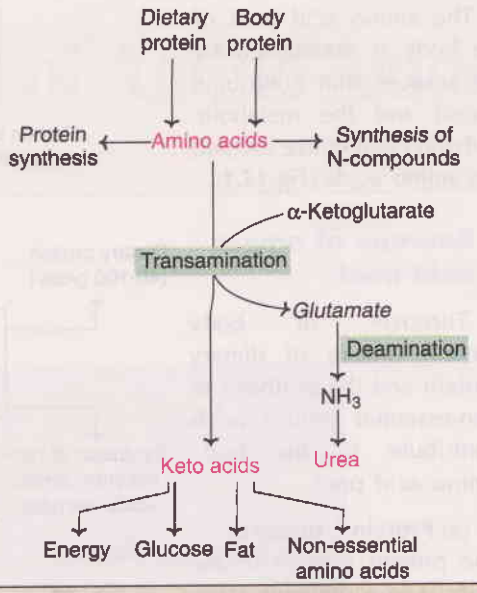


Fig. 15.2 : An overview of amino acid metabolism.

Salient features of transamination

1. All transaminases require ***pyridoxal phosphate*** (PLP), a coenzyme derived from vitamin B_6.

2. Specific transaminases exist for each pair of amino and keto acids. However, only two—namely, aspartate transaminase and alanine transaminase—make a significant contribution for transamination.

3. There is no free NH_3 liberated, only the transfer of amino group occurs.

4. Transamination is ***reversible*** (***Fig.15.3***).

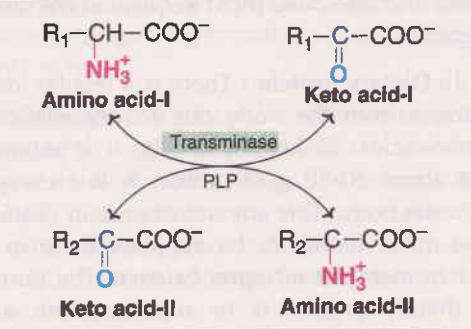


Fig. 15.3 : Transamination reaction.

5. Transamination is very important for the redistribution of amino groups and ***production of non-essential amino acids***, as per the requirement of the cell. It involves both catabolism (degradation) and anabolism (synthesis) of amino acids.

6. Transamination diverts the excess amino acids towards ***energy generation***.

7. The amino acids undergo transamination to finally concentrate nitrogen in glutamate. ***Glutamate*** is the only amino acid that undergoes oxidative deamination to a significant extent to liberate free NH_3 for urea synthesis.

8. All amino acids except lysine, threonine, proline and hydroxyproline participate in transamination.

9. Transamination is not restricted to α-amino groups only. For instance, δ-amino group of ornithine is transaminated.

10. Serum transaminases are important for diagnostic and prognostic purposes. (***Refer Chapter 6***).

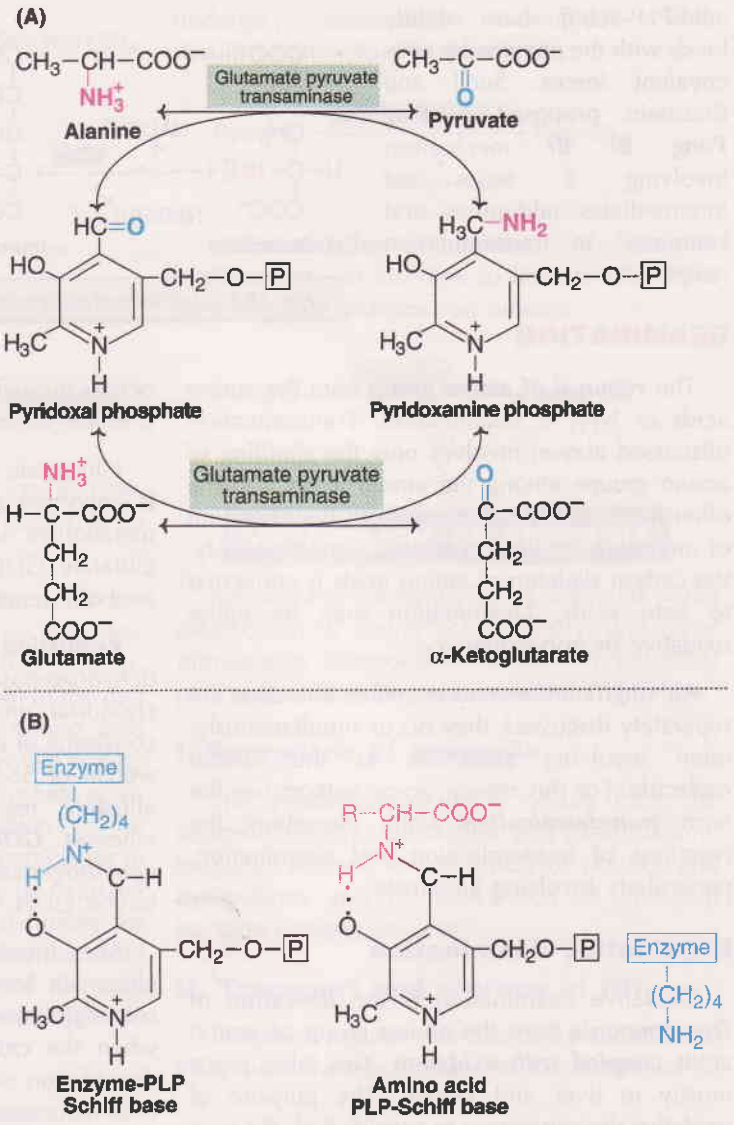


Fig. 15.4 : *Mechanism of transamination—(**A**) Involvement of pyridoxal phosphate (PLP) in the transfer of amino group, (**B**) Formation of enzyme-PLP-Schiff base and amino acid-PLP-Schiff base. Note that when the amino acid binds, enzyme separates.*

Mechanism of transamination

Transamination occurs in two stages (***Fig.15.4***)

1. Transfer of the amino group to the coenzyme pyridoxal phosphate (bound to the coenzyme) to form pyridoxamine phosphate.

2. The amino group of pyridoxamine phosphate is then transferred to a keto acid to produce a new amino acid and the enzyme with PLP is regenerated.

All the transaminases require ***pyridoxal phosphate*** (PLP), a derivative of vitamin B_6. The aldehyde group of PLP is linked with ε-amino group of lysine residue, at the active site of the enzyme forming a ***Schiff base*** (imine linkage). When an amino acid (substrate) comes in contact with the enzyme, it displaces lysine and a new Schiff base linkage is formed. The amino

acid-PLP-Schiff base tightly binds with the enzyme by non-covalent forces. Snell and Braustein proposed a ***Ping Pong Bi Bi*** mechanism involving a series of intermediates (aldimines and ketimines) in transamination reaction.

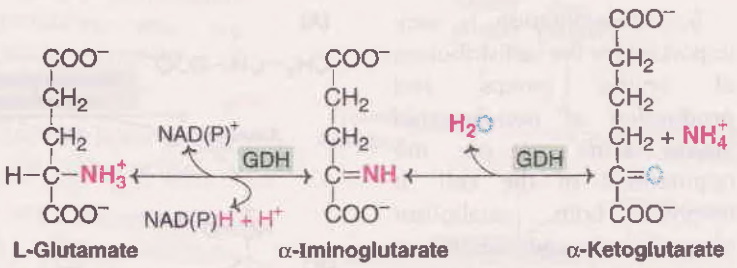


Fig. 15.5 : Oxidation of glutamate by glutamate dehydrogenase (GDH).

DEAMINATION

The ***removal of amino group*** from the amino acids as NH_3 is deamination. Transamination (discussed above) involves only the shuffling of amino groups among the amino acids. On the other hand, deamination results in the liberation of ammonia for urea synthesis. Simultaneously, the carbon skeleton of amino acids is converted to keto acids. Deamination may be either oxidative or non-oxidative.

Although transamination and deamination are separately discussed, they occur simultaneously, often involving glutamate as the central molecule. For this reason, some authors use the term ***transdeamination*** while describing the reactions of transamination and deamination, particularly involving glutamate.

I. Oxidative deamination

Oxidative deamination is the ***liberation of free ammonia*** from the amino group of amino acids ***coupled with oxidation.*** This takes place mostly in liver and kidney. The purpose of oxidative deamination is to provide NH_3 for urea synthesis and α-keto acids for a variety of reactions, including energy generation.

Role of glutamate dehydrogenase : In the process of transamination, the amino groups of most amino acids are transferred to α-ketoglutarate to produce glutamate. Thus, ***glutamate*** serves as a '***collection centre' for amino groups*** in the biological system. Glutamate rapidly undergoes oxidative deamination, catalysed by glutamate dehydrogenase (GDH) to liberate ammonia. This enzyme is unique in that it can utilize either NAD^+ or $NADP^+$ as a coenzyme. Conversion of glutamate to α-ketoglutarate occurs through the formation of an intermediate, α-iminoglutarate (***Fig.15.5***).

Glutamate dehydrogenase catalysed reaction is important as it reversibly links up glutamate metabolism with TCA cycle through α-ketoglutarate. GDH is involved in both catabolic and anabolic reactions.

Regulation of GDH activity : Glutamate dehydrogenase is a zinc containing mitochondrial enzyme. It is a complex enzyme consisting of six identical units with a molecular weight of 56,000 each. GDH is controlled by allosteric regulation. ***GTP*** and ***ATP inhibit***—whereas ***GDP*** and ***ADP activate***—glutamate dehydrogenase. Steroid and thyroid hormones inhibit GDH.

After ingestion of a protein-rich meal, liver glutamate level is elevated. It is converted to α-ketoglutarate with liberation of NH_3. Further, when the cellular energy levels are low, the degradation of glutamate is increased to provide α-ketoglutarate which enters TCA cycle to liberate energy.

Oxidative deamination by amino acid oxidases : L-Amino acid oxidase and D-amino acid oxidase are flavoproteins, possessing FMN and FAD, respectively. They act on the corresponding amino acids (L or D) to produce α-keto acids and NH_3. In this reaction, oxygen is reduced to H_2O_2, which is later decomposed by catalase (***Fig.15.6***).

The activity of ***L-amino acid oxidase*** is much low while that of ***D-amino acid oxidase*** is high in tissues (mostly liver and kidney). L-Amino acid oxidase does not act on glycine and dicarboxylic

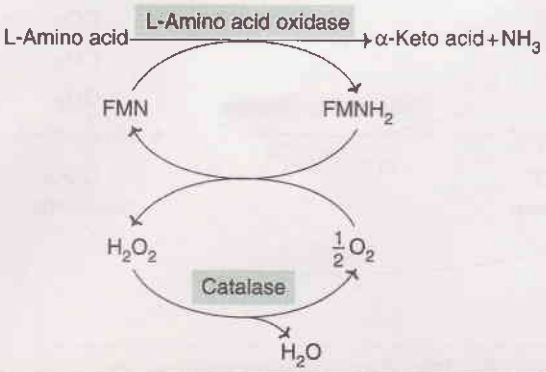


Fig. 15.6 : Oxidative deamination of amino acids.

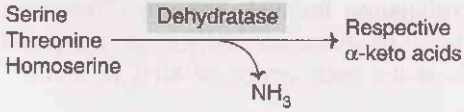
acids. This enzyme, due to its very low activity, does not appear to play any significant role in the amino acid metabolism.

Fate of D-amino acids : D-Amino acids are found in plants and microorganisms. They are, however, not present in the mammalian proteins. But D-amino acids are regularly taken in the diet and metabolized by the body. D-Amino acid oxidase converts them to the respective α-keto acids by oxidative deamination. The α-keto acids so produced undergo transamination to be converted to L-amino acids which participate in various metabolisms. Keto acids may be oxidized to generate energy or serve as precursors for glucose and fat synthesis. Thus, D-amino acid oxidase is important as it initiates the first step for the conversion of unnatural D-amino acids to L-amino acids in the body (***Fig.15.7***).

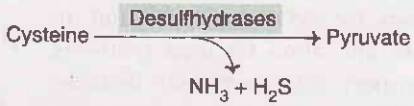
II. Non-oxidative deamination

Some of the amino acids can be deaminated to liberate NH_3 without undergoing oxidation

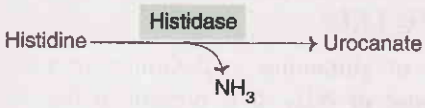
(a) **Amino acid dehydrases :** Serine, threonine and homoserine are the hydroxy amino acids. They undergo non-oxidative deamination catalysed by PLP-dependent dehydrases (dehydratases).



(b) **Amino acid desulfhydrases :** The sulfur amino acids, namely cysteine and homocysteine, undergo deamination coupled with desulfhydration to give keto acids.



(c) **Deamination of histidine :** The enzyme histidase acts on histidine to liberate NH_3 by a non-oxidative deamination process.



METABOLISM OF AMMONIA

Ammonia is constantly being liberated in the metabolism of amino acids (mostly) and other nitrogenous compounds. At the physiological pH, ***ammonia exists as ammonium (NH_4^+) ion.***

I. Formation of ammonia

The production of NH_3 occurs from the amino acids (transamination and deamination), biogenic amines, amino group of purines and pyrimidines and by the action of intestinal bacteria (urease) on urea.

II. Transport and storage of NH_3

Despite a regular and constant production of NH_3 from various tissues, its concentration in

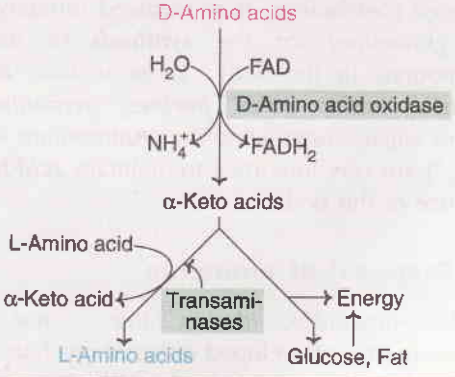


Fig. 15.7 : Metabolic fate of D-amino acids

the circulation is surprisingly low (normal plasma 10-20 mg/dl). This is mostly because the body has an efficient mechanism for NH_3 transport and its immediate utilization for urea synthesis. The transport of ammonia between various tissues and the liver mostly occurs in the form of ***glutamine*** or ***alanine*** and not as free ammonia. Alanine is important for NH_3 transport from muscle to liver by glucose-alanine cycle (***Refer Fig.13.13***).

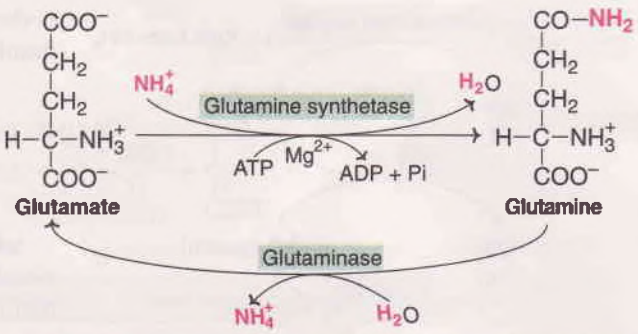


*Fig. 15.8 : Synthesis of glutamine and its conversion to glutamate. (**Note** : The reactions are independent and irreversible).*

Role of glutamine : Glutamine is a ***storehouse of NH_3***. It is present at the highest concentration (8 mg/dl in adults) in blood among the amino acids. Glutamine serves as a storage and transport form of NH_3. Its synthesis mostly occurs in liver, brain and muscle. Ammonia is removed from the brain predominantly as glutamine. Glutamine is ***freely diffusible*** in tissues, hence easily transported.

Glutamine synthetase (a mitochondrial enzyme) is responsible for the synthesis of glutamine from glutamate and ammonia. This reaction is unidirectional and requires ATP and Mg^{2+} ions.

Glutamine can be deaminated by hydrolysis to release ammonia by glutaminase (***Fig.15.8***) an enzyme mostly found in kidney and intestinal cells.

III. Functions of ammonia

Ammonia is not just a waste product of nitrogen metabolism. It is involved (directly or via glutamine) for the ***synthesis*** of many compounds in the body. These include ***non-essential amino acids, purines, pyrimidines,*** amino sugars, asparagine etc. Ammonium ions (NH_4^+) are very important to maintain ***acid-base balance*** of the body.

IV. Disposal of ammonia

The organisms, during the course of evolution, have developed different mechanisms for the disposal of ammonia from the body. The animals in this regard are of three different types

(a) **Ammoniotelic** : The aquatic animals dispose off NH_3 into the surrounding water.

(b) **Uricotelic** : Ammonia is converted mostly to uric acid e.g. reptiles and birds.

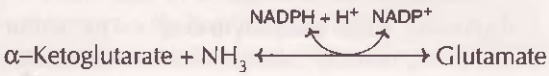
(c) **Ureotelic** : The mammals including man convert NH_3 to urea. Urea is a non-toxic and soluble compound, hence easily excreted.

V. Toxicity of ammonia

Even a marginal elevation in the blood ammonia concentration is ***harmful to the brain.*** Ammonia, when it accumulates in the body, results in ***slurring of speech and blurring of the vision*** and causes tremors. It may lead to coma and, finally, death, if not corrected.

Hyperammonemia : Elevation in blood NH_3 level may be genetic or acquired. Impairment in urea synthesis due to a defect in any one of the five enzymes is described in urea synthesis. All these disorders lead to hyperammonemia and cause mental retardation. The acquired hyperammonemia may be due to hepatitis, alcoholism etc. where the urea synthesis becomes defective, hence NH_3 accumulates.

Explanation for NH_3 toxicity : The reaction catalysed by glutamate dehydrogenase probably explains the toxic affects of NH_3 in brain



Accumulation of NH_3 shifts the equilibrium to the right with more glutamate formation, hence more utilization of α-ketoglutarate. α-Ketoglutarate is a key intermediate in TCA cycle and its depleted levels impair the TCA cycle. The net result is that production of energy (ATP) by the brain is reduced. The ***toxic effects of NH_3*** on brain are, therefore, ***due to impairment in ATP formation.***

Trapping and elimination of ammonia : When the plasma level of ammonia is highly elevated, intravenous administration of sodium benzoate and phenyllactate is done. These compounds can respectively condense with glycine and glutamate to form water soluble products that can be easily excreted. By this way, ammonia can be trapped and removed from the body. In some instances of toxic hyperammonemia, hemodialysis may become necessary.

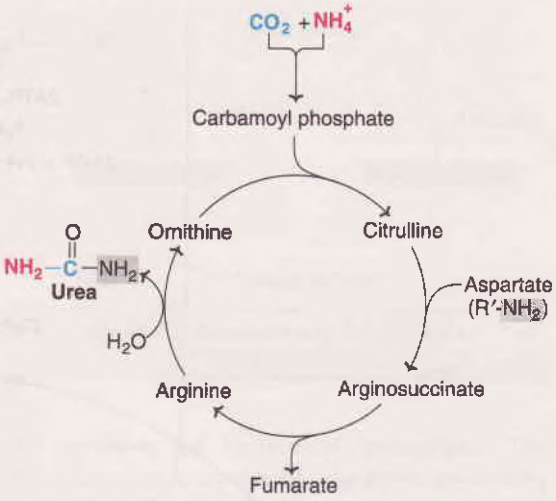


*Fig. 15.9 : Outline of urea cycle. (**Note :** In the synthesis of urea one amino group comes from ammonium ion while the other is from aspartate; carbon is derived from CO_2. This is represented in colours.)*

UREA CYCLE

Urea is the ***end product of protein metabolism*** (amino acid metabolism). The nitrogen of amino acids, converted to ammonia (as described above), is toxic to the body. It is converted to urea and detoxified. As such, urea accounts for 80-90% of the nitrogen containing substances excreted in urine.

Urea is ***synthesized in liver*** and transported to kidneys for excretion in urine. Urea cycle is the ***first metabolic cycle*** that was elucidated by Hans Krebs and Kurt Henseleit (1932), hence it is known as ***Krebs-Henseleit cycle***. The individual reactions, however, were described in more detail later on by Ratner and Cohen.

Urea has ***two amino ($-NH_2$) groups***, one derived ***from NH_3*** and the other ***from aspartate.*** Carbon atom is supplied by CO_2. Urea synthesis is a five-step cyclic process, with five distinct enzymes. The first ***two enzymes*** are present in ***mitochondria*** while the ***rest*** are localized in ***cytosol.*** The details of urea cycle are described (***Figs.15.9 and 15.10***).

1. **Synthesis of carbamoyl phosphate :** Carbamoyl phosphate synthase I (CPS I) of mitochondria catalyses the condensation of NH_4^+ ions with CO_2 to form carbamoyl phosphate. This step consumes two ATP and is ***irreversible***, and ***rate-limiting.*** CPS I requires ***N-acetylglutamate*** for its activity. Another enzyme, carbamoyl phosphate synthase II (CPS II)—involved in pyrimidine synthesis—is present in cytosol. It accepts amino group from glutamine and does not require N-acetylglutamate for its activity.

2. **Formation of citrulline :** Citrulline is synthesized from carbamoyl phosphate and ornithine by ornithine transcarbamoylase. Ornithine is regenerated and used in urea cycle. Therefore, its role is comparable to that of oxaloacetate in citric acid cycle. Ornithine and citrulline are basic amino acids. (They are never found in protein structure due to lack of codons). Citrulline produced in this reaction is transported to cytosol by a transporter system.

3. **Synthesis of arginosuccinate :** Argino-succinate synthase condenses citrulline with aspartate to produce arginosuccinate. The second amino group of urea is incorporated in this reaction. This step requires ATP which is cleaved to AMP and pyrophosphate (PPi). The latter is immediately broken down to inorganic phosphate (Pi).

(b) Many important nitrogenous compounds (porphyrins, purines, pyrimidines, etc.) are produced from the amino acids. About 30 g protein is daily utilized for this purpose.

(c) Generally, about 10-15% of bo requirements are met from the am

(d) The amino acids a carbohydrates and fa predominant when the excess of the body

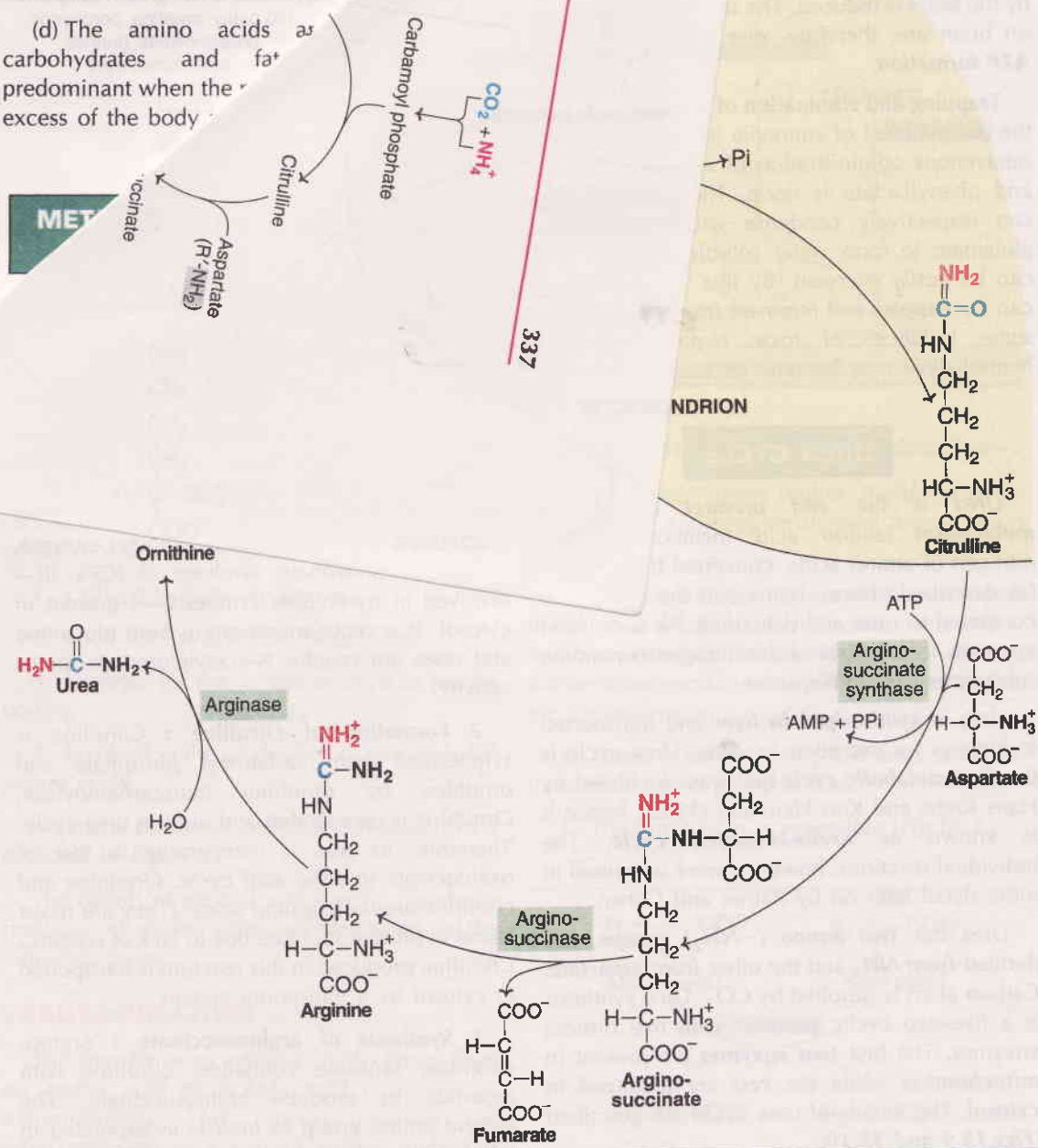


*Fig. 15.10 : Reactions of urea cycle (NAG—N-acetylglutamate; in the formation of urea, one amino group is derived from free ammonium ion while the other is from aspartate; carbon is obtained from CO_2; * mitochondrial enzymes, the rest of the enzymes are cytosomal).*

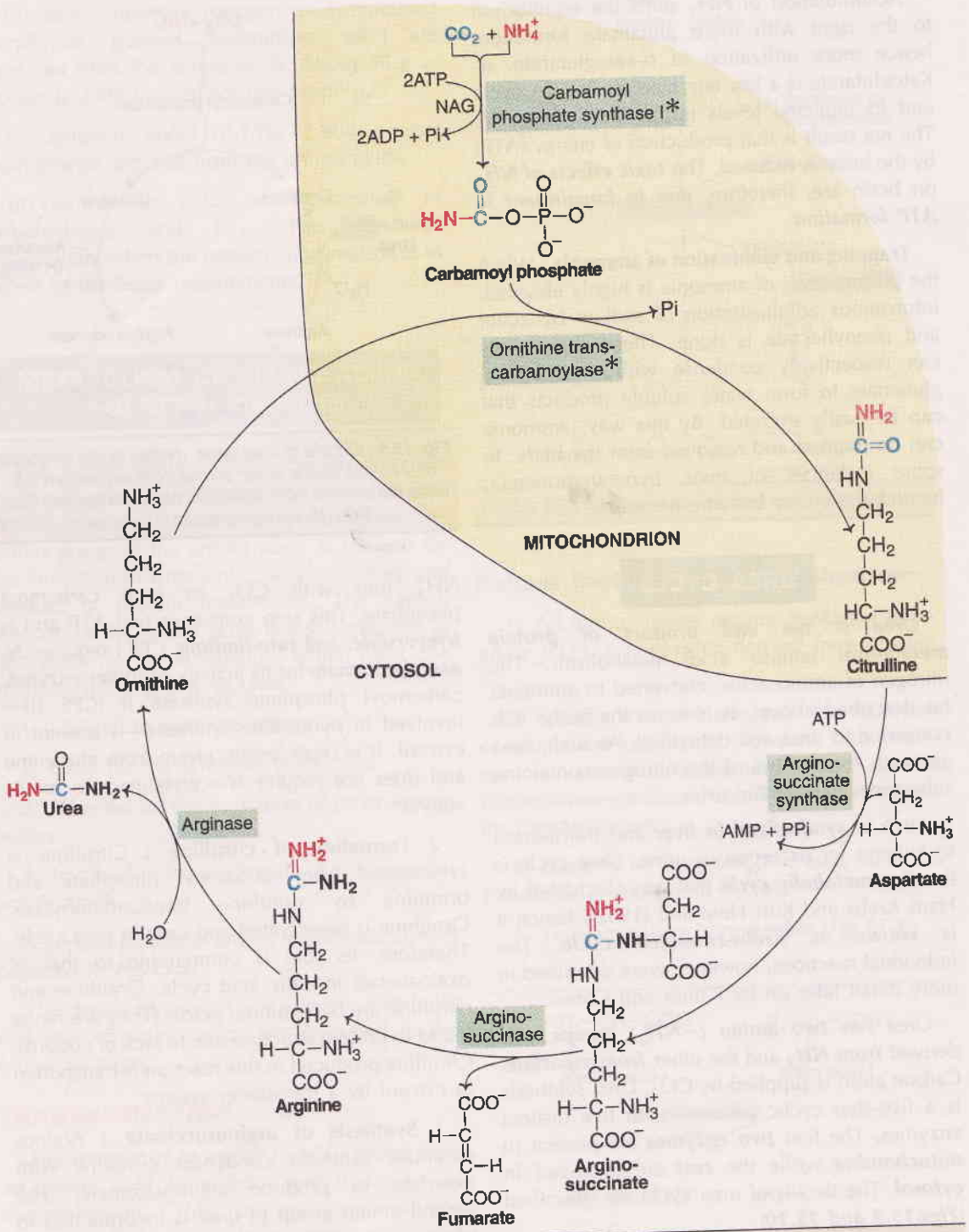


*Fig. 15.10 : Reactions of urea cycle (NAG—N-acetylglutamate; in the formation of urea, one amino group is derived from free ammonium ion while the other is from aspartate; carbon is obtained from CO_2; * mitochondrial enzymes, the rest of the enzymes are cytosomal).*

4. **Cleavage of arginosuccinate :** Arginosuccinase cleaves arginosuccinate to give arginine and fumarate. Arginine is the immediate precursor for urea. Fumarate liberated here provides a connecting link with TCA cycle, gluconeogenesis etc.

5. **Formation of urea :** Arginase is the fifth and final enzyme that cleaves arginine to yield urea and ornithine. Ornithine, so regenerated, enters mitochondria for its reuse in the urea cycle. Arginase is activated by Co^{2+} and Mn^{2+}. Ornithine and lysine compete with arginine (competitive inhibition). ***Arginase*** is mostly found in the liver, while the rest of the enzymes (four) of urea cycle are also present in other tissues. For this reason, arginine synthesis may occur to varying degrees in many tissues. But only the liver can ultimately produce urea.

Overall reaction and energetics

The urea cycle is irreversible and consumes 4 ATP. Two ATP are utilized for the synthesis of carbamoyl phosphate. One ATP is converted to AMP and PPi to produce arginosuccinate which equals to 2 ATP. Hence ***4 ATP*** are actually ***consumed***.

$NH_4^+ + CO_2$ + Aspartate + 3ATP $\longrightarrow$ Urea + Fumarate + 2 ADP + 2 Pi + AMP + PPi

Regulation of urea cycle

The first reaction catalysed by ***carbamoyl phosphate synthase I*** (CPS I) is ***rate-limiting*** reaction or committed step in urea synthesis. CPS I is allosterically activated by N-acetylglutamate (NAG). It is synthesized from glutamate and acetyl CoA by synthase and degraded by a hydrolase (***Fig.15.11***).

The rate of urea synthesis in liver is correlated with the concentration of N-acetylglutamate. High concentrations of arginine increase NAG. The consumption of a protein-rich meal increases the level of NAG in liver, leading to enhanced urea synthesis.

Carbamoyl phosphate synthase I and glutamate dehydrogenase are localized in the mitochondria. They coordinate with each other in the formation of NH_3, and its utilization for the synthesis of carbamoyl phosphate. The remaining four enzymes of urea cycle are mostly controlled by the concentration of their respective substrates.

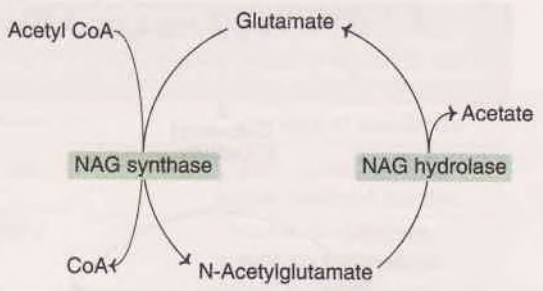


Fig. 15.11 : *Formation and degradation of N-acetylglutamate.*

Disposal of urea

Urea produced in the liver freely diffuses and is transported in blood to ***kidneys***, and excreted. A small amount of urea enters the intestine where it is broken down to CO_2 and NH_3 by the bacterial enzyme urease. This ammonia is either lost in the feces or absorbed into the blood. In renal failure, the blood urea level is elevated (uremia), resulting in diffusion of more urea into intestine and its breakdown to NH_3. Hyperammonemia (increased blood NH_3) is commonly seen in patients of kidney failure. For these patients, oral administration of antibiotics (neomycin) to kill intestinal bacteria is advised.

Integration between urea cycle and TCA cycle

Urea cycle is linked with TCA cycle in three different ways (***Fig.15.12***). This is regarded as ***bicyclic integration*** between the two cycles.

1. The production of ***fumarate*** in urea cycle is the most important integrating point with TCA cycle. Fumarate is converted to malate and then to oxaloacetate in TCA cycle. Oxaloacetate undergoes transamination to produce aspartate which enters urea cycle. Here, it combines with citrulline to produce arginosuccinate. Oxaloacetate is an important metabolite which can combine with acetyl CoA to form citrate and get

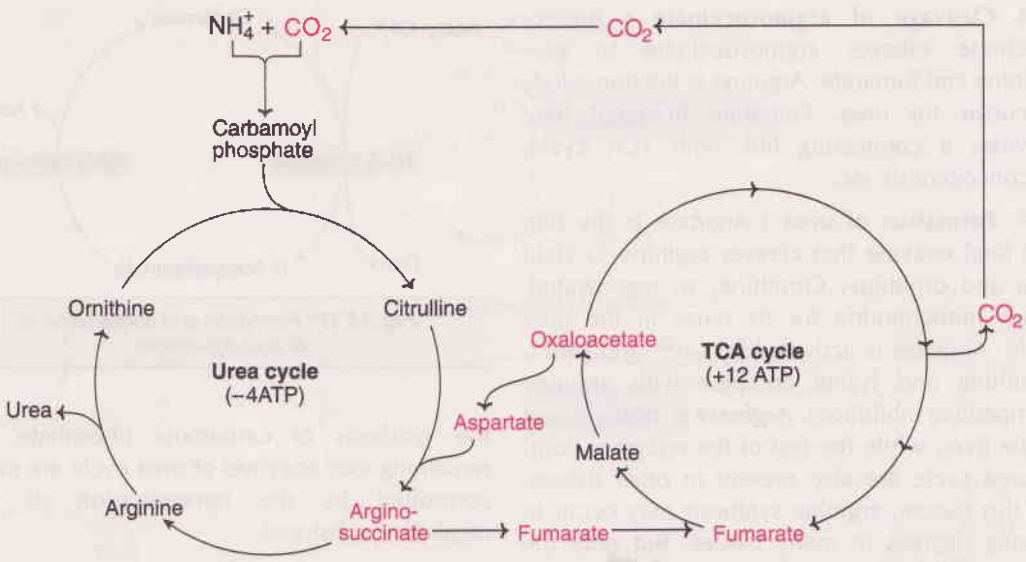


Fig. 15.12 : Interrelation between urea and tricarboxylic acid (TCA) cycle (Depicted in blue colour).

finally oxidized. Oxaloacetate can also serve as a precursor for the synthesis of glucose (gluconeogenesis).

2. ATP (12) are generated in the TCA cycle while ***ATP*** (4) are utilized for urea synthesis.

3. Citric acid cycle is an important metabolic pathway for the complete oxidation of various metabolites to CO_2 and H_2O. The ***CO_2*** liberated in TCA cycle (in the mitochondria) can be utilized in urea cycle.

Metabolic disorders of urea cycle

Metabolic defects associated with each of the five enzymes of urea cycle have been reported (***Table 15.1***). All the disorders invariably lead to a build-up in blood ammonia ***(hyperammonemia)***, leading to toxicity. Other metabolites of urea cycle also accumulate which, however, depends on the specific enzyme defect. The clinical symptoms associated with defect in urea cycle enzymes include vomiting, lethargy, irritability, ataxia and mental retardation.

Blood urea—clinical importance

In healthy people, the normal blood urea concentration is 10-40 mg/dl. Higher protein intake marginally increases blood urea level, however this is well within normal range. About 15-30 g of urea (7-15 g nitrogen) is excreted in urine per day.

Blood urea estimation is widely used as a screening test for the evaluation of ***kidney*** (renal) ***function***. It is estimated in the laboratory either by urease method or diacetyl monoxime (DAM) procedure. Elevation in blood urea may be broadly classified into three categories.

1. **Pre-renal** : This is associated with ***increased protein breakdown***, leading to a negative nitrogen balance, as observed after major surgery, prolonged fever, diabetic coma, thyrotoxicosis etc. In leukemia and bleeding disorders also, blood urea is elevated.

Table 15.1 Metabolic defects in urea cycle

Defect	*Enzyme involved*
Hyperammonemia type I	Carbamoyl phosphate synthase I
Hyperammonemia type II	Ornithine transcarbamoylase
Citrullinemia	Arginosuccinate synthase
Arginosuccinic aciduria	Arginosuccinase
Hyperargininemia	Arginase

2. **Renal :** In renal disorders like ***acute glomerulonephritis***, chronic nephritis, nephrosclerosis, polycystic kidney, blood urea is increased.

3. **Post-renal :** Whenever there is an ***obstruction*** in the ***urinary tract*** (e.g. tumors, stones, enlargement of prostate gland etc.), blood urea is elevated. This is due to increased reabsorption of urea from the renal tubules.

The term '***uremia***' is used to indicate increased blood urea levels due to renal failure. ***Azotemia*** reflects a condition with elevation in blood urea/or other nitrogen metabolites which may or may not be associated with renal diseases.

Non-protein nitrogen (NPN)

As is obvious from the name, the term NPN refers to all the nitrogen-containing substances other than proteins. These include urea (most abundant), creatinine, creatine, uric acid, peptides, amino acids etc. In healthy persons, NPN concentration in blood is 20-40 mg/dl.

The molecular weight of urea is 60 and about half of it (28) is contributed by the two nitrogen atoms. Thus, if blood urea concentration is 60 mg, then about half of it—28 mg—is ***blood urea nitrogen (BUN)***. Therefore,

$$BUN = \frac{1}{2} NPN$$

$$NPN = 2\ BUN$$

In some countries, estimations of BUN or NPN are used rather than blood urea for assessing kidney function.

METABOLISM OF INDIVIDUAL AMINO ACIDS

In the preceding pages, the general aspects of amino acid metabolism have been discussed. A summary of the biologically important or specialized products obtained from or contributed by the amino acids is given in the ***Table 15.2***. The metabolism of individual amino acids with special emphasis on the specialized products is described next.

Table 15.2 A summary of the specialized products formed/contributed by amino acids

Amino acid	*Specialized product(s)*
Glycine	Creatine, glutathione, heme, purines, conjugated bile acids.
Tyrosine	Thyroxine, triiodothyronine, epinephrine, norepinephrine, dopamine, melanin.
Tryptophan	NAD^+, $NADP^+$ (coenzymes of niacin), serotonin, melatonin.
Methionine	Active methionine, creatine, epinephrine, polyamines.
Cysteine	Glutathione, taurine, coenzyme A, active sulfate.
Histidine	Histamine
Arginine	Creatine, nitric oxide
Lysine	Carnitine
Glutamate	γ-Amino butyric acid, glutathione, γ-carboxyglutamate.
Glutamine	Purines, pyrimidines, amino sugars.
Aspartate	Purines, pyrimidines
Serine	Phosphatidylserine, sphingomyelins, choline.
β-Alanine	Coenzyme A

GLYCINE

Glycine (Gly, G) is a non-essential, optically inactive and ***glycogenic*** (precursor for glucose) amino acid. It is indispensable for chicks. The outline of glycine metabolism is depicted in ***Fig.15.13***. Glycine is actively involved in the synthesis of many specialized products (heme, purines, creatine etc.) in the body, besides its incorporation into proteins, synthesis of serine and glucose and participation in one-carbon metabolism.

Glycine is the most abundant amino acid normally excreted into urine (0.5–1.0 g/g creatinine).

Glycine in proteins

Glycine is one among the commonest amino acids found in protein structure. Being small and non-polar, glycine is mostly present in the

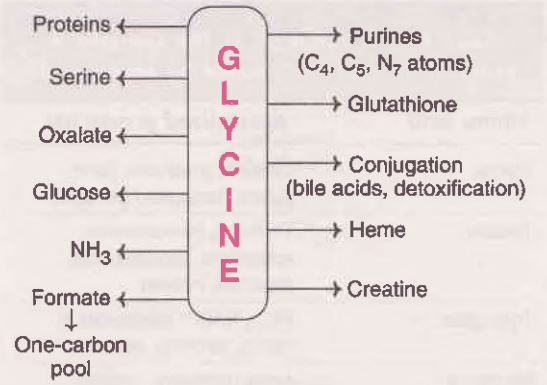


Fig. 15.13 : Overview of glycine metabolism.

interior structure of protein. Collagen contains very high (about 30%) content of glycine.

Synthesis of glycine

Glycine is synthesized from serine by the enzyme serine hydroxymethyl transferase which is dependent on tetrahydrofolate (THF). Glycine can also be obtained from threonine, catalysed by threonine aldolase. Glycine synthase can convert a one-carbon unit (N^5, N^{10}-methylene THF), CO_2 and NH_3 to glycine.

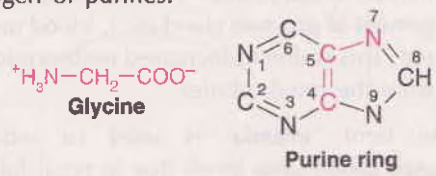
Degradation of glycine

Glycine undergoes oxidative deamination by glycine synthase to liberate NH_4^+, CO_2 and one-carbon fragment as N^5, N^{10}-methylene THF. This provides a major route for glycine breakdown in mammals. Glycine synthase is a multienzyme complex and requires PLP, NAD^+ and THF for its activity. This reaction is reversible and, therefore, glycine can be generated from one-carbon unit (methylene fragment of THF).

Glycine is reversibly converted to serine by THF dependent serine hydroxymethyl transferase. Pyruvate produced from serine by serine dehydratase, serves as a precursor for glucose. Serine is degraded to glyoxylate which undergoes transamination to give back glycine. Glyoxylate is also converted to oxalate, an excretory product and formate which enters one-carbon pool (***Fig.15.14***).

Synthesis of specialized products

1. **Formation of purine ring :** The entire molecule of glycine is utilized for the formation of positions 4 and 5 of carbon and position 7 of nitrogen of purines.



2. **Synthesis of glutathione :** Glutathione is a tripeptide (γ-glutamyl-cysteinyl-glycine) and requires three amino acids for its formation (***Fig.15.15***).

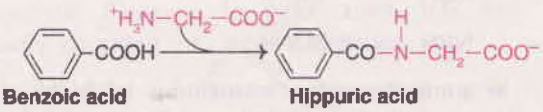
3. **Conjugation reactions :** As a conjugating agent, glycine performs two important functions

(a) The bile acids—cholic acid and chenodeoxy cholic acid—are conjugated with glycine.

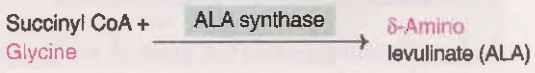
Cholic acid + Glycine ⟶ Glycocholic acid

Chenodeoxycholic acid + Glycine ⟶ Glycochenodeoxy cholic acid

(b) Glycine is important for ***detoxification*** of benzoic acid (commonly used as a food preservative) to hippuric acid.



4. **Synthesis of heme :** Glycine condenses with succinyl CoA to form δ-amino levulinate which serves as a precursor for heme synthesis (details given in porphyrin metabolism—***Chapter 10***).



5. **Biosynthesis of creatine :** Creatine is present in the tissues (muscle, brain, blood etc.) as the high energy compound, phosphocreatine and as free creatine. Three amino acids—***glycine***, ***arginine*** and ***methionine***—are required for creatine formation (***Fig.15.16***). The first

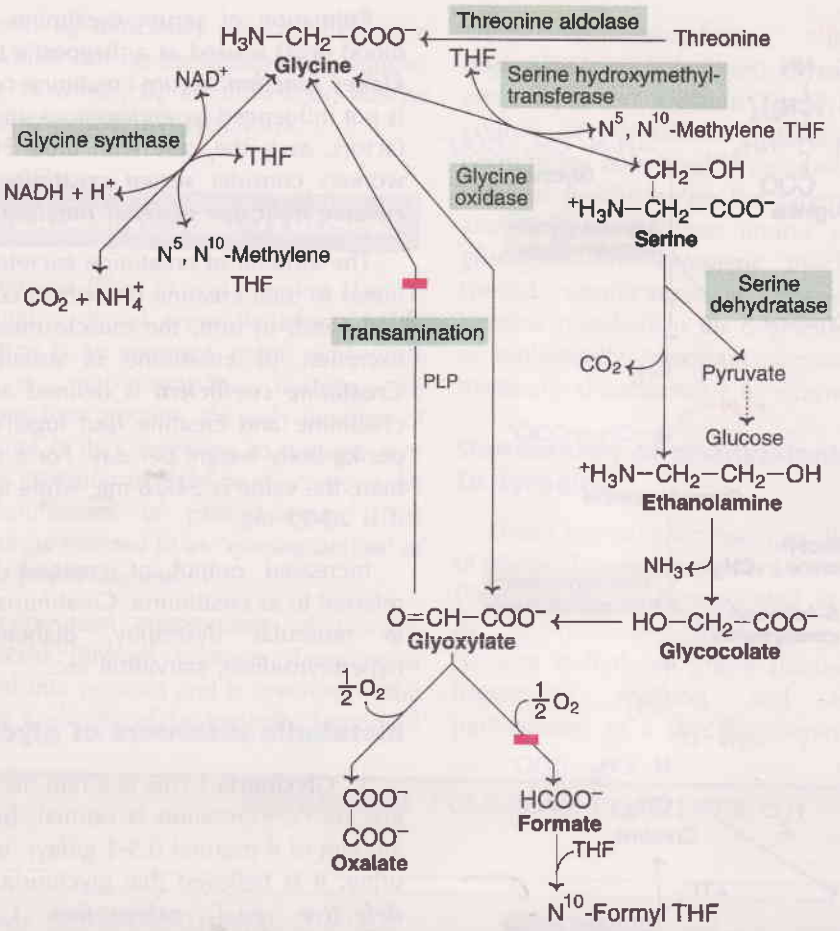


Fig. 15.14 : *Metabolism of glycine (THF–Tetrahydrofolate; PLP–Pyridoxal phosphate; ▬ –Block in primary hyperoxaluria).*

reaction occurs in the kidney. It involves the transfer of guanidino group of arginine to glycine, catalysed by arginine-glycine transamidinase to produce guanidoacetate (glycocyamine). S-Adenosylmethionine (active methionine) donates methyl group to glycocyamine to produce creatine. This reaction occurs in liver. Creatine is reversibly phosphorylated to phosphocreatine (creatine phosphate) by creatine kinase. It is stored in muscle as high energy phosphate.

Creatinine is an anhydride of creatine. It is formed by spontaneous cyclization of creatine or creatine phosphate. Creatinine is excreted in urine.

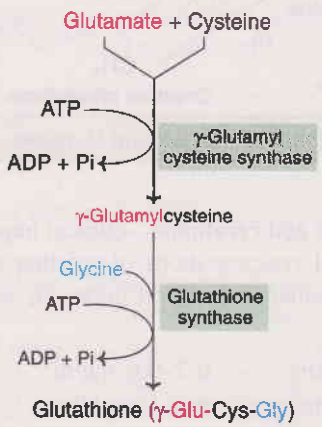


Fig. 15.15 : *Outline of glutathione synthesis.*

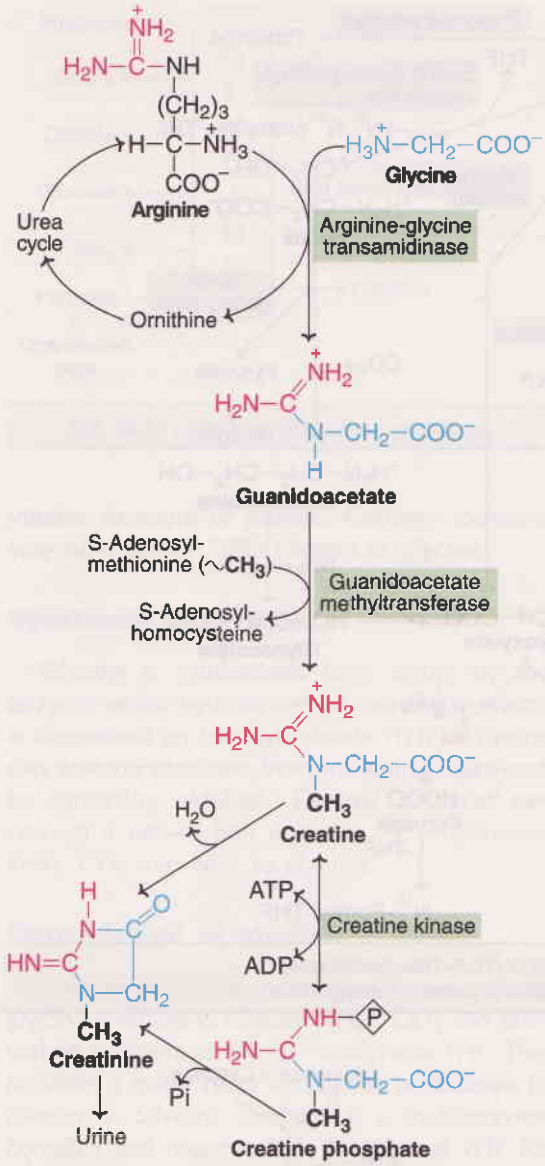


Fig. 15.16 : Metabolism of creatine.

Creatine and creatinine—clinical importance : The normal concentrations of creatine and creatinine in human serum and urine are as follows

Serum

Creatine — 0.2–0.6 mg/dl
Creatinine — 0.6–1 mg/dl

Urine

Creatine — 0–50 mg/day
Creatinine — 1–2 g/day

Estimation of serum creatinine (along with blood urea) is used as a diagnostic test to assess ***kidney function.*** Serum creatinine concentration is not influenced by endogenous and exogenous factors, as is the case with urea. Hence, some workers consider ***serum creatinine*** as a ***more reliable indicator of renal function.***

The amount of creatinine excreted is proportional to total creatine phosphate content of the body and, in turn, the muscle mass. The daily excretion of creatinine is usually constant. ***Creatinine coefficient*** is defined as the mg of creatinine and creatine (put together) excreted per kg body weight per day. For a normal adult man, the value is 24-26 mg, while for a woman, it is 20-22 mg.

Increased output of creatine in urine is referred to as creatinuria. Creatinuria is observed in muscular dystrophy, diabetes mellitus, hyperthyroidism, starvation etc.

Metabolic disorders of glycine

1. **Glycinuria :** This is a rare disorder. Serum glycine concentration is normal, but very high amount of it (normal 0.5-1 g/day) is excreted in urine. It is believed that glycinuria is due to a ***defective renal reabsorption.*** Glycinuria is characterized by increased tendency for the formation of oxalate renal stones. However, urinary oxalate level is normal in these patients.

2. **Primary hyperoxaluria :** This disorder is characterized by increased urinary oxalate resulting in oxalate stones. Deposition of oxalate (***oxalosis***) in various tissues is observed. The urinary oxalate is of endogenous origin and not due to dietary consumption of oxalate. Primary hyperoxaluria is due to a defect in ***glycine transaminase*** coupled with impairment in glyoxalate oxidation to formate.

It is now known that primary hyperoxaluria is mainly due to a ***defect in protein targeting*** (i.e. defect in transport of protein from one compartment to another). As a result, the enzyme glycine transaminase is found in mitochondria instead of its normal distribution in peroxisomes.

In vitamin ***B_6 deficiency,*** urinary ***oxalate*** is ***elevated*** which can be corrected by B_6 supplementation. However, B_6 administration has no effect on endogenous hyperoxaluria.

PHENYLALANINE AND TYROSINE

Phenylalanine (Phe, F) and tyrosine (Tyr, Y) are structurally related ***aromatic amino acids.*** Phenylalanine is an essential amino acid while tyrosine is non-essential. Besides its incorporation into proteins, the only function of phenylalanine is its conversion to tyrosine. For this reason, ingestion of tyrosine can reduce the dietary requirement of phenylalanine. This phenomenon is referred to as ***'sparing action' of tyrosine on phenylalanine.***

The predominant metabolism of phenylalanine occurs through tyrosine. Tyrosine is incorporated into proteins and is involved in the synthesis of a variety of biologically important compounds—***epinephrine, norepinephrine, dopamine*** (catecholamines), ***thyroid hormones***—and the pigment ***melanin*** (***Fig.15.17***). During the course of degradation, phenylalanine and tyrosine are converted to metabolites which can serve as precursors for the synthesis of glucose and fat. Hence, these amino acids are both ***glucogenic*** and ***ketogenic***. Biochemists attach special significance to phenylalanine and tyrosine metabolism for two reasons—synthesis of biologically important compounds and the metabolic disorders due to enzyme defects.

Conversion of phenylalanine to tyrosine

Under normal circumstances, the degradation of phenylalanine mostly occurs through tyrosine. Phenylalanine is hydroxylated at para-position by phenylalanine hydroxylase to produce tyrosine (p-hydroxy phenylalanine). This is an irreversible reaction and requires the participation of a specific coenzyme ***biopterin***

BIOMEDICAL / CLINICAL CONCEPTS

- *About 300–400 g of protein per day is constantly degraded and synthesized in the human body.*
- *The amino acids are mainly utilized for protein biosynthesis, production of specialized products (creatine, porphyrin, amines, purines, pyrimidines) and generation of energy.*
- *Glutamate is the collection centre for the amino groups in the biological system while glutamine is the storehouse of NH_3. Free NH_3 can be liberated predominantly from glutamate.*
- *Ammonia accumulation in blood is toxic to brain causing slurring of speech, blurring of vision, tremors and even death. Mammals convert NH_3 to urea, a non-toxic excretory product. Metabolic defects in urea cycle enzymes result in hyperammonemia.*
- *Dietary consumption of a protein rich meal increases the level of N-acetylglutamate in liver which enhances urea production.*
- *Primary hyperoxaluria—a metabolic disorder due to a defect in the enzyme glycine transaminase—is characterized by elevated urinary oxalate and the formation of oxalate stones.*
- *Blood urea estimation is commonly used to assess renal function. Elevation of blood urea level (normal 10–40 mg/dl) is associated with several disorders which may be pre-renal (diabetic coma), renal (acute glomerulonephritis) and post-renal (tumors or stones in the urinary tract).*
- *Estimation of serum creatinine (normal < 1 mg/dl) is considered to be a more reliable indicator for the evaluation of kidney function.*

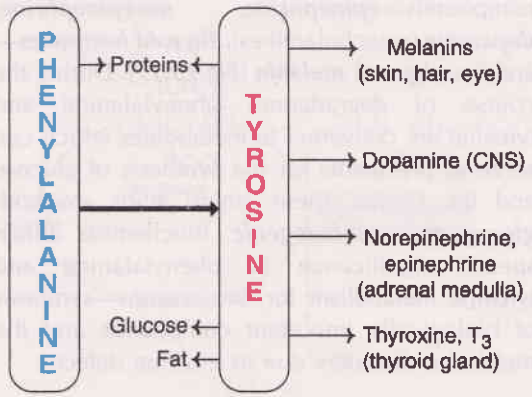


Fig. 15.17 : Overview of phenylalanine and tyrosine metabolism (CNS–Central nervous system; T_3–Triiodothyronine).

(containing pteridine ring) which is structurally related to folate. The active form of biopterin is tetrahydrobiopterin (H_4-biopterin). In the phenylalanine hydroxylase reaction, tetrahydrobiopterin is oxidized to dihydrobiopterin (H_2-biopterin). Tetrahydrobiopterin is then regenerated by an NADPH-dependent dihydrobiopterin reductase (***Fig.15.18***).

The enzyme phenylalanine hydroxylase is present in the liver. In the conversion of phenylalanine to tyrosine, the reaction involves the incorporation of one atom of molecular oxygen (O_2) into the para position of phenylalanine while the other atom of O_2 is reduced to form water. It is the tetrahydrobiopterin that supplies the reducing equivalents which, in turn, are provided by NADPH. Due to a defect in ***phenylalanine hydroxylase***, the conversion of phenylalanine to tyrosine is blocked resulting in the disorder ***phenylketonuria*** (PKU).

DEGRADATION OF TYROSINE (PHENYLALANINE)

The metabolism of phenylalanine and tyrosine is considered together. The sequence of the reactions in the degradation of these amino acids, depicted in ***Fig.15.19***, is described hereunder

1. As phenylalanine is converted to tyrosine (details in ***Fig.15.18***), a single pathway is responsible for the degradation of both these amino acids, which occurs mostly in liver.

2. Tyrosine first undergoes transamination to give p-hydroxyphenylpyruvate. This reaction is catalysed by tyrosine transaminase (PLP dependent).

3. p-Hydroxyphenylpyruvate hydroxylase (or dioxygenase) is a copper-containing enzyme. It catalyses oxidative decarboxylation as well as hydroxylation of the phenyl ring of p-hydroxyphenylpyruvate to produce homogentisate. This reaction involves a shift in hydroxyl group from para position to meta position, and incorporates a new hydroxyl group at para position. This step in tyrosine metabolism requires ascorbic acid.

4. Homogentisate oxidase (iron metalloprotein) cleaves the benzene ring of homogentisate to form 4-maleylacetoacetate.

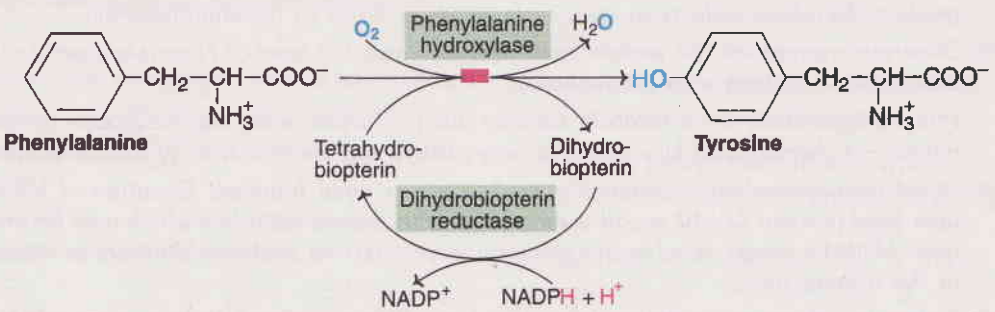


Fig. 15.18 : Synthesis of tyrosine from phenylalanine (■—Block in phenylketonuria).

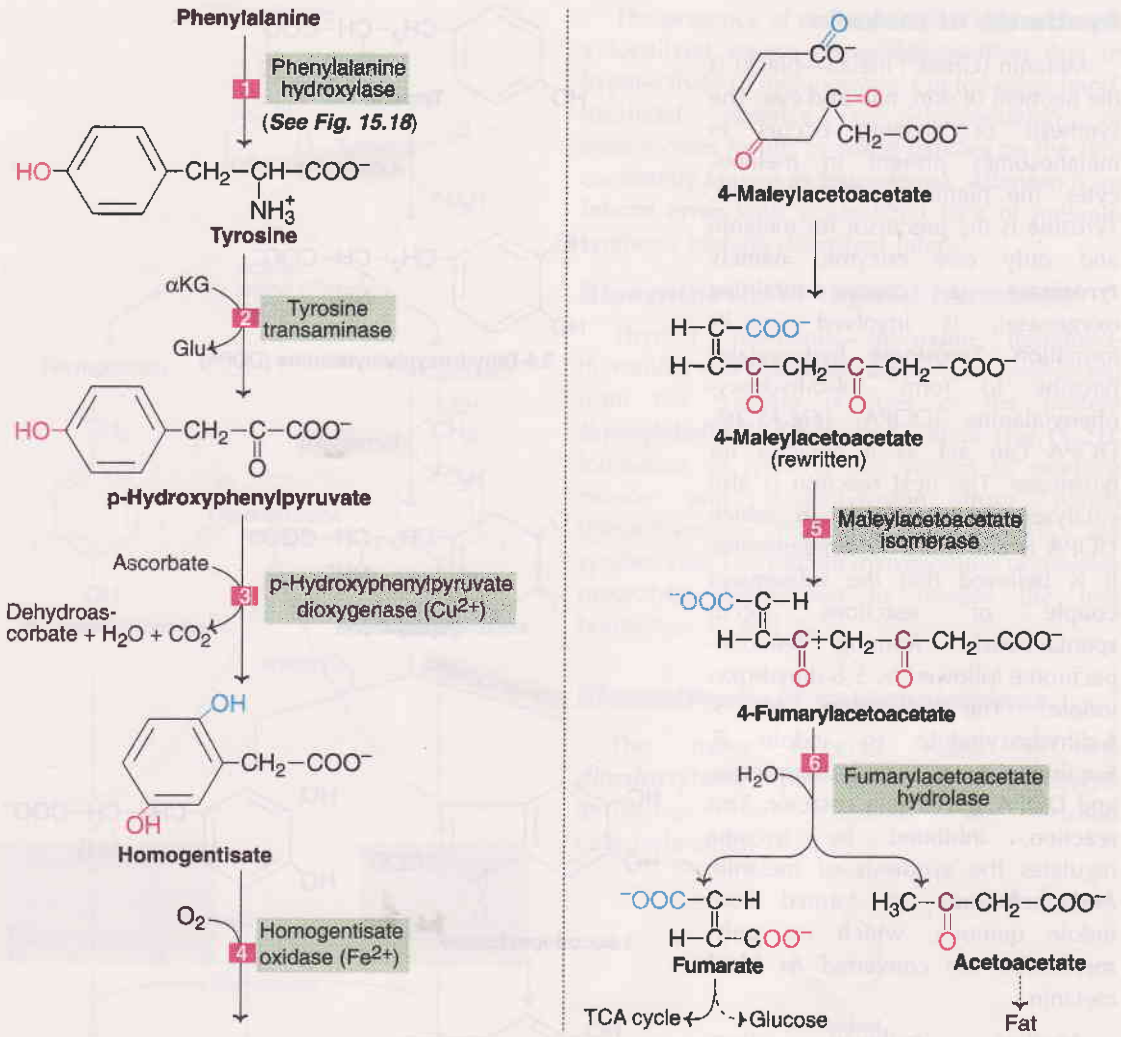


Fig. 15.19 contd. next column

Fig. 15.19 : Tyrosine metabolism—degradative pathway [αKG–α-Ketoglutarate; Glu-Glutamate; The circled numbers indicate metabolic defects (1) Phenylketonuria; (2) Tyrosinemia type II; (3) Neonatal tyrosinemia; (4) Alkaptonuria; (5) and (6) Tyrosinosis (tyrosinemia, type I)].

Molecular oxygen is required for this reaction to break the aromatic ring.

5. Maleylacetoacetate undergoes isomerization to form 4-fumaryl acetoacetate and this reaction is catalysed by maleylacetoacetate isomerase.

6. Fumaryl acetoacetase (fumaryl acetoacetate hydrolase) brings about the hydrolysis of fumaryl acetoacetate to liberate fumarate and acetoacetate.

Fumarate is an intermediate of citric acid cycle and can also serve as precursor for gluconeogenesis. Acetoacetate is a ketone body from which fat can be synthesized. Phenylalanine and tyrosine are, therefore, both glucogenic and ketogenic.

The inborn errors of phenylalanine and tyrosine metabolism are indicated in ***Fig.15.19***. Detailed information on these disorders is given later.

Synthesis of melanin

Melanin (*Greek* : melan—black) is the pigment of skin, hair and eye. The synthesis of melanin occurs in melanosomes present in melanocytes, the pigment-producing cells. Tyrosine is the precursor for melanin and only one enzyme, namely ***tyrosinase*** (a copper-containing oxygenase), is involved in its formation. Tyrosinase hydroxylates tyrosine to form 3,4-dihydroxyphenylalanine (DOPA) (***Fig.15.20***). DOPA can act as a cofactor for tyrosinase. The next reaction is also catalysed by tyrosinase in which DOPA is converted to dopaquinone. It is believed that the subsequent couple of reactions occur spontaneously, forming leucodopachrome followed by 5,6-dihydroxyindole. The oxidation of 5, 6-dihydroxyindole to indole 5, 6-quinone is catalysed by tyrosinase, and DOPA serves as a cofactor. This reaction, inhibited by tyrosine regulates the synthesis of melanin. ***Melanochromes*** are formed from indole quinone, which on ***polymerization*** are converted ***to black*** melanin.

Another pathway from dopaquinone is also identified. Cysteine condenses with dopaquinone and in the next series of reactions results the synthesis of red melanins. The structure of melanin pigments is not clearly known.

Melanin—the colour pigment : The skin colour of the individual is determined by the relative concentrations of black and red melanins. This, in turn, is dependent on many factors, both genetic and environmental. These include the activity of tyrosinase, the density of melanocytes, availability of tyrosine etc.

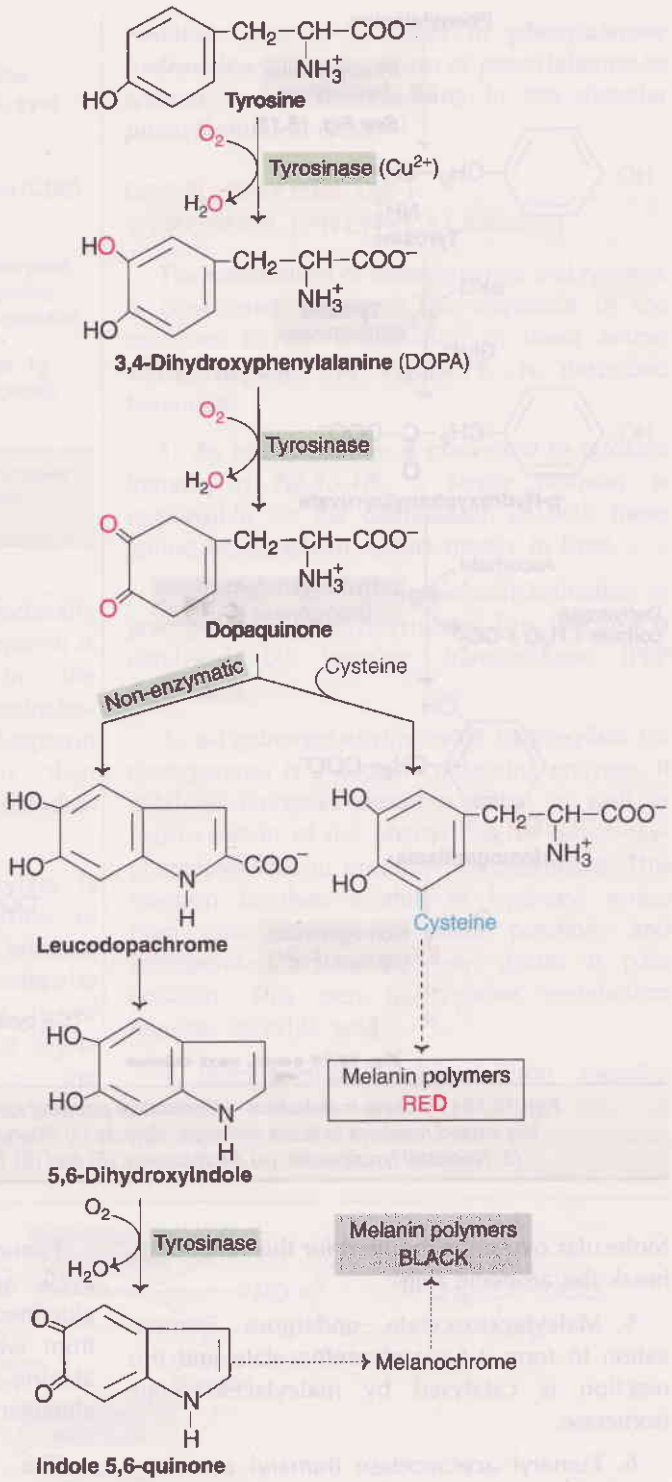


Fig. 15.20 : Metabolism of tyrosine—biosynthesis of melanin (Defect in tyrosinase causes albinism).

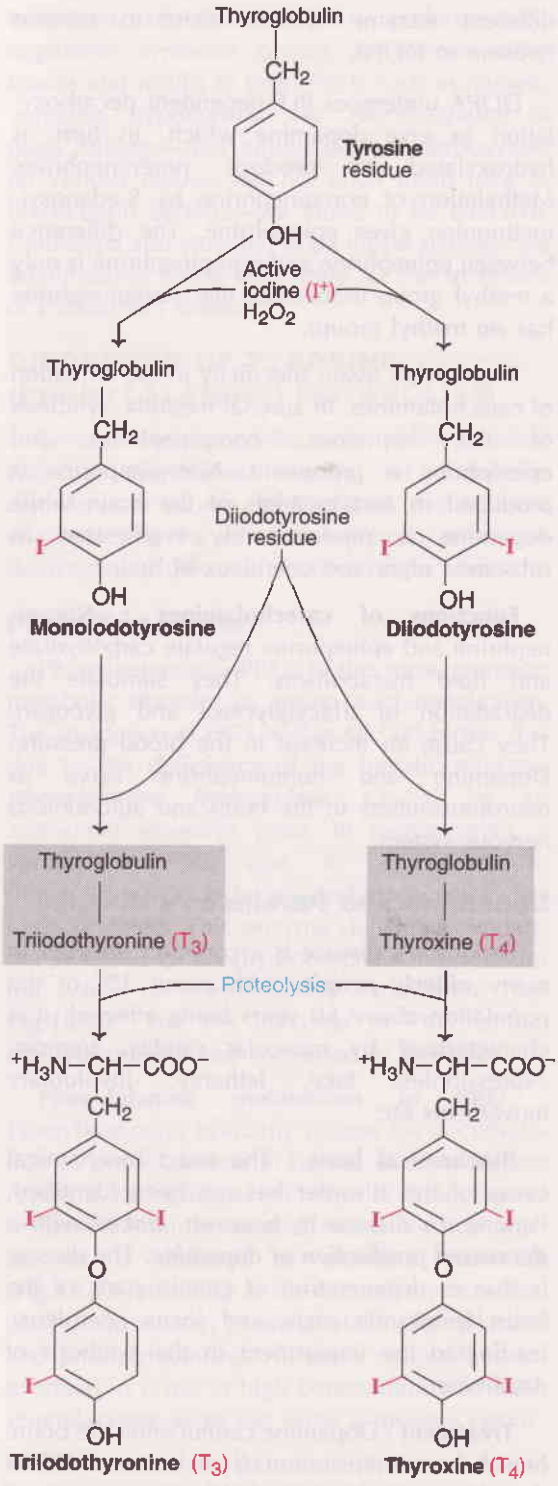


Fig. 15.21 : Metabolism of tyrosine-synthesis of thyroid hormones.

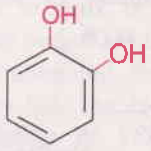
The presence of ***moles*** on the body represents a localized severe ***hyperpigmentation*** due to hyperactivity of melanocytes. On the other hand, localized absence or degeneration of melanocytes results in ***white patches*** on the skin commonly known as ***leucoderma***. ***Albinism*** is an ***inborn error*** with generalized lack of melanin synthesis (details described later).

Biosynthesis of thyroid hormones

Thyroid hormones—***thyroxine*** (tetraiodothyronine) and ***triiodothyronine***—are synthesized from the tyrosine residues of the protein ***thyroglobulin*** and activated iodine (***Fig.15.21***). Iodination of tyrosine ring occurs to produce mono- and diiodotyrosine from which triiodothyronine (T_3) and thyroxine (T_4) are synthesized. The protein thyroglobulin undergoes proteolytic breakdown to release the free hormones, T_3 and T_4.

Biosynthesis of catecholamines

The name catechol refers to the ***dihydroxylated phenyl ring***. The amine derivatives of catechol are called catecholamines.



Catechol

Tyrosine is the precursor for the synthesis of catecholamines, namely ***dopamine***, ***norepinephrine*** (noradrenaline) and ***epinephrine*** (adrenaline).

The conversion of tyrosine to catecholamines occurs in adrenal medulla and central nervous system involving the following reactions (***Fig.15.22***).

Tyrosine is hydroxylated to 3,4-dihydroxyphenylalanine (DOPA) by ***tyrosine hydroxylase***. This enzyme catalyses the ***rate limiting*** reaction and requires tetrahydrobiopterin as coenzyme (like phenylalanine hydroxylase). In contrast to this enzyme, tyrosinase present in melanocytes converts tyrosine to DOPA. Hence, two

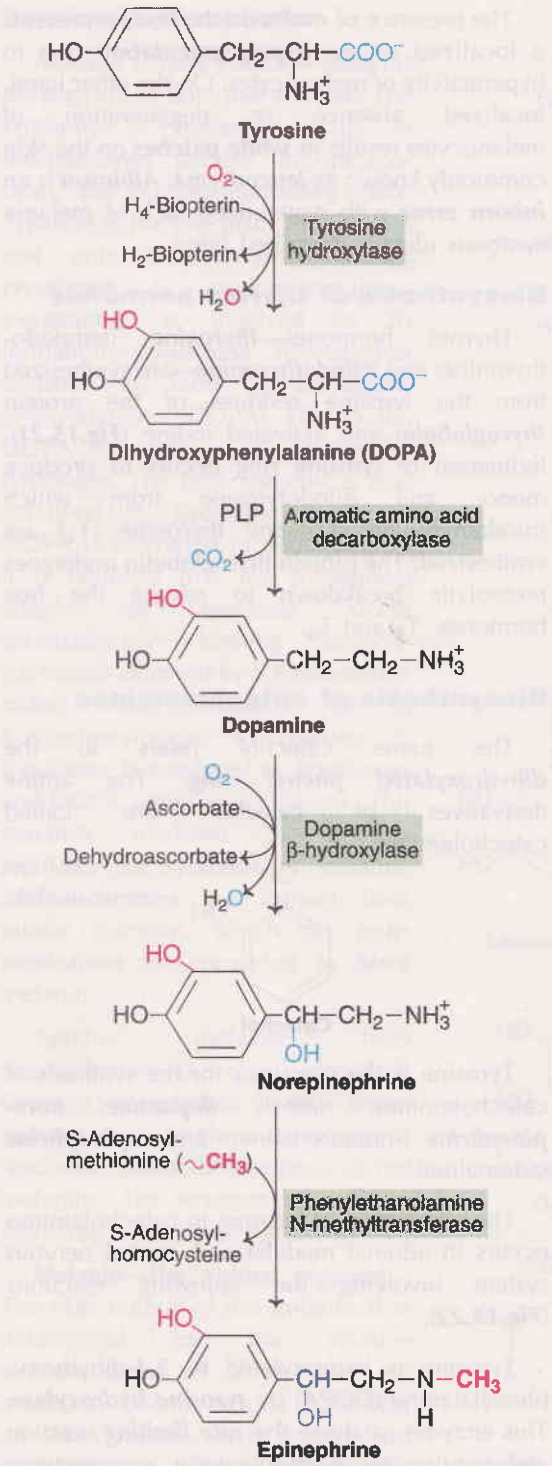


Fig. 15.22 : Metabolism of tyrosine-synthesis of catecholamines (dopamine, norepinephrine, epinephrine; PLP–pyridoxal phosphate).

different enzyme systems exist to convert tyrosine to DOPA.

DOPA undergoes PLP-dependent decarboxylation to give dopamine which, in turn, is hydroxylated to produce norepinephrine. Methylation of norepinephrine by S-adenosylmethionine gives epinephrine. The difference between epinephrine and norepinephrine is only a methyl group (remember that ***nor***epinephrine has ***no*** methyl group).

There exists tissue specificity in the formation of catecholamines. In adrenal medulla, synthesis of the hormones, norepinephrine and epinephrine is prominent. Norepinephrine is produced in certain areas of the brain while dopamine is predominantly synthesized in substantia nigra and coeruleus of brain.

Functions of catecholamines : Norepinephrine and epinephrine regulate carbohydrate and lipid metabolisms. They stimulate the degradation of triacylglycerol and glycogen. They cause an increase in the blood pressure. Dopamine and norepinephrine serve as neurotransmitters in the brain and autonomous nervous system.

Dopamine and Parkinson's disease

Parkinson's disease is a common ***disorder*** in many ***elderly people***, with about 1% of the population above 60 years being affected. It is characterized by muscular rigidity, tremors, expressionless face, lethargy, involuntary movements etc.

Biochemical basis : The exact biochemical cause of this disorder has not been identified. Parkinson's disease is, however, ***linked with a decreased production of dopamine***. The disease is due to degeneration of certain parts of the brain (substantia nigra and locus coeruleus), leading to the impairment in the synthesis of dopamine.

Treatment : Dopamine cannot enter the brain, hence its administration is of no use. ***DOPA*** (levodopa or L-dopa) is used in the treatment of Parkinson's disease. In the brain, DOPA is decarboxylated to dopamine which alleviates the

symptoms of this disorder. Unfortunately, dopamine synthesis occurs in various other tissues and results in side-effects such as nausea, vomiting, hypretension etc. Administration of dopa analogs—that inhibit dopa decarboxylase (in various tissues) but not enter brain (due to blood-brain barrier)—are found to be effective. Carbidopa and γ-methyl-dopa (dopa analogs) are administered along with dopa for the treatment of Parkinson's disease.

DISORDERS OF TYROSINE (PHENYLALANINE) METABOLISM

Several enzyme defects in phenylalanine/tyrosine degradation leading to metabolic disorders are known. In ***Fig.15.19***, the deficient enzymes and the respective inborn errors are depicted and they are discussed here under.

Phenylketonuria

Phenylketonuria (PKU) is the most common metabolic disorder in amino acid metabolism. The incidence of PKU is 1 in 10,000 births. It is due to the ***deficiency*** of the hepatic enzyme, ***phenylalanine hydroxylase***, caused by an autosomal recessive gene. In recent years, a variant of PKU—due to a defect in dihydrobiopterin reductase (relatively less)—has been reported. This enzyme deficiency impairs the synthesis of tetrahydrobiopterin required for the action of phenylalanine hydroxylase (***See Fig.15.18***). The net outcome in PKU is that phenylalanine is not converted to tyrosine.

Phenylalanine metabolism in PKU : Phenylketonuria primarily causes the accumulation of phenylalanine in tissues and blood, and results in its increased excretion in urine. Due to disturbances in the routine metabolism, phenylalanine is diverted to alternate pathways (***Fig.15.23***), resulting in the excessive production of phenylpyruvate, phenylacetate, phenyllactate and phenylglutamine. All these metabolites are excreted in urine in high concentration in PKU. Phenylacetate gives the urine a mousey odour.

The name phenylketonuria is coined due to the fact that the metabolite ***phenylpyruvate*** is a ***keto*** acid ($C_6H_5CH_2-CO-COO^-$) excreted in urine in high amounts.

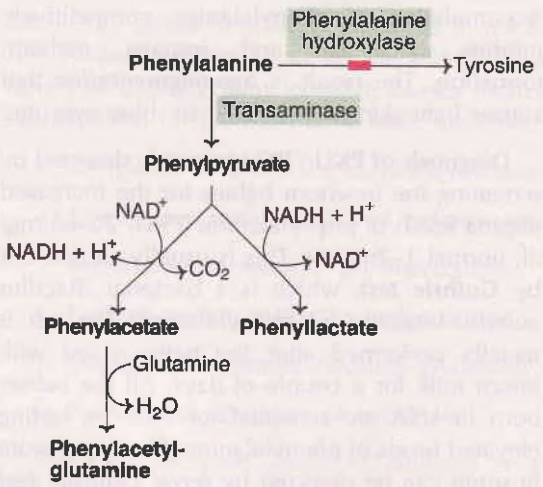


Fig. 15.23 : Metabolites that accumulate in phenylketonuria (▬—Block in phenylketonuria).

Clinical/biochemical manifestations of PKU : The disturbed metabolism of phenylalanine—resulting in the increased concentration of phenylalanine and its metabolites in the body—causes many clinical and biochemical manisfestations.

1. **Effects on central nervous system :** Mental retardation, failure to walk or talk, failure of growth, seizures and tremor are the characteristic findings in PKU. If untreated, the patients show very low IQ (below 50). The biochemical basis of ***mental retardation*** in PKU is not well understood. There are, however, many explanations offered

- Accumulation of phenylalanine in brain impairs the transport and metabolism of other aromatic amino acids (tryptophan and tyrosine).
- The synthesis of ***serotonin*** (an excitatory neurotransmitter) from tryptophan is insufficient. This is due to the competition of phenylalanine and its metabolites with tryptophan that impairs the synthesis of serotonin.
- Defect in ***myelin*** formation is observed in PKU patients.

2. **Effect on pigmentation :** Melanin is the pigment synthesized from tyrosine by tyrosinase.

Accumulation of phenylalanine competitively inhibits tyrosinase and impairs melanin formation. The result is ***hypopigmentation*** that causes light skin colour, fair hair, blue eyes etc.

Diagnosis of PKU : PKU is mostly detected by screening the newborn babies for the increased plasma levels of phenylalanine (PKU, 20–65 mg/dl; normal 1–2mg/dl). This is usually carried out by ***Guthrie test***, which is a bacterial *(Bacillus subtilis)* bioassay for phenylalanine. The test is usually performed after the baby is fed with breast milk for a couple of days. All the babies born in USA are screened for PKU by testing elevated levels of phenylalanine. Phenylpyruvate in urine can be detected by ***ferric chloride test*** (a green colour is obtained). This test is not specific, since many other compounds give a false positive test.

Treatment of PKU : The maintenance of ***plasma phenylalanine*** concentration ***within*** the ***normal range*** is a challenging task in the treatment of PKU. This is done by selecting foods with low phenylalanine content and/or feeding synthetic amino acid preparations, low in phenylalanine. Dietary intake of phenylalanine should be adjusted by measuring plasma levels. Early diagnosis (in the first couple of months of baby's life) and treatment for 4–5 years can prevent the damage to brain. However, the restriction to protein diet should be continued for many more years in life. Since the amino acid ***tyrosine*** cannot be synthesized in PKU patients, it becomes ***essential*** and should be provided in the diet in sufficient quantity.

In some seriously affected PKU patients, treatment includes administration of 5-hydroxytryptophan and dopa to restore the synthesis of serotonin and catecholamines.

Tyrosinemia type II

This disorder—also known as ***Richner-Hanhart syndrome***, is due to a defect in the enzyme ***tyrosine transaminase.*** The result is a blockade in the routine degradative pathway of tyrosine. Accumulation and excretion of tyrosine and its metabolites—namely p-hydroxyphenylpyruvate, p-hydroxyphenyllactate, phydroxyphenylacetate, N-acetyltyrosine—and tyramine are observed.

Tyrosinemia type II is characterized by skin (dermatitis) and eye lesions and, rarely, mental retardation. A disturbed self-coordination is seen in these patients.

Neonatal tyrosinemia

The absence of the enzyme ***p-hydroxyphenylpyruvate dioxygenase*** causes neonatal tyrosinemia. This is mostly a temporary condition and usually responds to ascorbic acid. It is explained that the substrate inhibition of the enzyme is overcome by the presence of ascorbic acid.

Alkaptonuria (Black urine disease)

Alkaptonuria has great historical importance. It was first described by Lusitanus in 1649 and characterized in 1859. Garrod conceived the idea of inborn errors of metabolism from his observation on alkaptonuria. The prevalance of this autosomal recessive disorder is 1 in 25,000.

Enzyme defect : The defective enzyme in alkaptonuria is ***homogentisate oxidase*** in tyrosine metabolism (***See Fig.15.19***). Homogentisate accumulates in tissues and blood, and is excreted into urine. Homogentisate, on standing, gets oxidized to the corresponding quinones, which polymerize to give black or brown colour. For this reason, the ***urine*** of alkaptonuric patients resembles ***coke in colour.***

Biochemical manifestations : Homogentisate gets oxidized by polyphenol oxidase to benzoquinone acetate which undergoes polymerization to produce a pigment called ***alkapton*** (***Fig.15.24***). Alkapton deposition occurs in connective tissue, bones and various organs (nose, ear etc.) resulting in a condition known as ***ochronosis.*** Many alkaptonuric patients suffer from arthritis and this is believed to be due to the deposition of pigment alkapton (in the joints), produced from homogentisate.

Diagnosis : Change in colour of the urine on standing to brown or dark has been the simple traditional method to identify alkaptonuria. The

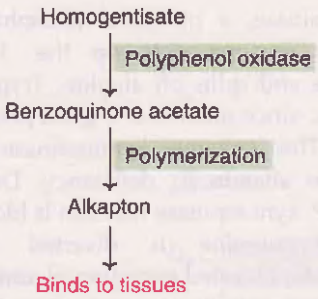


Fig. 15.24 : Conversion of homogentisate to alkapton.

urine gives a positive test with ferric chloride and silver nitrate. This is due to the strong reducing activity of homogentisate. Benedict's test—employed for the detection of glucose and other reducing sugars—is also positive with homogentisate.

Treatment : Alkaptonuria is ***not*** a ***dangerous disorder*** and, therefore, does not require any specific treatment. However, consumption of protein diet with relatively low phenylalanine content is recommended.

Tyrosinosis or tyrosinemia type I

This is due to the deficiency of the enzymes ***fumarylacetoacetate hydroxylase*** and/or ***maleylacetoacetate isomerase***. Tyrosinosis is a rare but serious disorder. It causes liver failure, rickets, renal tubular dysfunction and polyneuropathy. Tyrosine, its metabolites and many other amino acids are excreted in urine.

In acute tyrosinosis, the infant exhibits diarrhea, vomiting, and 'cabbage-like' odor. Death may even occur due to liver failure within one year. For the treatment, diets low in tyrosine, phenylalanine and methionine are recommended.

Albinism

Albinism (*Greek*: albino—white) is an inborn error, due to the ***lack of*** synthesis of the pigment ***melanin***. It is an autosomal recessive disorder with a frequency of 1 in 20,000.

Biochemical basis : The colour of skin and hair is controlled by a large number of genes. About 150 genes have been identified in mice. The melanin synthesis can be influenced by a variety of factors. Many possible causes (rather explanations) for albinism have been identified

1. Deficiency or lack of the enzyme tyrosinase.
2. Decrease in melanosomes of melanocytes.
3. Impairment in melanin polymerization.
4. Lack of protein matrix in melanosomes.
5. Limitation of substrate (tyrosine) availability.
6. Presence of inhibitors of tyrosinase.

The most common cause of albinism is a ***defect in tyrosinase***, the enzyme most responsible for the synthesis of melanin (***See Fig.15.20***).

Clinical manifestations : The most important function of melanin is the protection of the body from sun radiation. Lack of melanin in albinos makes them sensitive to sunlight. Increased susceptibility to ***skin cancer*** (carcinoma) is observed. ***Photophobia*** (intolerance to light) is associated with lack of pigment in the eyes. However, there is no impairment in the eyesight of albinos.

Hypopigmentation

In some individuals, a reduced synthesis of melanin (instead of total lack) is often observed. Hypopigmentation disorders may be either diffuse or localized.

A good example of diffuse hypopigmentation is ***oculocutaneous albinism*** which is mostly due to mutations in the tyrosinase gene. The degree of hypopigmentation depends on the type and severity of mutated genes.

Vitiligo and ***leukoderma*** are the important among the localized hypopigmentation disorders. Vitiligo is an acquired progressive disease with loss of pigmentation around mouth, nose, eyes and nipples. Leukoderma is comparable with vitiligo, but lack of pigmentation usually begins with hands and then spreads.

Greying of hair is due to lack of melanin synthesis which usually occurs as a result of disappearance of melanocytes from the hair roots.

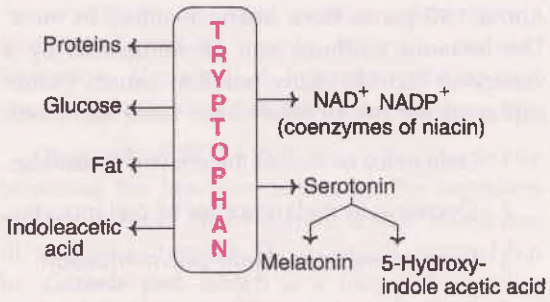


Fig. 15.25 : Overview of tryptophan metabolism.

TRYPTOPHAN

Tryptophan (Trp, W) was the first to be identified as an ***essential amino acid***. It contains an indole ring and chemically it is α-amino β-indole propionic acid. Tryptophan is both ***glucogenic*** and ***ketogenic*** in nature. It is a precursor for the synthesis of important compounds, namely ***NAD+*** and ***NADP+*** (coenzymes of niacin), ***serotonin*** and ***melatonin*** (***Fig.15.25***).

The metabolism of tryptophan is divided into

I. Kynurenine (kynurenine-anthranilate) pathway;

II. Serotonin pathway.

I. Kynurenine pathway

This pathway mostly occurs in liver leading to oxidation of tryptophan and the synthesis of NAD^+ and $NADP^+$ (***Fig.15.26***).

Tryptophan pyrrolase or oxygenase cleaves the five-membered ring of the indole nucleus to produce formylkynurenine. Tryptophan pyrrolase is a metalloprotein containing an iron porphyrin ring. It is a substrate inducible enzyme and is controlled by feedback regulation (by NADPH and other niacin derivatives). Tryptophan pyrrolase activity is also elevated by corticosteroids. Formamidase hydrolyses formylkynurenine and liberates formate which enters the one carbon pool. Kynurenine formed in this reaction is a branch point with different fates. In the prominent pathway, kynurenine undergoes NADPH-dependent hydroxylation to give 3-hydroxykynurenine.

Kynureninase, a pyridoxal phosphate (PLP)-dependent enzyme acts on the 3-hydroxykynurenine and splits off alanine. Tryptophan is glucogenic, since alanine is a good precursor for glucose. The enzyme kynureninase is very sensitive to vitamin B_6 deficiency. Due to the lack of PLP, kynureninase reaction is blocked and 3-hydroxykynurenine is diverted to form xanthurenate. Elevated excretion of ***xanthurenate*** serves as an indication of ***B_6 deficiency.*** Administration of isoniazid, an antituberculosis drug—induces B_6 deficiency and results in xanthurenate excretion in urine. Defects in the activity of kynureninase (in B_6 deficiency) cause reduced synthesis of NAD^+ and $NADP^+$ from tryptophan. The symptoms of pellagra —observed in B_6 deficiency—are explained on this basis.

The enzyme kynurenine hydroxylase is inhibited by estrogen, hence women, in general, are more susceptible to pellagra.

3-Hydroxyanthranilate is cleaved by an oxidase (Fe^{2+} dependent) to form an unstable intermediate, 2-amino 3-carboxy muconate semialdehyde. This compound has three fates.

1. It undergoes spontaneous cyclization to form quinolinate for NAD^+ synthesis.

2. Picolinate carboxylase decarboxylates the intermediate which cyclizes to produce picolinate. This enzyme competes with the formation of quinolinate. High activity of picolinate carboxylase in some animals (e.g. cat) deprives them of NAD^+ synthesis from tryptophan. In other words, cat is exclusively dependent on niacin for its coenzymes (NAD^+, $NADP^+$), since tryptophan cannot serve as a precursor.

3. The intermediate undergoes decarboxylation, catalysed by amino carboxysemialdehyde decarboxylase to produce 2-aminomuconate semialdehyde that enters glutarate pathway. The semialdehyde is converted to 2-aminomuconate by a dehydrogenase. The aminomuconate, in a series of reactions involving reduction, deamination, decarboxylation etc., is converted to glutaryl CoA and finally to acety CoA. The latter is either completely oxidized via TCA cycle or converted to fat (hence tryptophan is ketogenic).

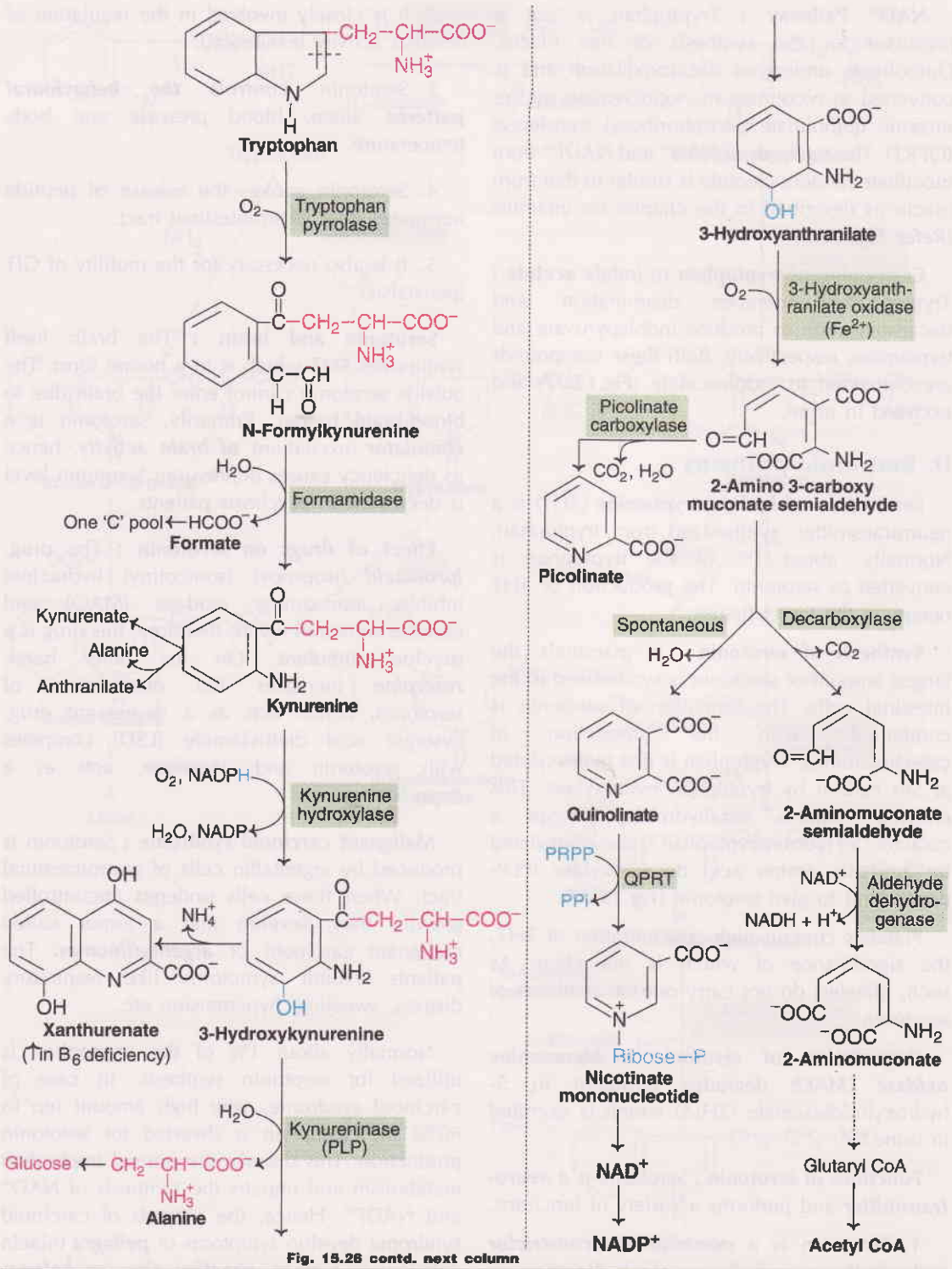


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Fig. 15.26 : Metabolism of tryptophan-kynurenine pathway (PLP–Pyridoxal phosphate; QPRT–Quinolinate phosphoribosyl transferase; PRPP–Phosphoribosyl pyrophosphate).

NAD^+ Pathway : Tryptophan is not a precursor for the synthesis of free niacin. Quinolinate undergoes decarboxylation and is converted to nicotinate mononucleotide by the enzyme quinolinate phosphoribosyl transferase (QPRT). The synthesis of NAD^+ and $NADP^+$ from nicotinate mononucleotide is similar to that from niacin as described in the chapter on vitamins (***Refer Fig.7.21***).

Conversion of tryptophan to indole acetate : Tryptophan undergoes deamination and decarboxylation to produce indolepyruvate and tryptamine, respectively. Both these compounds are converted to indoleacetate (***Fig.15.27***) and excreted in urine.

II. Serotonin pathway

Serotonin or ***5-hydroxytryptamine*** (5HT) is a neurotransmitter, synthesized from tryptophan. Normally, about 1% of the tryptophan is converted to serotonin. The production of 5HT occurs in the target tissues.

Synthesis of serotonin : In mammals, the largest amount of serotonin is synthesized in the intestinal cells. The formation of serotonin is comparable with the production of catecholamines. Tryptophan is first hydroxylated at 5th carbon by tryptophan hydroxylase. This enzyme requires tetrahydrobiopterin as a cofactor. 5-Hydroxytryptophan is decarboxylated by aromatic amino acid decarboxylase (PLP-dependent) to give serotonin (***Fig.15.27***).

Platelets contain high concentration of 5HT, the significance of which is not clear. As such, platelets do not carry out the synthesis of serotonin.

Degradation of serotonin : ***Monoamine oxidase*** (***MAO***) degrades serotonin to 5-hydroxyindoleacetate (5HIA) which is excreted in urine.

Functions of serotonin : Serotonin is ***a neurotransmitter*** and performs a variety of functions.

1. Serotonin is a powerful ***vasoconstrictor*** and results in smooth muscle contraction in bronchioles and arterioles.

2. It is closely involved in the regulation of cerebral activity (excitation).

3. Serotonin ***controls the behavioural patterns***, sleep, blood pressure and body temperature.

4. Serotonin evokes the release of peptide hormones from gastrointestinal tract.

5. It is also necessary for the motility of GIT (peristalsis).

Serotonin and brain : The brain itself synthesizes 5HT which is in a bound form. The outside serotonin cannot enter the brain due to blood-brain barrier. Primarily, serotonin is a ***stimulator*** (excitation) ***of brain activity***, hence its deficiency causes depression. Serotonin level is decreased in psychosis patients.

Effect of drugs on serotonin : The drug, ***iproniazid*** (isopropyl isonicotinyl hydrazine) inhibits monoamine oxidase (MAO) and elevates serotonin levels, therefore, this drug is a psychic stimulant. On the other hand, ***reserpine*** increases the degradation of serotonin, hence acts as a depressant drug. Lysergic acid diethylamide (LSD) competes with serotonin and, therefore, acts as a depressant.

Malignant carcinoid syndrome : Serotonin is produced by argentaffin cells of gastrointestinal tract. When these cells undergo uncontrolled growth, they develop into a tumor called malignant carcinoid or ***argentaffinomas.*** The patients exhibit symptoms like respiratory distress, sweating, hypertension etc.

Normally about 1% of the tryptophan is utilized for serotonin synthesis. In case of carcinoid syndrome, very high amount (up to 60%) of tryptophan is diverted for serotonin production. This disturbs the normal tryptophan metabolism and impairs the synthesis of NAD^+ and $NADP^+$. Hence, the patients of carcinoid syndrome develop symptoms of ***pellagra*** (niacin deficiency). Further, ***negative nitrogen balance*** is also observed.

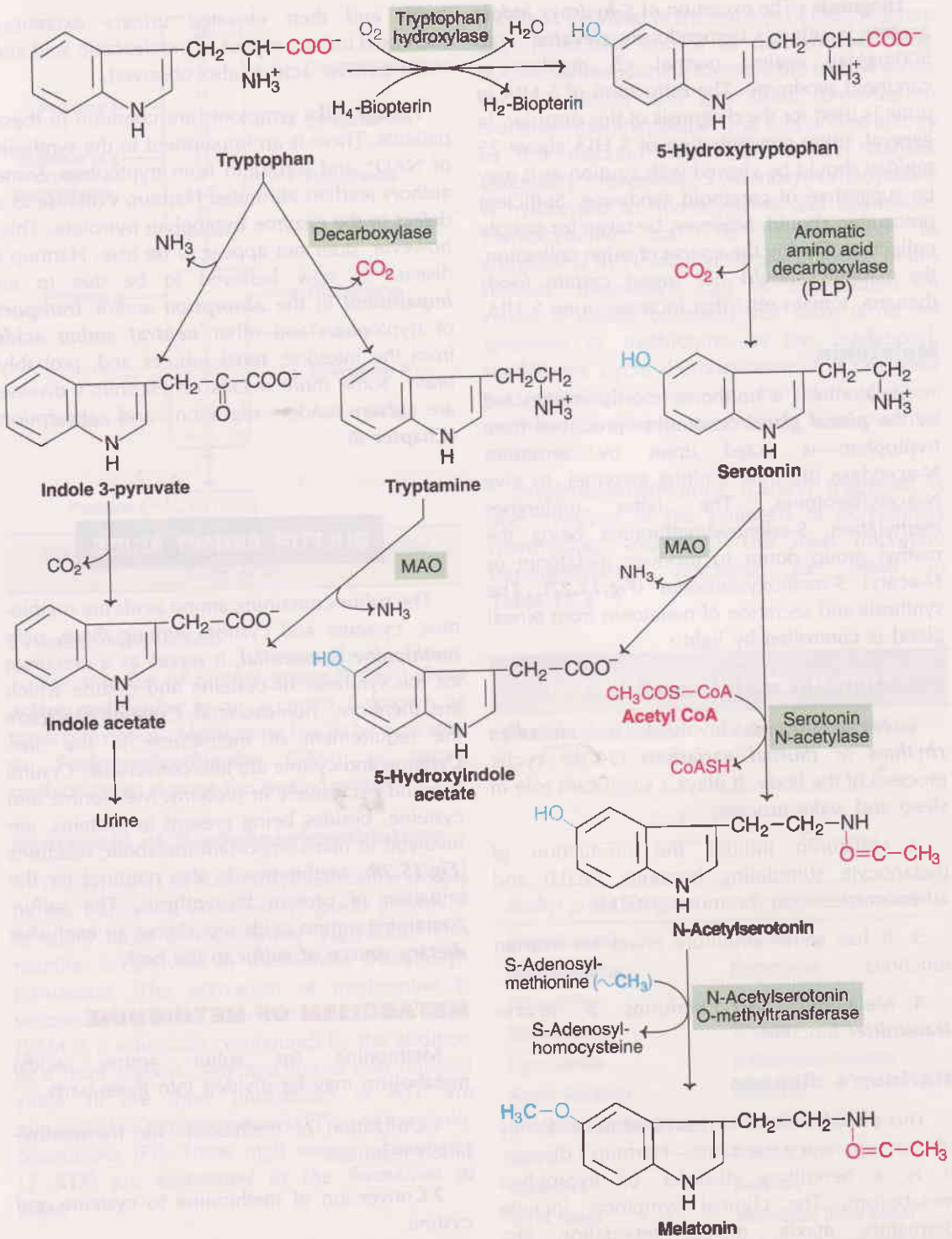


Fig. 15.27 : Metabolism of tryptophan-serotonin and melatonin synthesis (PLP–Pyridoxal phosphate; MAO–Monoamine oxidase).

Diagnosis : The excretion of ***5-hydroxy indole acetate*** in urine is tremendously ***elevated*** (upto 500mg/day against normal <5 mg/day) in carcinoid syndrome. The estimation of 5 HIA in urine is used for the diagnosis of this disorder. In general, urine concentration of 5 HIA above 25 mg/day should be viewed with caution as it may be suggestive of carcinoid syndrome. Sufficient precaution should, however, be taken for sample collection. During the course of urine collection, the patients should not ingest certain foods (banana, tomato etc.) that increase urine 5 HIA.

Melatonin

Melatonin is a hormone, mostly synthesized by the ***pineal gland.*** Serotonin—produced from tryptophan—is acted upon by serotonin N-acetylase (the rate limiting enzyme), to give N-acetylserotonin. The latter undergoes methylation, S-adenosylmethionine being the methyl group donor to produce melatonin or N-acetyl 5-methoxyserotonin (***Fig.15.27***). The synthesis and secretion of melatonin from pineal gland is controlled by light.

Functions of melatonin

1. Melatonin is involved in ***circadian rhythms*** or ***diurnal variations*** (24 hr cyclic process) of the body. It plays a significant role in sleep and wake process.

2. Melatonin inhibits the production of melanocyte stimulating hormone (MSH) and adrenocorticotropic hormone (ACTH).

3. It has some inhibitory effect on ovarian functions.

4. Melatonin also performs a ***neuro-transmitter*** function.

Hartnup's disease

This disorder was first described in the family of Hartnup, hence the name—Hartnup's disease. It is a hereditary disorder of tryptophan metabolism. The clinical symptoms include dermatitis, ataxia, mental retardation etc. Hartnup's disease is characterized by ***low plasma levels of tryptophan and other neutral amino acids*** and their elevated urinary excretion. Increased urinary output of indoleacetic acid and indolepyruvic acid is also observed.

Pellagra-like symptoms are common in these patients. There is an impairment in the synthesis of NAD^+ and serotonin from tryptophan. Some authors (earlier) attributed Hartnup's disease to a defect in the enzyme tryptophan pyrrolase. This, however, does not appear to be true. Hartnup's disease is now believed to be due to an ***impairment*** in the ***absorption*** and/or ***transport*** of tryptophan and other ***neutral amino acids*** from the intestine, renal tubules and, probably brain. Some more details on Hartnup's disease are given under digestion and absorption (***Chapter 8***).

SULFUR AMINO ACIDS

The sulfur-containing amino acids are methionine, cysteine and cystine. Among these, only ***methionine*** is ***essential***. It serves as a precursor for the synthesis of cysteine and cystine which are, therefore, non-essential. Cysteine can spare the requirement of methionine in the diet. Cysteine and cystine are interconvertible. Cystine is found exclusively in proteins. Methionine and cysteine, besides being present in proteins, are involved in many important metabolic reactions (***Fig.15.28***). Methionine is also required for the initiation of protein biosynthesis. The sulfur-containing amino acids are almost an exclusive ***dietary source of sulfur*** to the body.

METABOLISM OF METHIONINE

Methionine (or sulfur amino acids) metabolism may be divided into three parts.

1.Utilization of methionine for transmethylation reactions.

2.Conversion of methionine to cysteine and cystine.

3.Degradation of cysteine and its conversion to specialized products.

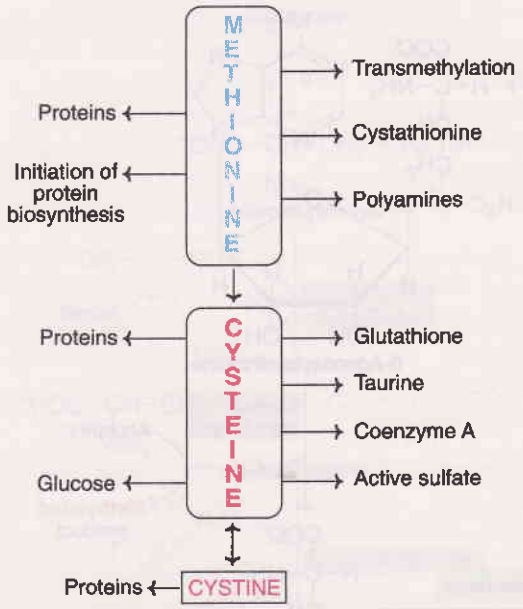


***Fig. 15.28** : Overview of the metabolism of sulfur amino acids.*

Transmethylation

The ***transfer of methyl group*** ($-CH_3$) ***from active methionine*** to an acceptor is known as transmethylation. Methionine has to be activated to ***S-adenosylmethionine*** (SAM) or active methionine to donate the methyl group.

Synthesis of S-adenosylmethionine

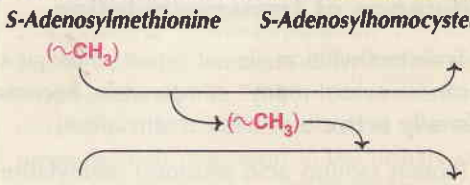
The synthesis of S-adenosylmethionine occurs by the transfer of an adenosyl group from ATP to sulfur atom of methionine (***Fig.15.29***). This reaction is catalysed by methionine S-adenosyltransferase. The activation of methionine is unique as the sulfur becomes a sulfonium atom (SAM is a sulfonium compound) by the addition of a third carbon. This reaction is also unusual since all the three phosphates of ATP are eliminated as pyrophosphates (PPi) and inorganic phosphates (Pi). Three high energy phosphates (***3 ATP***) are ***consumed in the formation of SAM.***

Functions of S-adenosylmethionine

S-Adenosylmethionine is highly reactive due to the presence of a positive charge. The enzymes involved in the transfer of methyl group are collectively known as ***methyltransferases.*** S-Adenosylmethionine transfers the methyl group to an acceptor and gets itself converted to S-adenosylhomocysteine. The loss of free energy in this reaction makes the methyl transfer essentially irreversible. S-Adenosylhomocysteine is hydrolysed to homocysteine and adenosine. Homocysteine can be remethylated to methionine by N^5-methyl tetrahydrofolate. In this manner, methionine can be regenerated for reuse. It should be noted that there is no net synthesis of methionine in the S-adenosylmethionine cycle (homocysteine, the precussor for methionine has to be derived from methionine). Hence, methionine is an essential amino acid.

S-Adenosylmethionine (carbon fragment) is also involved in the synthesis of polyamines (spermidine, spermine). The most important transmethylation reactions are listed in ***Table 15.3***.

Table 15.3 Transmethylation reactions



Methyl group acceptor	*Methylated product*
Guanidoacetate	Creatine
Norepinephrine	Epinephrine
Epinephrine	Metanephrine
Ethanolamine	Choline
Nicotinamide	N-Methylnicotinamide
Acetyl serotonin	Melatonin
Phosphatidylethanolamine	Phosphatidylcholine
Serine	Choline
Carnosine	Anserine
tRNA bases	Methylated tRNA bases
Protein-amino acid residues (histidine, lysine, arginine etc.)	Protein-methylated amino acids (methylhistidine, methyllysine, methylarginine etc.)

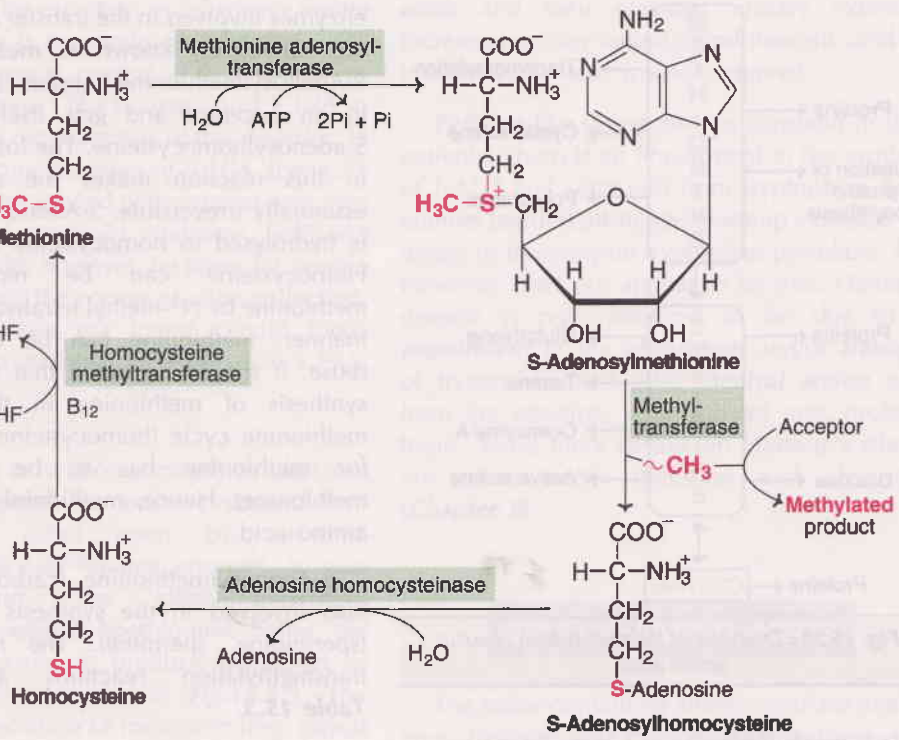


Fig. 15.29 : S-Adenosylmethionine cycle–synthesis, utilization and regeneration.

Significance of transmethylation

1. Transmethylation is of great biological significance since ***many compounds become functionally active*** only after methylation.

2. Protein (amino acid residues) methylation helps to control protein turnover. In general, ***methylation protects*** the ***proteins*** from immediate ***degradation.***

3. In plants, S-adenosylmethionine is the precursor for the synthesis of a plant hormone, ***ethylene***, which regulates plant growth and development and is involved in the ripening of fruits.

Synthesis of cysteine

Homocysteine formed from methionine is a precursor for the synthesis of cysteine (***Fig.15.30***). Homocysteine condenses with serine to form cystathionine. This reaction is catalysed by a PLP-dependent cystathionine synthase. The enzyme cystathioninase (PLP-dependent) cleaves and deaminates cystathionine to cysteine and α-ketobutyrate. The sum of the reactions catalysed by cystathionine synthase and cystathioninase is a good example of ***transsulfuration*** (transfer of sulfur from one compound to another). It should be noted that only the ***sulfur atom of cysteine*** comes from homocysteine (originally methionine) while the ***rest of the molecule is from serine.***

Homocysteine and heart attacks

Homocysteine is an intermediate in the synthesis of cysteine from methionine (***Fig.15.30***), Elevation in plasma homocysteine (normal < 15 μ mol/l) has been implicated in coronary artery diseases, although the mechanism is not known. It is believed that homocysteine reacts with collagen to produce reactive free radicals, besides interfering with collagen cross links. Homocysteine is also involved in the aggregation of LDL particles. All this leads to an increased tendency for atherogenesis, and consequently heart complications.

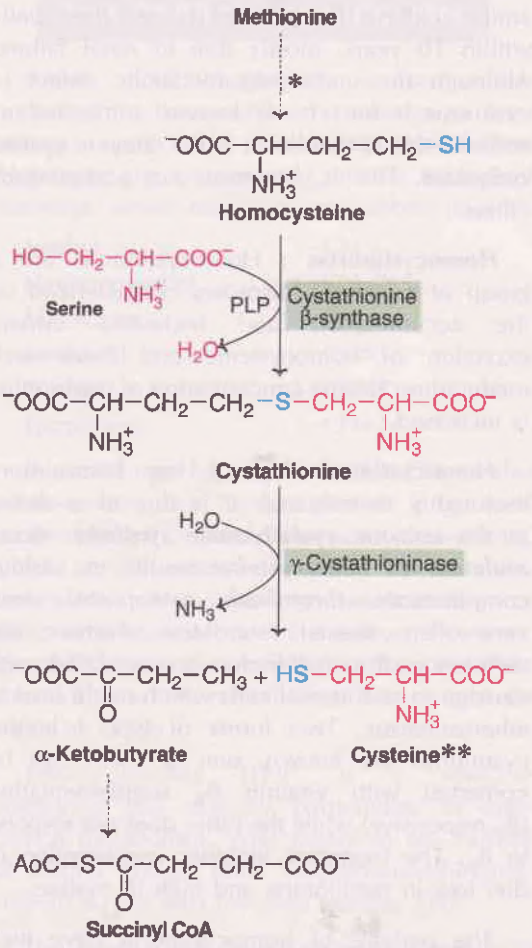


Fig. 15.30 : Synthesis of cysteine from methionine (–For reactions of methionine conversion to homocysteine see Fig. 15.29) (**–In cysteine synthesis, only sulfur is obtained from homocysteine, the rest of the molecule is from serine).*

Supplementation of diet with folic acid, vitamin B_{12} and vitamin B_6 have some beneficial affects in lowering plasma homocysteine levels (***Refer Chapter 7***).

Degradation of cysteine

Cystine and cysteine are interconvertible by an NAD^+-dependent cystine reductase. Cysteine on decarboxylation produces ***mercapto-ethanolamine*** which is involved in the biosynthesis of ***coenzyme A*** from the vitamin pantothenic acid.

The enzyme cysteine dioxygenase oxidizes cysteine to cysteine sulfinate which, on further oxidation, is converted to cysteic acid. The latter undergoes decarboxylation to produce ***taurine*** which conjugates with ***bile acids***. Cysteic acid can also be degraded to pyruvate, which is glycogenic (***Fig.15.31***).

Cysteine sulfinate cleaves off alanine to produce sulfite which is converted to sulfate and excreted in urine. Some amount of sulfate condenses with ATP to form active sulfate or ***3′-phosphoadenosine 5′-phosphosulfate*** (PAPS). Active sulfate (PAPS) is utilized for the synthesis of mucopolysaccharides (sulfation), besides being used in detoxification. Sulfate is also a structural component of some proteins, lipids etc.

Cysteine can be degraded by desulfhydrase to liberate sulfur (as H_2S), ammonia and pyruvate.

Cysteine is a component of tripeptide glutathione (synthesis described in glycine metabolism).

Inborn errors of sulfur amino acid metabolism

Cystinuria (cystine-lysinuria) : Cystinuria is one of the most ***common inherited diseases*** with a frequency of 1 in 7,000. It is primarily characterized by increased excretion of cystine (25-40 times normal). Elevation in the urinary output of lysine, arginine and ornithine is also observed. A specific carrier system exists in kidney tubules for the reabsorption of amino acids, namely cysteine, ornithine, arginine and lysine (remember COAL to recall). In cystinuria, this ***carrier system*** becomes ***defective*** leading to the excretion of all these four amino acids in urine.

Cystine is relatively insoluble and increase in its concentration leads to precipitation and formation of cystine stones in kidney and urinary tract. Cystinuria is usually identified in the laboratory by cyanide nitroprusside test. The treatment includes restricted ingestion of dietary cystine and high intake of fluids.

Cystinosis (cystine storage disease) : Cystine crystals are deposited in many tissues and organs

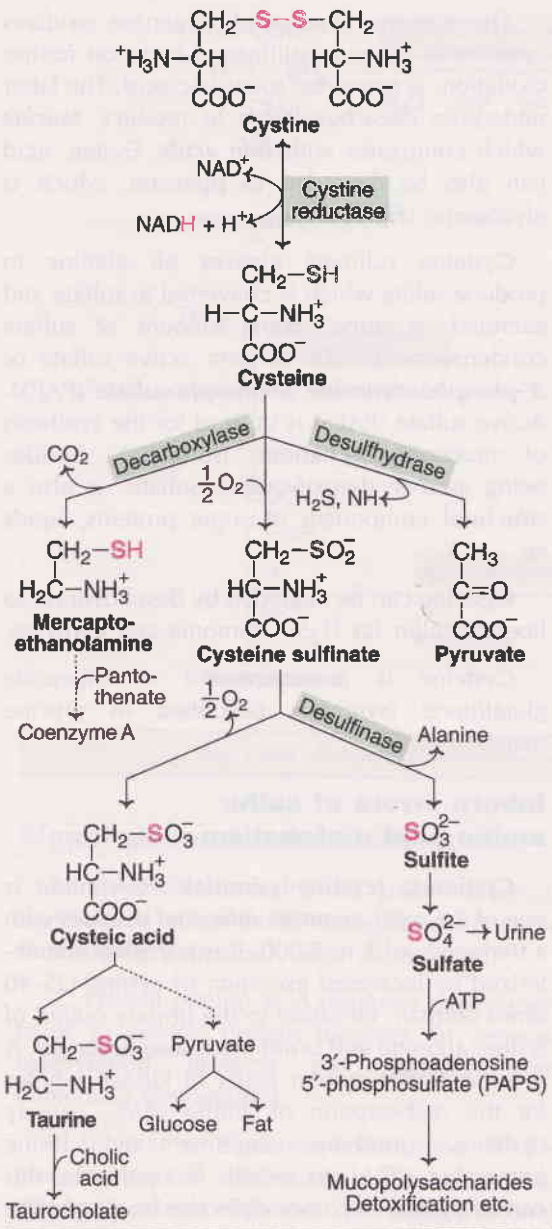


Fig. 15.31 : Metabolism of cysteine and cystine.

of reticuloendothelial system throughout the body. These include spleen, lymph nodes, liver, kidney, bone marrow etc. A ***defect in the lysosomal function*** is said to be the primary cause of this disorder. In fact, cystine accumulates in the lysosomes of various tissues. Impairment in renal function is commonly seen in cystinosis. It is characterized by generalized amino aciduria. The affected patients die usually within 10 years, mostly due to renal failure. Although the underlying metabolic defect in cystinosis is not clearly known, some authors attribute this to the defect in the enzyme ***cystine reductase.*** This is, however, not accepted by others.

Homocystinurias : Homocystinurias are a group of metabolic disorders characterized by the accumulation and increased urinary excretion of homocysteine and S-adenosyl-methionine. Plasma concentration of methionine is increased.

Homocystinuria type I has been more thoroughly investigated. It is due to a defect in the enzyme ***cystathionine synthase. Accumulation of homocysteine*** results in various complications—***thrombosis***, osteoporosis and, very often, mental retardation. Further, the deficiency of cystathionine is associated with damage to endothelial cells which might lead to atherosclerosis. Two forms of type I homocystinurias are known, one of them can be corrected with vitamin B_6 supplementation (B_6 responsive) while the other does not respond to B_6. The treatment includes consumption of diet low in methionine and high in cystine.

The patients of homocystinuria have high levels of homocysteine, and usually die of myocardial infarction, stroke, or pulmonary embolism.

The other homocystinurias are associated with enzyme defects (as stated below) in the conversion of homocysteine to methionine by remethylation.

Homocystinuria II : N^5-N^{10}—Methylene THF reductase.

Homocystinuria III : N^5-N^{10}—Methyl THF-homocysteine methyltransferase. This is mostly due to impairment in the synthesis of methyl-cobalamin.

Homocystinuria IV : N^5—Methyl THF homocysteine methyl transferase. This is primarily due to a defect in the intestinal absorption of vitamin B_{12}.

ONE-CARBON METABOLISM

Amino acid metabolism is particularly important for the transfer or exchange of one-carbon units. The following ***one-carbon fragments*** are encountered in the biological reactions, which constitute one-carbon pool

Methyl	($-CH_3$)
Hydroxymethyl	($-CH_2OH$)
Methylene	($=CH_2$)
Methenyl	($-CH=$)
Formyl	($-CH=O$)
Formimino	($-CH=NH$)

[**Note :** It may be stated here that CO_2 is also a one-carbon unit. Carbon dioxide is involved (carboxylation) in many biochemical reactions, which are dependent on biotin. For instance, conversion of pyruvate to oxaloacetate in gluconeogenesis. Most of the authors, however, ignore CO_2 as one-carbon unit and do not even consider it worth mentioning. This would be unfair to CO_2!]

Tetrahydrofolate (THF) is a versatile coenzyme that actively participates in one-carbon metabolism. With regard to the transfer of methyl groups from S-adenosylmethionine, vitamin B_{12} is also involved besides THF.

The one-carbon unit covalently binds with THF at position N^5 or N^{10} or on both N^5 and N^{10} of pteroyl structure of folate. The details of different one-carbon units binding with THF and the structures of THF derivatives are given under vitamin-folic acid (***Chapter 7***).

The one-carbon metabolism is rather complex, involving many reactions. For the sake of better understanding, it is divided into generation and utilization of one-carbon units, and the role of methionine and vitamin B_{12}.

I. Generation of one-carbon units

Many compounds (particularly amino acids) act as donors of one-carbon fragments

1. The formate released from glycine and tryptophan metabolism combines with THF to form N^{10}-formyl THF.

2. Histidine contributes formimino fragment to produce N^5-formimino THF.

3. When serine is converted to glycine, N^5, N^{10}-methylene THF is formed. This is the most predominant entry of one carbon units into one carbon pool.

4. Choline and betaine contribute to the formation of N^5-methyl THF.

The different derivatives of THF carrying one-carbon units are interconvertible, and this is metabolically significant for the continuity of one- carbon pool (***Fig.15.32***).

II. Utilization of one-carbon moieties

One-carbon fragments from THF are used for the synthesis of a wide variety of compounds. These include purines, formylmethionine tRNA (required for initiation of protein synthesis), glycine, pyrimidine nucleotide (thymidylate) etc.

III. Role of methionine and B_{12} in one-carbon metabolism

Methyl ($-CH_3$) group is an important one-carbon unit. The role of active methionine as methyl donor in transmethylation reactions is already described. After the release of methyl group, methionine is converted to homocysteine. For the regeneration of methionine, free homocysteine and N^5-methyl THF are required and this reaction is dependent on methylcobalamin (vitamin B_{12}). The one-carbon pool, under the control of THF, is linked with methionine metabolism (transmethylation) through vitamin B_{12}. Hence vitamin B_{12} is also involved in one-carbon metabolism.

BRANCHED CHAIN AMINO ACIDS

Valine, leucine and ***isoleucine*** are the branched chain and ***essential*** amino acids. These three amino acids initially undergo a common pathway and then diverge to result in different end products. Based on the products obtained from the carbon skeleton, the branched

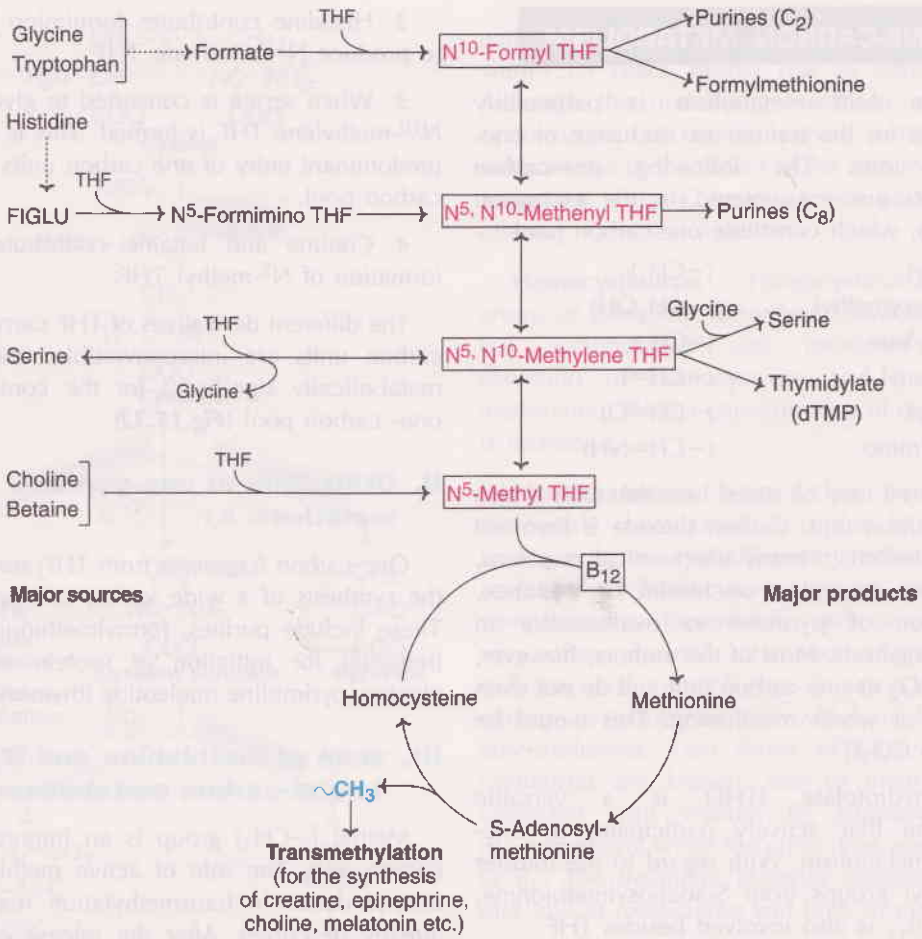


Fig. 15.32 : Summary of one-carbon metabolism (THF–Tetrahydrofolate; ~CH_3–Active methyl group).

chain amino acids are either glycogenic or ketogenic

Valine – glycogenic
Leucine – ketogenic
Isoleucine – glycogenic and ketogenic.

The first three metabolic reactions are common to the branched chain amino acids (***Fig.15.33***).

1. **Transamination :** The three amino acids undergo a reversible transamination to form their respective keto acids.

2. **Oxidative decarboxylation :** α-Keto acid dehydrogenase is a complex mitochondrial enzyme. It is comparable in function to pyruvate dehydrogenase complex and employs 5 coenzymes—TPP, lipoamide, FAD, coenzyme A and NAD^+—for its action. α-Keto acid dehydrogenase catalyses oxidative decarboxylation of the keto acids to the corresponding acyl CoA thioesters. This is a regulatory enzyme in the catabolism of branched chain amino acids.

3. **Dehydrogenation :** The dehydrogenation is similar to that in fatty acid oxidation. FAD is the coenzyme and there is an incorporation of a double bond. It is now believed that there are two enzymes responsible for dehydrogenation.

After the initial three common reactions, the metabolism of branched chain amino acids diverges and takes independent routes. In a series of reactions that follow, valine is converted

to propionyl CoA, a precursor for glucose. Leucine produces acetyl CoA and acetoacetate, the substrates for fatty acid synthesis. Isoleucine is degraded to propionyl CoA and acetyl CoA. Thus, valine is glycogenic and leucine is ketogenic while isoleucine is both glycogenic and ketogenic.

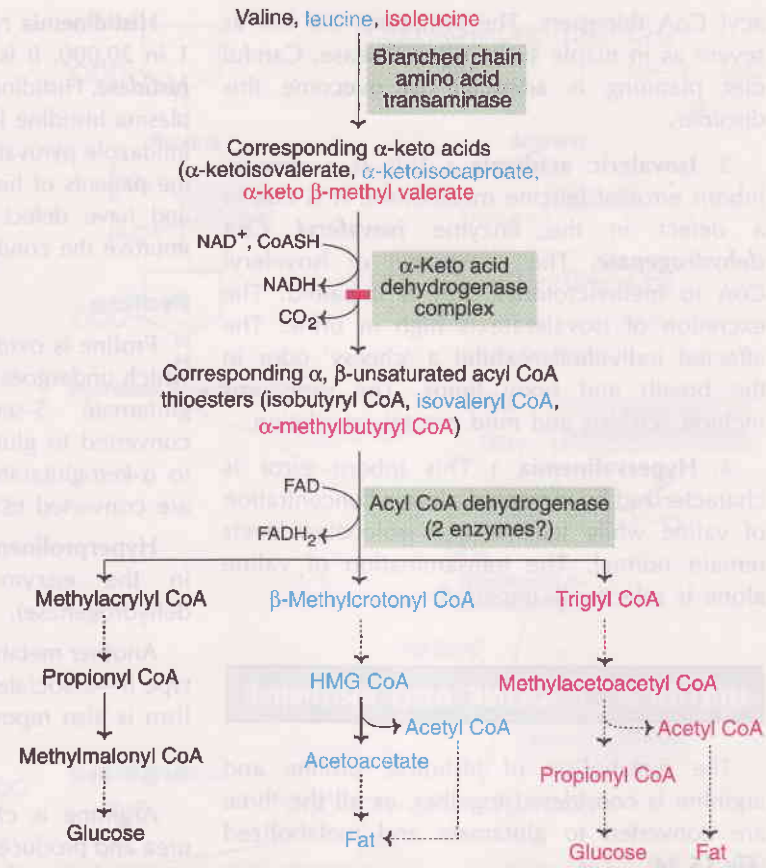


Fig. 15.33 : Summary of branched chain amino acid metabolism (▬–Defect in α-keto acid dehydrogenase results in maple syrup urine disease; HMG CoA–β-Hydroxy β-methyl-glutaryl CoA; Valine is glycogenic, leucine is ketogenic, while isoleucine is both).

Metabolic defects of branched chain amino acids

1. **Maple syrup urine disease :** This is a metabolic disorder of branched chain amino acids. The ***urine*** of the affected individuals ***smells*** like maple syrup or ***burnt sugar***—hence the name.

Enzyme defect : Maple syrup urine disease is due to a defect in the enzyme ***branched chain α-keto acid dehydrogenase.*** This causes a blockade in the conversion of α-keto acids to the respective acyl CoA thioesters. The plasma and urine concentrations of branched amino acids and their keto acids are highly elevated. This disease is also known as ***branched chain ketonuria.***

Biochemical complications and symptoms :

- Accumulation of branched chain amino acids causes an impairment in transport and function of other amino acids.
- Protein biosynthesis is reduced.
- Branched chain amino acids competitively inhibit glutamate dehydrogenase.
- The disease results in acidosis, lethargy, convulsions, mental retardation, coma and, finally, death within one year after birth.

Diagnosis and treatment : An early diagnosis by enzyme analysis—preferably within the first week of life—is ideal. Estimation of urinary branched amino acids and keto acids will also help in diagnosis.

The treatment is to feed a diet with low (or no) content of branched amino acids. The plasma levels of branched amino acids should be constantly monitored for adjusting their dietary intake.

2. **Intermittent branched chain ketonuria :** This is a less severe variant form of maple syrup urine disease. The enzyme defect is the same—***α-keto acid dehydrogenase.*** As such, there is an impairment and no total blockade in the conversion of α-keto acids to their respective

acyl CoA thioesters. The symptoms are not as severe as in maple syrup urine disease. Careful diet planning is adequate to ovecome this disorder.

3. **Isovaleric acidemia :** This is a specific inborn error of leucine metabolism. It is due to a defect in the enzyme ***isovaleryl CoA dehydrogenase.*** The conversion of isovaleryl CoA to methylcrotonyl CoA is impaired. The excretion of isovalerate is high in urine. The affected individuals exhibit a 'cheesy' odor in the breath and body fluids. The symptoms include acidosis and mild mental retardation.

4. **Hypervalinemia :** This inborn error is characterized by increased plasma concentration of valine while leucine and isoleucine levels remain normal. The transamination of valine alone is selectively impaired.

HISTIDINE, PROLINE AND ARGININE

The metabolism of histidine, proline and arginine is considered together, as all the three are converted to glutamate and metabolized (***Fig.15.34***).

Histidine

The metabolism of histidine is important for the generation of ***one-carbon*** unit, namely formimino group. The enzyme histidase acts on histidine to split off ammonia. Urocanate formed in this reaction is acted upon by urocanase to produce 4-imidazole 5-propionate. Imidazole ring of the product is cleaved by a hydrolase to give N-formiminoglutamate (FIGLU). Tetrahydrofolate (THF) takes up the formimino group to form N^5-formimino THF, and glutamate is liberated. ***Deficiency of folate*** blocks this reaction and causes ***elevated excretion of FIGLU*** in urine. Histidine loading test is commonly employed to assess folate deficiency.

Histidine, on decarboxylation, gives the corresponding amine—***histamine.*** Histamine regulates HCl secretion by gastric mucosa. Excessive production of histamine causes asthma and allergic reactions.

Histidinemia : The frequency of histidinemia is 1 in 20,000. It is due to a defect in the enzyme ***histidase.*** Histidinemia is characterized by elevated plasma histidine levels and increased excretion of imidazole pyruvate and histidine in urine. Most of the patients of histidinemia are mentally retarded and have defect in speech. No treatment will improve the condition of the patients.

Proline

Proline is oxidized to pyrroline 5-carboxylate which undergoes a non-enzymatic conversion to glutamate 5-semialdehyde. The latter is converted to glutamate and then transaminated to α-ketoglutarate. The five carbons of proline are converted to α-ketoglutarate.

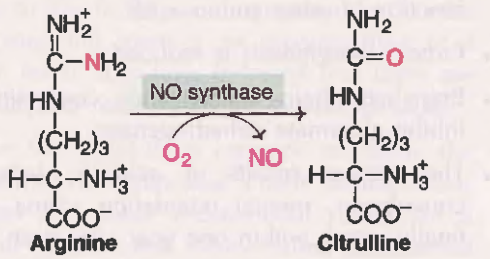
Hyperprolinemia type I : It is due to a defect in the enzyme ***proline oxidase*** (proline dehydrogenase).

Another metabolic disorder—hyperprolinemia type II—associated with hydroxyproline metabolism is also reported.

Arginine

Arginine is cleaved by arginase to liberate urea and produce ornithine. Ornithine undergoes transamination of δ-amino group to form glutamate γ-semialdehyde which is converted to glutamate. ***Hyperargininemia*** is an inborn error in arginine metabolism due to a defect in the enzyme ***arginase.***

Nitric oxide (NO) : Arginine is the substrate for the production of nitric oxide (NO), a wonder molecule with a wide range of functions. The enzyme ***nitric oxide synthase*** (three isoenzymes known) cleaves the nitrogen from the guanidino group of arginine to form NO. This reaction requires NADPH, FMN, FAD, heme and tetrahydrobiopterin. NO has a very short half-life (about 5 seconds).



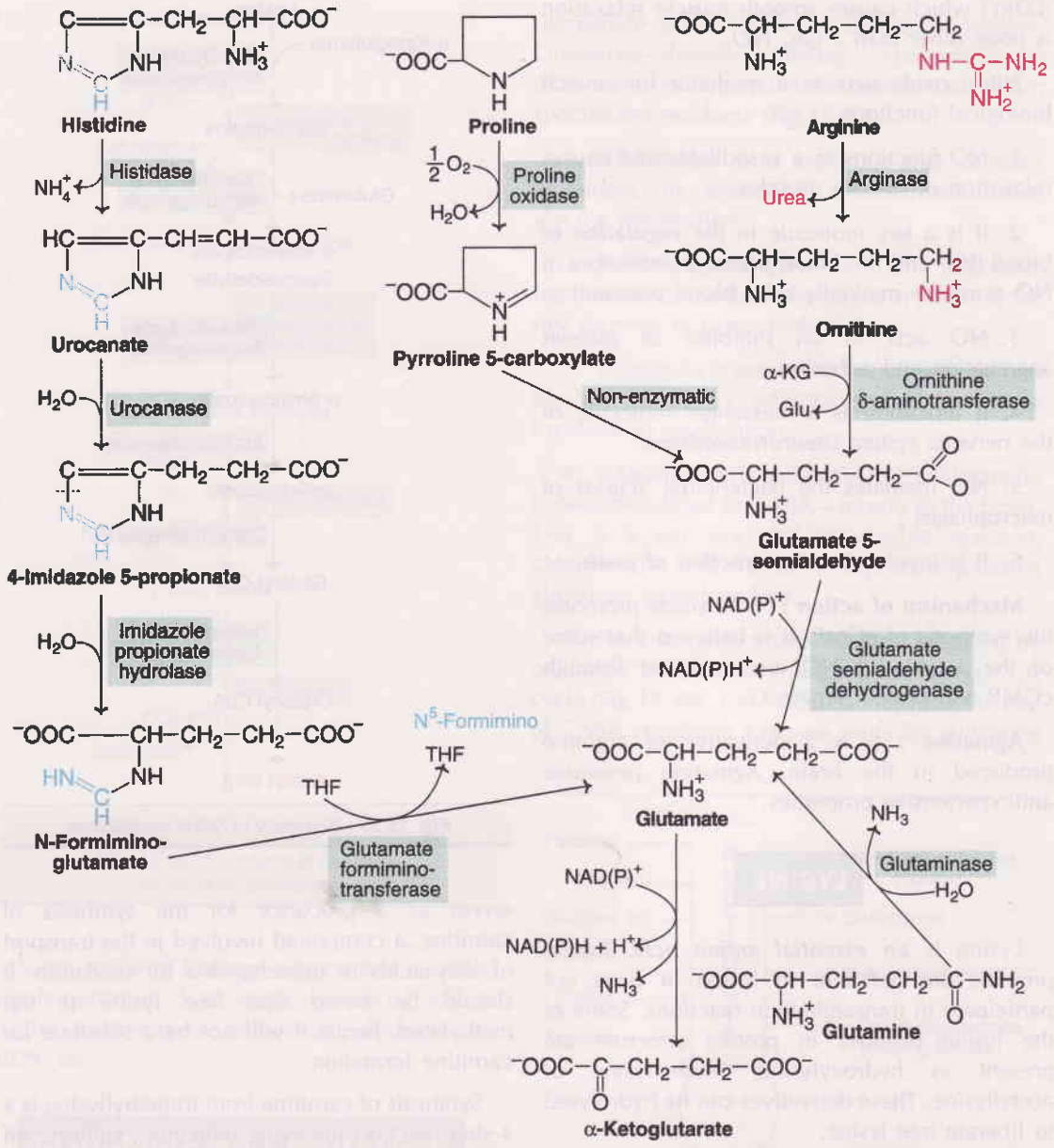


Fig. 15.34 : Metabolism of histidine, proline, arginine, glutamate and glutamine (THF–Tetrahydrofolate; α-KG–α-Ketoglutarate; Glu–Glutamate).

The occurrence of high concentrations of citrulline in human brain has been known for several years. Only recently it is realized that the citrulline is formed during the course of NO synthesis [**Note :** Nitric oxide (NO) should not be confused with nitrous oxide (N_2O)—laughing gas—used as an anesthetic].

Functions of NO : The role of nitric oxide as a therapeutic drug (in the form of nitroglycerine and amyl nitrate) for the treatment of angina pectoris has been known since 1867. However, it is only recently that *in vivo* production and the biological importance of NO are recognized. In fact, the endothelium derived releasing factor

(EDRF) which causes smooth muscle relaxation is none other than a gas, NO.

Nitric oxide acts as a mediator for several biological functions.

1. NO functions as a vasodilator and causes relaxation of smooth muscles.

2. It is a key molecule in the ***regulation of*** blood flow and the ***blood pressure*** (inhibitors of NO synthesis markedly raise blood pressure).

3. NO acts as an inhibitor of platelet aggregation and adhesion.

4. It functions as a messenger molecule of the nervous system (***neurotransmitter***).

5. NO mediates the bactericidal actions of macrophages.

6. It is involved in the ***erection of penis***.

Mechanism of action : Nitric oxide promotes the synthesis of cGMP. It is believed that some of the actions of NO are mediated through cGMP and protein kinase G.

Agmatine : It is a derivative of arginine produced in the brain. Agmatine possesses antihypertensive properties.

LYSINE

Lysine is an ***essential amino acid***. Cereal proteins are deficient in lysine. It does not participate in transamination reactions. Some of the lysine residues in protein structure are present as hydroxylysine, methyllysine or acetyllysine. These derivatives can be hydrolysed to liberate free lysine.

Lysine is a ***ketogenic*** amino acid. The summary of lysine metabolism is depicted in ***Fig.15.35***.

Synthesis of carnitine

Some of the lysine residues in proteins are found in methylated form. The methyl groups are obtained from active methionine (SAM). Such proteins on degradation (by proteolysis) will release the methyllysines. The trimethyllysine

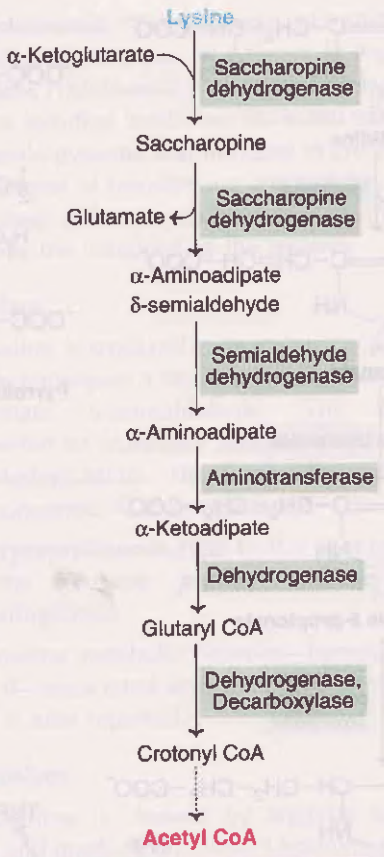


Fig. 15.35 : Summary of lysine metabolism.

serves as a precursor for the synthesis of carnitine, a compound involved in the transport of fatty acids to mitochondria for oxidation. It should be noted that free lysine is not methylated, hence it will not be a substrate for carnitine formation.

Synthesis of carnitine from trimethyllysine is a 4-step reaction involving oxidation, splitting off glycine residue, dehydrogenation and, finally, oxidation (***Fig.15.36***).

Biochemical importance of carnitine

Carnitine plays a key role in the fatty acid oxidation (***Chapter 15***).

Human requirements of carnitine are usually met with the endogeneous biosynthesis and the dietary supply. Good sources of carnitine include meat, fish, poultry and dairy products.

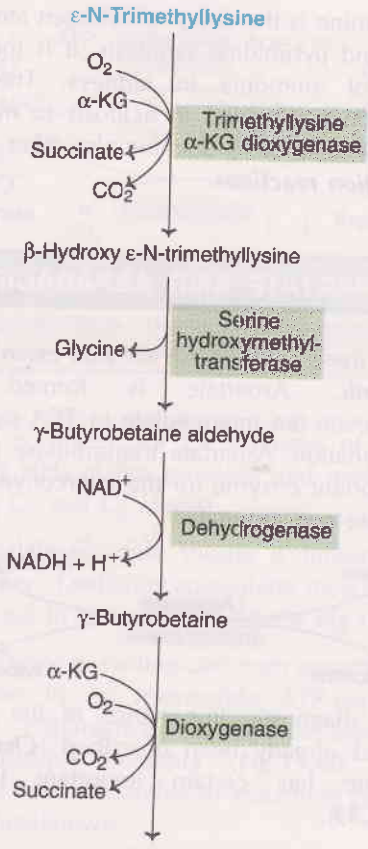


Fig. 15.36 : Synthesis of carnitine (α-KG-α-Ketoglutarate).

Some research findings suggest that carnitine supplementation has some beneficial effects in the treatment of myocardial dysfunctions, AIDS, etc.

GLUTAMATE AND GLUTAMINE

Glutamate and glutamine are ***non-essential glycogenic*** amino acids. Both of them play a predominant role in the amino acid metabolism, and are directly involved in the final transfer of *amino group for urea synthesis.*

The amino acids—histidine, proline and arginine—are converted in their metabolism to glutamate (***See Fig.15.34***). α-Ketoglutarate—an intermediate in TCA cycle—serves as an immediate precursor for glutamate formation. Glutamate—besides being converted to glutamine—is involved in the synthesis of certain specialized products (***Fig.15.37***).

1. **Glutathione** is a tripeptide that contains glutamate. Its formation is described under glycine metabolism.

2. **N-Acetylglutamate** is an allosteric regulator of carbamoyl phosphate synthase I, the first enzyme in urea synthesis.

3. Glutamate is present in the ***clotting factors*** (II, VII, IX, X) as γ-carboxyglutamate and is involved in coagulation.

4. **γ-Aminobutyric acid (GABA) :** Glutamate is decarboxylated to GABA—mostly in the brain. This is a sensitive and irreversible reaction, catalysed by a pyridoxal phosphate-dependent glutamate decarboxylase.

GABA undergoes transamination followed by oxidation to form succinate which enters TCA cycle (***Fig.15.38***). The reactions involving the fate of GABA constitute a bypass route for glutamate

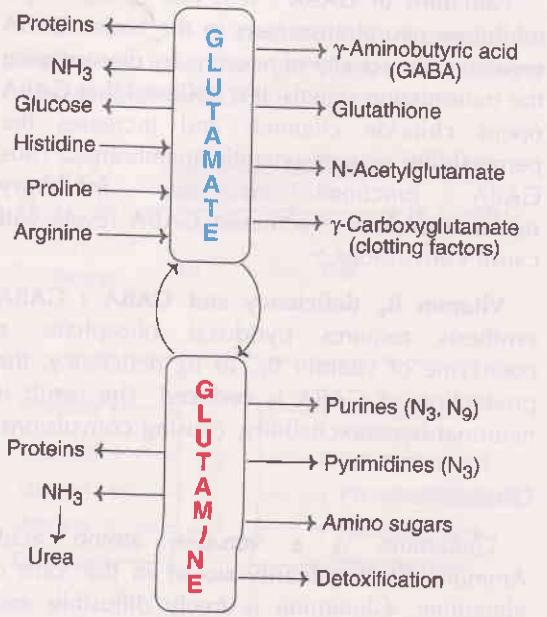


Fig. 15.37 : Overview of glutamate and glutamine metabolism.

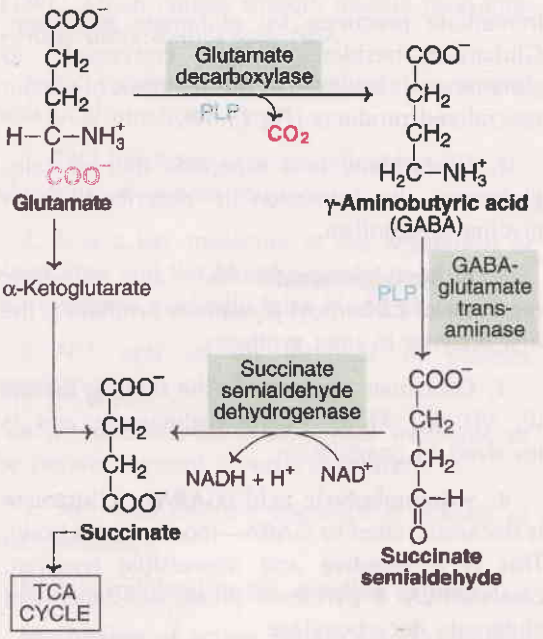


Fig. 15.38 : Metabolism of γ-aminobutyric acid (PLP–Pyridoxal phosphate).

to enter TCA cycle, which is known as ***GABA shunt.***

Functions of GABA : It is one of the major inhibitory neurotransmitters in the brain. GABA regulates the activity of neurons by discouraging the transmission signals. It is believed that GABA opens chloride channels and increases the permeability of post-synaptic membranes. Thus GABA functions as an ***inhibitory neurotransmitter.*** Decreased GABA levels will cause convulsions.

Vitamin B_6 deficiency and GABA : GABA synthesis requires pyridoxal phosphate, a coenzyme of vitamin B_6. In B_6 deficiency, the production of GABA is ***reduced***. The result is neuronal hyperexcitability, causing convulsions.

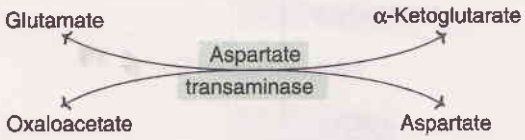
Glutamine

Glutamine is a versatile amino acid. Ammonia is temporarily stored in the form of glutamine. Glutamine is freely diffusible and, hence, easily transported. The synthesis and degradation of glutamine are described (***See Fig.15.8***).

Glutamine is the donor of nitrogen atoms for purine and pyrimidine synthesis. It is the chief source of ammonia in kidneys. The NH_3 production is elevated in acidosis to maintain ***acid-base balance.*** Glutamine also takes part in ***conjugation reactions.***

ASPARTATE AND ASPARAGINE

Both these amino acids are ***non-essential*** and ***glycogenic***. Aspartate is formed from oxaloacetate (an intermediate in TCA cycle) by transamination. Aspartate transaminase (AST) is an important enzyme for the interconversion of glutamate and aspartate.



The diagnostic importance of the enzyme AST has already been described (***Chapter 6***). Aspartate has certain important functions (***Fig.15.39***).

1. It donates one amino group for the ***synthesis of urea*** (the other amino group in urea directly comes from ammonia).

2. Aspartate forms a connecting link between urea cycle and TCA cycle (via oxaloacetate).

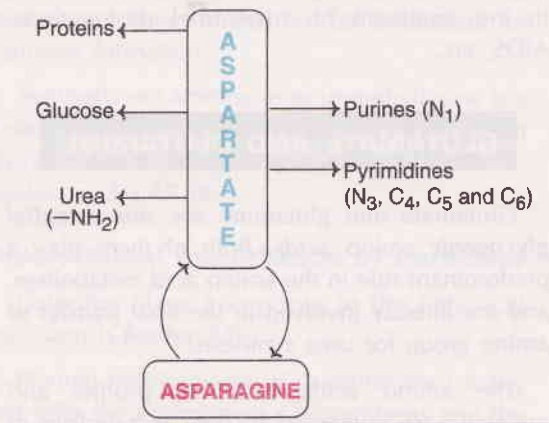


Fig. 15.39 : Overview of aspartate and asparagine metabolism.

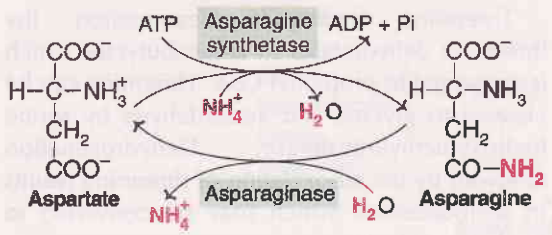


*Fig. 15.40 : Synthesis of asparagine and its conversion to aspartate (**Note :** The reactions are independent and irreversible).*

3. It is utilized for the synthesis of ***purines*** (N_1 and NH_2 at 6th position) and ***pyrimidines*** (N_3, C_4 C_5 and C_6 atoms).

4. Malate-aspartate shuttle is important for the transfer of reducing equivalents (NADH) from the cytosol to mitochondria (***Refer Fig.11.13***).

Asparagine is synthesized from aspartate by a synthetase in an irreversible ATP-dependent reaction. Asparaginase hydrolyses asparagine and liberates ammonia (***Fig.15.40***). These reactions are comparable to glutamine synthesis and its breakdown.

ALANINE

The ***non-essential*** amino acid alanine performs two important functions in the body

1. Incorporation into the structure of proteins;

2. Participation in transamination and NH_3 transport.

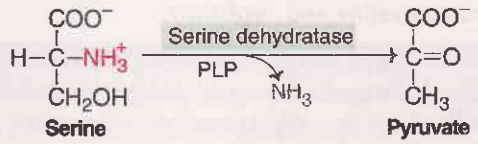
As already discussed, ammonia is toxic to the body, hence it cannot be transported in free form. Glutamate and glutamine shoulder the major burden of ammonia transport. Alanine is also important in this regard. In the peripheral tissues (most predominantly—muscle), pyruvate produced in glycolysis gets converted to alanine (by transamination) and is transported to liver. Pyruvate can be regenerated from alanine in liver and the pyruvate so produced serves as a precursor for glucose. Amino group is diverted for transamination or urea formation. This is an ***alanine-pyruvate shuttle for carrying nitrogen*** to be reutilized or converted to urea.

The amino acid β-alanine is a constituent of the vitamin pantothenic acid, and thus the coenzyme A.

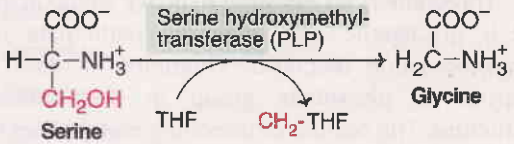
SERINE

Serine is a ***non-essential glycogenic amino acid***. As described in glycine metabolism, serine and glycine are interconvertible. Serine can be synthesized from the intermediates of glycolysis (3-phosphoglycerate). The metabolic reactions of serine are described hereunder (***Fig.15.41***)

1. Serine participates in transamination reactions. It undergoes deamination to form pyruvate.



2. Serine is involved in one-carbon metabolism. It donates methylene ($-CH_2$) moiety to tetrahydrofolate (THF).



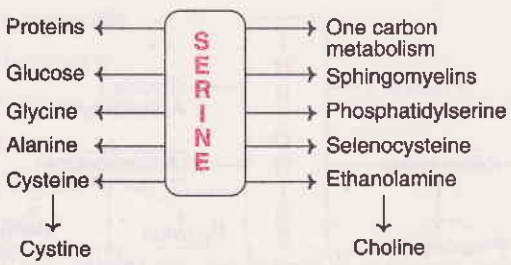
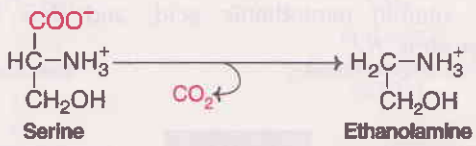


Fig. 15.41 : Overview of serine metabolism.

3. On decarboxylation (PLP-dependent) serine forms ethanolamine which is the precursor for choline synthesis.



4. Serine is utilized for the synthesis of cysteine (***See Fig.15.30***). It may be noted that the entire cysteine molecule is derived from serine except the sulfur that comes from homocysteine.

5. Serine is involved in the formation of ***selenocysteine***, the 21st amino acid found in certain proteins.

6. Serine directly participates in the synthesis of phospholipid-phosphatidyl serine (details described in lipid metabolism, ***Chapter 14***).

7. Serine is also involved in the synthesis of sphingomyelins and cephalins.

8. In the structure of proteins, serine (–OH group) serves as a carrier of phosphate which is involved in the regulation of many enzyme activities.

THREONINE

Threonine is an ***essential hydroxy*** amino acid. It is glycogenic and does not participate in transamination reactions. Threonine is often a carrier of phosphate group in the protein structure. The outline of threonine metabolism is depicted in ***Fig.15.42***.

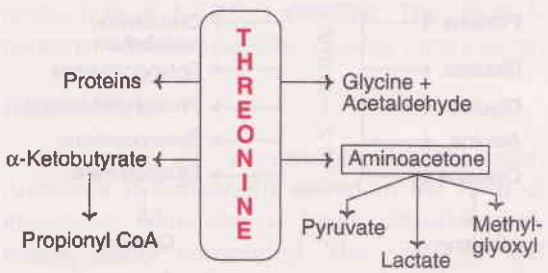


Fig. 15.42 : Overview of threonine metabolism.

Threonine undergoes deamination (by threonine dehydratase) to α-ketobutyrate which is converted to propionyl CoA. Threonine can be cleaved to glycine and acetaldehyde by serine hydroxymethyltransferase. Dehydrogenation followed by decarboxylation of threonine results in aminoacetone which may be converted to pyruvate or lactate.

FATE OF CARBON SKELETON OF AMINO ACIDS

The metabolic reactions of individual amino acids are described above. After the removal of amino groups, the carbon skeleton of amino acids is converted to intermediates of TCA cycle or their precursors. The carbon skeleton finally has one or more of the following fates

1. Oxidation via TCA cycle to produce energy (about 10-15% of body needs).

2. Synthesis of glucose.

3. Formation of lipids—fatty acids and ketone bodies.

4. Synthesis of non-essential amino acids.

The carbon skeletons of the 20 standard (or more) amino acids (or the amino acids of proteins) are degraded to one of the following seven products—***pyruvate***, ***α-ketoglutarate***, ***succinyl CoA***, ***fumarate***, ***oxaloacetate***, ***acetyl CoA*** and ***acetoacetate***. Some authors use the term amphibolic (*Greek*: amphiboles—uncertain) intermediates to these compounds due to their multiple metabolic functions.

The amino acids are classified into two groups, based on the nature of the metabolic end products of carbon skeleton.

1. **Glycogenic (glucogenic) amino acids :** These are the amino acids whose carbon skeleton is finally degraded to pyruvate or one of the intermediates of TCA cycle (α-ketoglutarate, succinyl CoA, fumarate and oxaloacetate). These intermediates serve as good substrates for gluconeogenesis leading to the ***formation of glucose*** or glycogen.

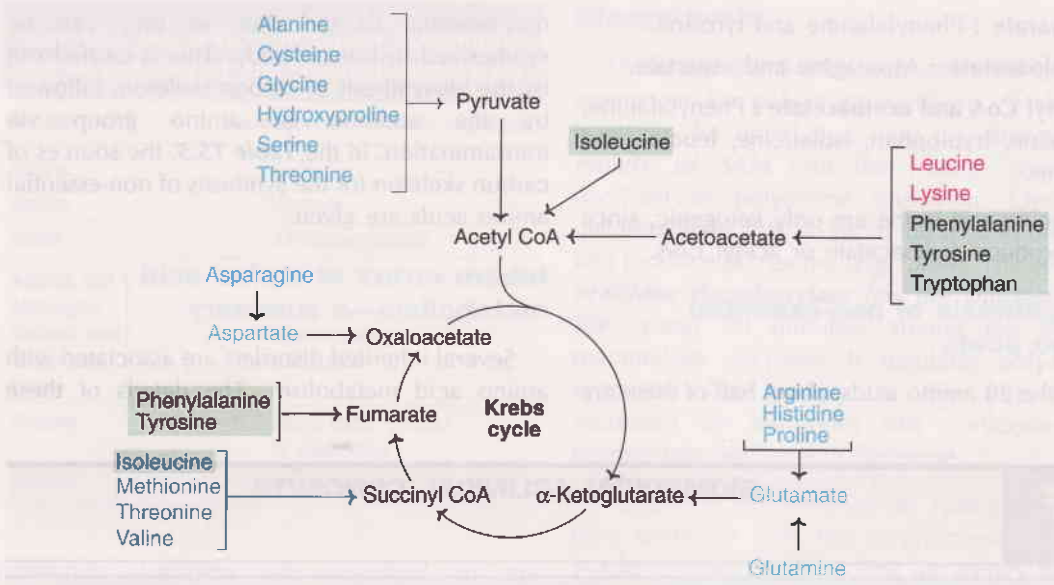


***Fig. 15.43** : Summary of the products formed from carbon skeleton of amino acids (colour indication, Blue–glucogenic; Green shade–glucogenic and ketogenic; Red–ketogenic).*

2. **Ketogenic amino acids :** The amino acids whose carbon skeleton is metabolized to acetyl CoA or acetoacetate can be ***converted to fat*** (i.e., fatty acids or ketone bodies). Acetoacetate is a ketone body (besides acetone and β-hydroxybutyrate).

Some of the amino acids are both glycogenic and ketogenic since they serve as precursors for glucose as well as fat.

The classification of amino acids (glycogenic, ketogenic, or both) is given in ***Table 15.4***. The various products obtained from the carbon skeleton of amino acids and their connection with the citric acid cycle is depicted in ***Fig.15.43***.

The details on the formation of amphibolic intermediates by the degradation of amino acids are given in the metabolism of respective amino acids. They are summarized hereunder

- **Pyruvate** : Alanine, cysteine, glycine, hydroxyproline, serine and threonine.
- **α-Ketoglutarate** : Glutamine, glutamate, arginine, histidine and proline.
- **Succinyl CoA** : Isoleucine, methionine, threonine and valine.

Table 15.4 Classification of amino acids based on the fate of carbon skeleton

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

** Essential amino acids; (Helpful tips to recall—ketogenic amino acids start with letter 'L'; PITT for glyco- and ketogenic amino acids; rest of the 20 amino acids are only glycogenic).*

- **Fumarate :** Phenylalanine and tyrosine.
- **Oxaloacetate :** Asparagine and aspartate.
- **Acetyl CoA and acetoacetate :** Phenylalanine, tyrosine, tryptophan, isoleucine, leucine and lysine.

Leucine and lysine are only ketogenic, since they produce acetoacetate or acetyl CoA.

Biosynthesis of non-essential amino acids

Of the 20 amino acids, about half of them are non-essential in the diet, as they can be synthesized in human body. This is carried out by the biosynthesis of carbon skeleton, followed by the addition of amino group via transamination. In the ***Table 15.5***, the sources of carbon skeleton for the synthesis of non-essential amino acids are given.

Inborn errors of amino acid metabolism—a summary

Several inherited disorders are associated with amino acid metabolism. The details of these

BIOMEDICAL / CLINICAL CONCEPTS

- *Melanin—the pigment of skin, hair and eyes—is produced from tyrosine. Lack of melanin synthesis (mostly due to a deficiency of tyrosinase) causes albinism.*
- *Parkinson's disease—a common disorder of the elderly—is linked with decreased synthesis of dopamine. It is characterized by muscular rigidity, tremors, lethargy etc.*
- *Phenylketonuria, due to a defect in the enzyme phenylalanine hydroxylase, is characterized by failure of growth, seizures and mental retardation (low IQ).*
- *Alkaptonuria causes the accumulation of homogentisate which undergoes oxidation followed by polymerization to produce the pigment alkapton. Deposition of alkapton in tissues (connective tissue, bones) causes ochronosis which is associated with arthritis.*
- *Serotonin, an excitatory neurotransmitter, is synthesized from tryptophan. Psychic stimulant drugs (iproniazid) elevate serotonin levels while depressant drugs (LSD) decrease.*
- *Malignant carcinoid syndrome, a tumor of argentaffin cells of gastrointestinal tract, is characterized by tremendously increased production of serotonin. This disorder can be diagnosed by the elevated levels of 5-hydroxyindoleacetate in urine.*
- *Melatonin, produced from serotonin, is involved in circadian rhythms or diurnal variations, i.e., maintenance of body's biological clock.*
- *Homocysteine has been implicated as a risk factor in the onset of coronary heart diseases.*
- *Histidine loading test, characterized by elevated excretion of N-formiminoglutamate (FIGLU) is commonly employed to assess the deficiency of the vitamin, folic acid.*
- *Nitric oxide (NO), synthesized from arginine, is involved in several biological functions—vasodilation, platelet aggregation, neurotransmission and bactericidal action.*
- *γ-Aminobutyric acid (GABA), produced from glutamate, is an inhibitory neurotransmitter. Low levels of GABA result in convulsions.*
- *The carbon skeleton of amino acids may be converted to glucose (glycogenic) or fat (ketogenic), besides being responsible for the synthesis of non-essential amino acids.*
- *Polyamines (spermine, putrescine) are involved in the synthesis of DNA, RNA and proteins and, thus, they are essential for cell growth and differentiation.*

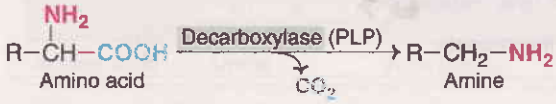
Table 15.5 The sources of carbon skeleton for the biosynthesis of non-essential amino acids

Amino acid	*Source(s) of carbon skeleton*
Glycine	Serine
Alanine	Pyruvate
Serine	3-Phosphoglycerate
Aspartic acid, Asparagine, Glutamic acid, Glutamine, Proline	Intermediates of Krebs cycle
Cysteine	Serine (sulfur donated by methionine)
Tyrosine	Phenylalanine

metabolic disorders are described in the respective amino acids. ***Table 15.6*** gives a summary of the inborn errors of amino acid metabolism.

BIOGENIC AMINES

In general, the decarboxylation of amino acids or their derivatives results in the formation of amines.



A summary of the biogenic amines derived from different amino acids and their major functions are given in ***Table 15.7***.

POLYAMINES

Polyamines (*Greek*: poly—many) possess multiple amino groups. ***Putrescine***, ***spermine*** and ***spermidine*** are the biologically important polyamines. Spermine and spermidine were originally detected in human semen (sperms), hence they are so named.

Biosynthesis

Ornithine and S-adenosylmethionine are the precursors for polyamine synthesis. It should, however, be noted that only the ***four-carbon moiety of SAM*** (not the methyl group) is involved in polyamine formation. Ornithine decarboxylase acts on ornithine to split off CO_2 and produce putrescine (***Fig.15.44***). The enzyme ***ornithine decarboxylase has the shortest half-life*** (about 10 minutes) among the known mammalian enzymes. It regulates polyamine synthesis. The activity of this enzyme is increased by hormones like corticosteroids, testosterone and growth hormone.

Putrescine is converted to spermidine and then spermine with the involvement of SAM. S-Adenosylmethionine is first decarboxylated to give decarboxylated SAM. SAM decarboxylase is a rare example of an enzyme that does not require pyridoxal phosphate as coenzyme. An amino acid residue bound to pyruvate is believed to function as a cofactor. The propylamino group of decarboxylated SAM is transferred to putrescine to give spermidine. Synthesis of spermine requires one more molecule of decarboxylated SAM and this reaction is catalysed by spermine synthase.

Degradation of polyamines

The enzyme polyamine oxidase (of liver peroxisomes) oxidizes spermine to spermidine and then to putrescine. Spermidine and putrescine are excreted in urine in a conjugated form, as acetylated derivatives. Some amount of putrescine is also oxidized to NH_3 and CO_2.

Functions of polyamines

1. Polyamines are basic in nature and possess multiple positive charges. Hence they are readily associated with nucleic acids (DNA and RNA).

2. They are involved in the ***synthesis of DNA, RNA and proteins***.

Table 15.6 Inborn errors of amino acid metabolism

Disorder	*Metabolic defect (enzyme/other)*
I. **Defects in urea synthesis** —Refer Table 15.1	
II. **Phenylalanine and tyrosine**	
1. Phenylketonuria	Phenylalanine hydroxylase
2. Tyrosinemia type II	Tyrosine transaminase
3. Neonatal tyrosinemia	p-Hydroxy phenylpyruvate dioxygenase
4. Alkaptonuria	Homogentisate oxidase
5. Tyrosinosis (tyrosinemia type I)	Maleyl acetoacetate isomerase or fumaryl acetoacetate hydrolase
6. Albinism	Tyrosinase
III. **Sulfur amino acids (methionine, cysteine and cystine)**	
7. Cystinuria	Defect in renal reabsorption
8. Cystinosis	Impairment in cystine utilization (defect in lysosomal function)
9. Homocystinuria type I	Cystathionine synthetase
10. Homocystinuria type II	N^5, N^{10}-Methylene THF reductase
11. Homocystinuria type III	N^5-Methyl THF-homocysteine methyltransferase
12. Cystathionuria	Cystathioninase
IV. **Glycine**	
13. Glycinuria	Defect in renal reabsorption
14. Primary hyperoxaluria	Glycine transaminase
V. **Tryptophan**	
15. Hartnup's disease	Defective intestinal absorption
VI. **Branched chain amino acids (valine, leucine and isoleucine)**	
16. Maple syrup urine disease	Branched chain α-keto acid dehydrogenase
17. Intermittent branched chain ketonuria	Variant of the above enzyme (less severe)
18. Hypervalinemia	Valine transaminase
19. Isovaleric acidemia	Isovaleryl CoA dehydrogenase
VII. **Histidine**	
20. Histidinemia	Histidase
VIII. **Proline**	
21. Hyperprolinemia type I	Proline oxidase

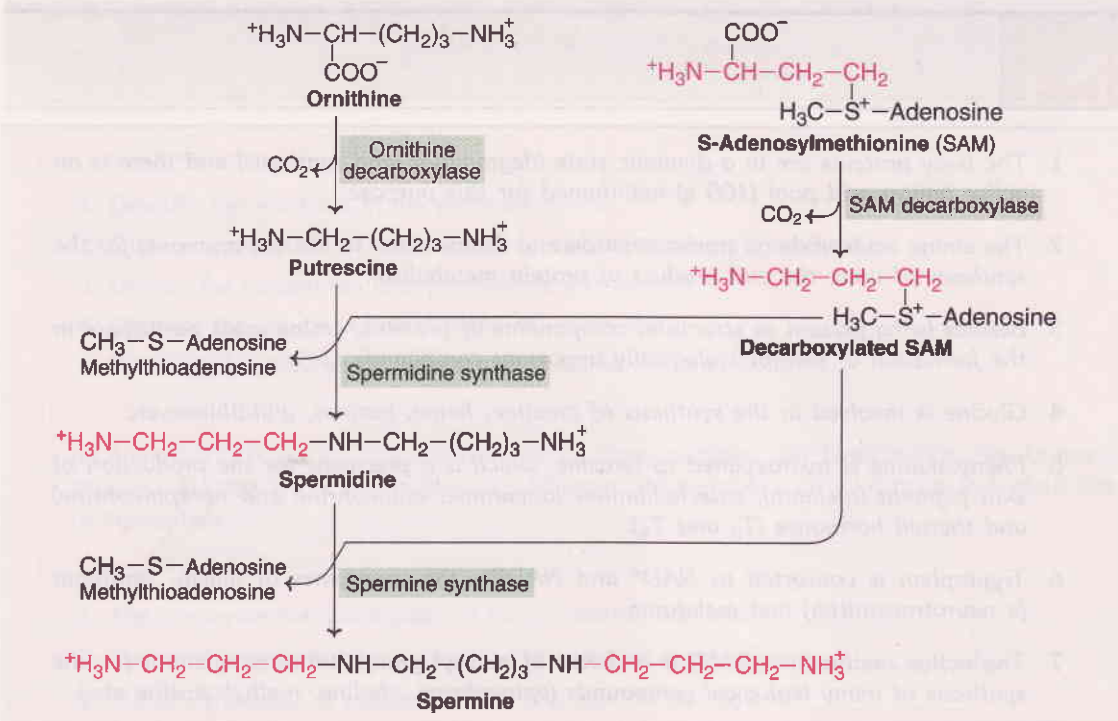


Fig. 15.44 : Biosynthesis of polyamines—putrescine, spermidine and spermine.

Table 15.7 A summary of the biogenic amines and their functions

Amino acid	*Amine*	*Function(s)*
Serine	Ethanolamine	Forms choline
Glutamate	γ-Aminobutyric acid	Inhibitory neurotransmitter
Histidine	Histamine	Vasodilator, promotes gastric HCl and pepsin synthesis
Phenylalanine	Dopamine	For the synthesis of norepinephrine and epinephrine
Tyrosine	Tyramine	Vasoconstrictor (increases blood pressure)
Tryptophan	Tryptamine	Elevates blood pressure
	Serotonin	Stimulates cerebral activity
	Melatonin	Circadian rhythms
Cysteine	Taurine	Constituent of bile acid (taurocholic acid)

3. They are essential for cell growth and proliferation.

4. Some enzymes are inhibited by polyamines, e.g. protein kinase.

5. They are believed to be involved in the stabilization of the membrane structure (cell and cellular organelles).

Clinical importance and polyamines

The ***excretion of polyamines*** is found to be ***elevated in*** almost all types of ***cancers***, e.g. leukemias; carcinoma of lungs, bladder, kidney etc. Diagnostically, putrescine is an ideal marker for cell proliferation whereas spermidine is suitable for the assessment of cell destruction.

SUMMARY

1. *The body proteins are in a dynamic state (degradation and synthesis) and there is an active amino acid pool (100 g) maintained for this purpose.*
2. *The amino acids undergo transamination and deamination to liberate ammonia for the synthesis of urea, the end product of protein metabolism.*
3. *Besides being present as structural components of proteins, amino acids participate in the formation of several biologically important compounds.*
4. *Glycine is involved in the synthesis of creatine, heme, purines, glutathione etc.*
5. *Phenylalanine is hydroxylated to tyrosine, which is a precursor for the production of skin pigment (melanin), catecholamines (dopamine, epinephrine and norepinephrine) and thyroid hormones (T_3 and T_4).*
6. *Tryptophan is converted to NAD^+ and $NADP^+$, the coenzymes of niacin, serotonin (a neurotransmitter) and melatonin.*
7. *The active methionine (SAM) is a donor of methyl group (transmethylation) for the synthesis of many biological compounds (epinephrine, choline, methylcytosine etc.).*
8. *Many amino acids contribute to one-carbon fragments (formyl, formimino, methylene etc.) for participation in one-carbon metabolism—which is mostly under the control of tetrahydrofolate.*
9. *The carbon skeleton of amino acids is involved either in the synthesis of glucose (glycogenic) or fat (ketogenic), or both-glucose and fat.*
10. *Many inborn errors (mostly due to enzyme defects) in amino acid metabolism have been identified. These include phenylketonuria (defect-phenylalanine hydroxylase), albinism (defect-tyrosinase), maple syrup urine disease (defect–α-keto acid dehydrogenase) etc.*

SELF-ASSESSMENT EXERCISES

I. Essay questions

1. Describe the reactions in the synthesis of urea.
2. Give an account of the formation of specialized products from glycine.
3. Discuss the metabolism of phenylalanine and tyrosine.
4. Describe the fate of carbon skeleton of amino acids.
5. Write briefly on various inborn errors of amino acid metabolism.

II. Short notes

(a) Amino acid pool, (b) Transmethylation, (c) Transamination, (d) Deamination, (e) Ammonia toxicity, (f) One-carbon metabolism, (g) Albinism, (h) Serotonin, (i) Glutamate and glutamine, (j) Polyamines.

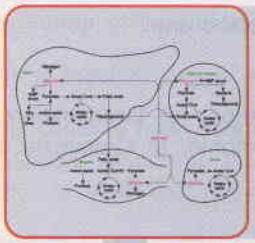
III. Fill in the blanks

1. The coenzyme that participates in transamination reactions is _____.
2. The most important enzyme involved in oxidative deamination is _____.
3. N-Acetylglutamate is required for the activation of the enzyme _____.
4. Primary hyperoxaluria is due to a defect in the enzyme _____.
5. The cofactor required for the conversion of phenylalanine to tyrosine is _____.
6. Parkinson's disease is linked with decreased synthesis of _____.
7. The metabolite excreted (elevated) in alkaptonuria is _____.
8. The disease in which very high amount of tryptophan (nearly 60%) is converted to serotonin is _____.
9. The mammalian enzyme with the shortest half-life (about 10 minutes) is _____.
10. The branched chain amino acid that is only ketogenic is _____.

IV. Multiple choice questions

11. The synthesis of urea occurs in
    (a) Kidney (b) Liver (c) Muscle (d) Brain.
12. The amino acid required for the formation of glutathione
    (a) Glycine (b) Cysteine (c) Glutamate (d) All of them.
13. In the synthesis of cysteine, the carbon skeleton is provided by
    (a) Serine (b) Methionine (c) Glutamate (d) Alanine.
14. The amino acids are said to be ketogenic when the carbon skeleton is finally degraded to
    (a) Succinyl CoA (b) Fumarate (c) Acetyl CoA (d) Pyruvate.
15. The amino acid that does not participate in transamination
    (a) Lysine (b) Glutamate (c) Alanine (d) Tryptophan.

16 Integration of Metabolism



The energy metabolism speaks :

"Carbohydrate, fat, protein metabolisms integrate;
Cells, tissues and organs coordinate;
To meet the body's fuel demands;
The essential requisite for existence."

Metabolism is a continuous process, with thousands of reactions, simultaneously occurring in the living cell. However, biochemists prefer to present metabolism in the form of reactions and metabolic pathways. This is done for the sake of convenience in presentation and understanding.

In the preceeding ***three chapters (13-15)***, we have learnt the metabolism of carbohydrates, lipids and amino acids. We shall now consider the organism as a whole and integrate the metabolism with particular reference to energy demands of the body.

Energy demand and supply

The organisms possess variable energy demands, hence the supply (input) is also equally variable. The consumed metabolic fuel may be burnt (oxidized to CO_2 and H_2O) or stored to meet the energy requirements as per the body needs. ***ATP*** serves as the ***energy currency of the cell*** in this process (***Fig.16.1***).

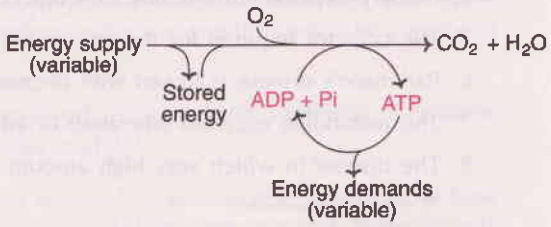


Fig. 16.1 : A summary of body's energy supply and demands. (Note : ATP serves as the energy currency).

The humans possess enormous capacity for food consumption. It is estimated that one can consume as much as 100 times his/her basal requirements! Obesity, a disorder of over-nutrition mostly prevalent in affluent societies, is primarily a consequence of overconsumption.

Integration of major metabolic pathways of energy metabolism

An overview of the interrelationship between the important metabolic pathways, concerned

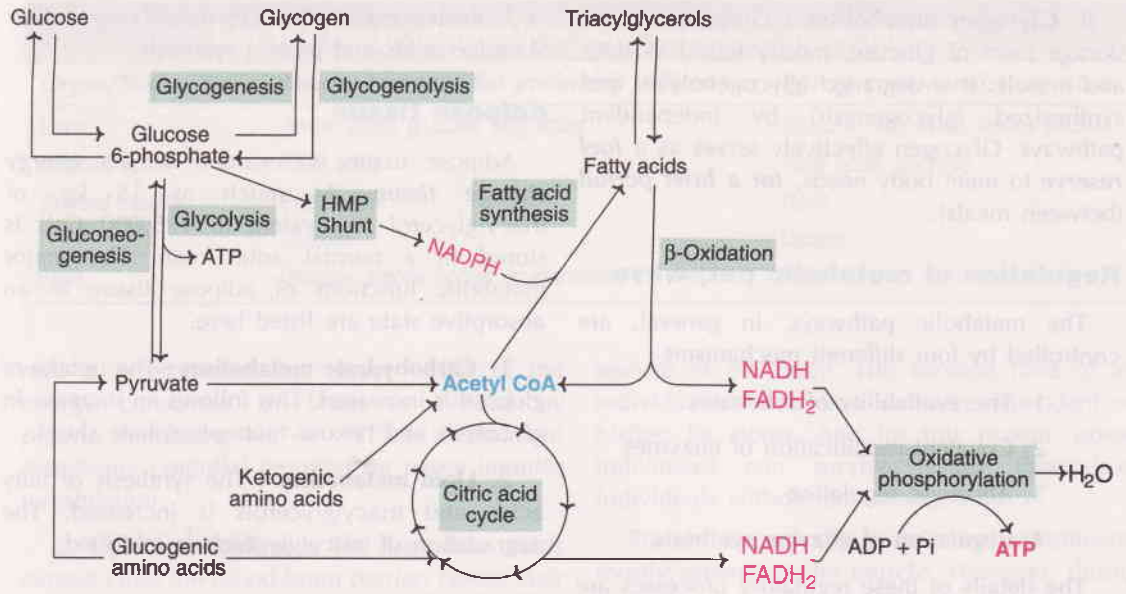


***Fig. 16.2** : An overview of integration of metabolic pathways of energy metabolism (HMP shunt–Hexose monophosphate shunt).*

with fuel metabolism depicted in ***Fig.16.2***, is briefly described here. For detailed information on these metabolic pathways, the reader must refer the respective chapters.

1. **Glycolysis :** The degradation of glucose to pyruvate (lactate under anaerobic condition) generates 8 ATP. Pyruvate is converted to acetyl CoA.

2. **Fatty acid oxidation :** Fatty acids undergo sequential degradation with a release of 2-carbon fragment, namely acetyl CoA. The energy is trapped in the form of NADH and $FADH_2$.

3. **Degradation of amino acids :** Amino acids, particularly when consumed in excess than required for protein synthesis, are degraded and utilized to meet the fuel demands of the body. The glucogenic amino acids can serve as precursors for the synthesis of glucose via the formation of pyruvate or intermediates of citric acid cycle. The ketogenic amino acids are the precursors for acetyl CoA.

4. **Citric acid cycle :** Acetyl CoA is the key and common metabolite, produced from different fuel sources (carbohydrates, lipids, amino acids). ***Acetyl CoA*** enters citric acid (Krebs) cycle and gets oxidized to CO_2. Thus, citric acid cycle is the final common metabolic pathway for the oxidation of all foodstuffs. 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 oxidized in the electron transport chain (ETC). The ETC is coupled with oxidative phosphorylation ***to generate ATP.***

6. **Hexose monophosphate shunt :** This pathway is primarily concerned with the liberation of NADPH and ribose sugar. NADPH is utilized for the biosynthesis of several compounds, including fatty acids. Ribose is an essential component of nucleotides and nucleic acids (***Note :*** DNA contains deoxyribose).

7. **Gluconeogenesis :** The synthesis of glucose from non-carbohydrate sources constitutes gluconeogenesis. Several compounds (e.g. pyruvate, glycerol, amino acids) can serve as precursors for gluconeogenesis.

8. **Glycogen metabolism :** Glycogen is the storage form of glucose, mostly found in liver and muscle. It is degraded (glycogenolysis) and synthesized (glycogenesis) by independent pathways. Glycogen effectively serves as a ***fuel reserve*** to meet body needs, ***for a brief period*** (between meals).

Regulation of metabolic pathways

The metabolic pathways, in general, are controlled by four different mechanisms

1. The availability of substrates
2. Covalent modification of enzymes
3. Allosteric regulation
4. Regulation of enzyme synthesis.

The details of these regulatory processes are discussed under the individual metabolic pathways, in the respective chapters.

ORGAN SPECIALIZATION AND METABOLIC INTEGRATION

The various tissues and organs of the body work in a well coordinated manner to meet its metabolic demands. The major organs along with their most important metabolic functions, in a well-fed absorptive state (usually 2-4 hours after food consumption), are described.

Liver

The liver is specialized to serve as the body's ***central metabolic clearing house.*** It 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 routes to other tissues. The major metabolic functions of liver, in an absorptive state, are listed

1. **Carbohydrate metabolism :** Increased glycolysis, glycogenesis and hexose monophosphate shunt and decreased gluconeogenesis.

2. **Lipid metabolism :** Increased synthesis of fatty acids and triacylglycerols.

3. **Protein metabolism :** Increased degradation of amino acids and protein synthesis.

Adipose tissue

Adipose tissue is regarded as the ***energy storage tissue.*** As much as 15 kg. of triacylglycerol (equivalent to 135,000 Cal) is stored in a normal adult man. The major metabolic functions of adipose tissue in an absorptive state are listed here.

1. **Carbohydrate metabolism :** The uptake of glucose is increased. This follows an increase in glycolysis and hexose monophosphate shunt.

2. **Lipid metabolism :** The synthesis of fatty acids and triacylglycerols is increased. The degradation of triacylglycerols is inhibited.

Skeletal muscle

The ***metabolism*** of skeletal muscle is rather ***variable*** depending on its needs. For instance, the resting muscle of the body utilizes about 30% of body's oxygen consumption. However, during strenuous exercise, this may be as high as 90%. The important metabolic functions of skeletal muscle in an absorptive state are listed.

1. **Carbohydrate metabolism :** The uptake of glucose is higher, and glycogen synthesis is increased.

2. **Lipid metabolism :** Fatty acids taken up from the circulation are also important fuel sources for the skeletal muscle.

3. **Protein metabolism :** Incorporation of amino acids into proteins is higher.

Brain

The human brain constitutes about 2% of the body's weight. But it ***utilizes*** as much as **20% *of the oxygen*** consumed by the body. Being a vital organ, special priority is given to the metabolic needs of the brain.

1. **Carbohydrate metabolism :** In an absorptive state, glucose is the only fuel source to the brain. About 120 g of glucose is utilized per day by an adult brain. This constitutes about 60% of the glucose consumed by the body at

Table 16.1 Energy relationship in major mammalian organs (tissues)

Organ/Tissue	*Energy compound(s) preferably utilized*	*Energy compound(s) exported*
Liver	Amino acids, glucose, fatty acids	Glucose, fatty acids, ketone bodies
Adipose tissue	Fatty acids	Fatty acids, glycerol.
Skeletal muscle	Fatty acids	None
	Glucose	Lactate
Brain	Glucose, ketone bodies (in starvation)	None

rest. It is estimated that about 50% of the energy consumed by brain is utilized by plasma membrane Na^+-K^+-ATPase to maintain membrane potential required for nerve impulse transmission.

2. **Lipid metabolism :** The free fatty acids cannot cross the blood-brain barrier, hence their contribution for the supply of energy to the brain is insignificant. Further, in a fed state, ketone bodies are almost negligible as fuel source to the brain. However, brain predominantly depends on ketone bodies during prolonged starvation (details given later).

The metabolic interrelationship among the major tissues in an absorptive state are given in ***Fig.16.3***. The fuel sources that are preferably utilized by the major organs and the compounds exported from them are listed in ***Table 16.1***.

METABOLISM IN STARVATION

Starvation may be due to food scarcity or the desire to rapidly lose weight or certain clinical conditions (e.g. surgery, burns etc.). Starvation is a metabolic stress which imposes certain metabolic compulsions on the organism. The metabolism is reorganized to meet the new demands of starvation.

Glucose is the fuel of choice for brain and muscle. Unfortunately, the carbohydrate reserve of the body is so low that it cannot meet the energy requirements even for a day. The fuel stores (or energy reserves) of a 70 kg normal man are given in ***Table 16.2***. Triacylglycerol (fat) of adipose tissue is the predominant energy reserve of the body. The survival time of an individual on starvation is mostly dependent on his/her fat stores. And for this reason, obese individuals can survive longer than lean individuals without consuming food.

Protein is basically a structural constituent, mostly present in the muscle. However, during starvation, protein can also meet the fuel demands of the body. It is estimated that about $^1/_3$rd of the body's protein can be utilized towards energy needs without compromising the vital functions.

Starvation is associated with a decrease in insulin level and an increase in glucagon. The metabolic changes during starvation are discussed with reference to the major organs/ tissues.

Liver in starvation

1. **Carbohydrate metabolism :** An important function of liver is to act as a ***blood glucose buffering organ.*** The action of liver is to suit the metabolic needs of the body. During starvation, increased gluconeogenesis and elevated glycogen degradation furnish glucose to the needy tissues (mostly brain).

Table 16.2 Energy reserves of a normal 70 kg man

Energy source (main storage tissue)	*Weight (kg)*	*Energy equivalent (in Cal)*
Triacylglycerol (adipose tissue)	15	135,000
Protein (muscle)	6	24,000
Glycogen (muscle, liver)	0.2	800

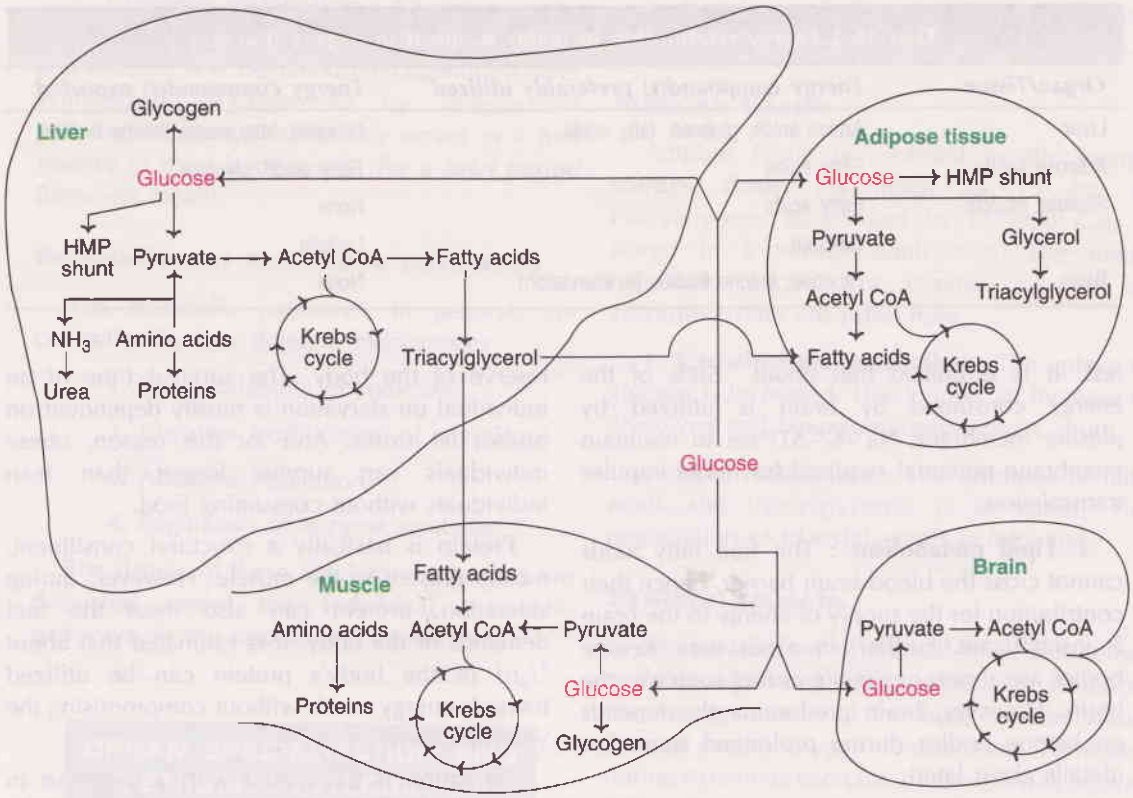


***Fig. 16.3** : Metabolic interrelationship among the major tissues in a well fed state (HMP shunt–Hexose monophosphate shunt).*

BIOMEDICAL / CLINICAL CONCEPTS

- *Biochemists, for their convenience, learn body chemical processes in terms of individual metabolic reactions and pathways, although thousands of reactions simultaneously occur in a living cell.*
- *The metabolic pathways in various tissues and organs are well coordinated to meet the demands of the body.*
- *Liver is appropriately regarded as the body's 'central metabolic clearing house' while adipose tissues constitute the energy (fat) storehouse.*
- *Brain is a vital metabolic organ that consumes about 20% of body's oxygen, although it constitutes only 2% of body weight.*
- *The metabolism in starvation is reorganized to meet the body's changed demands and metabolic compulsions.*
- *Under normal circumstances, glucose is the only fuel source to brain. However, during starvation, the brain slowly gets adapted to use ketone bodies for energy needs.*

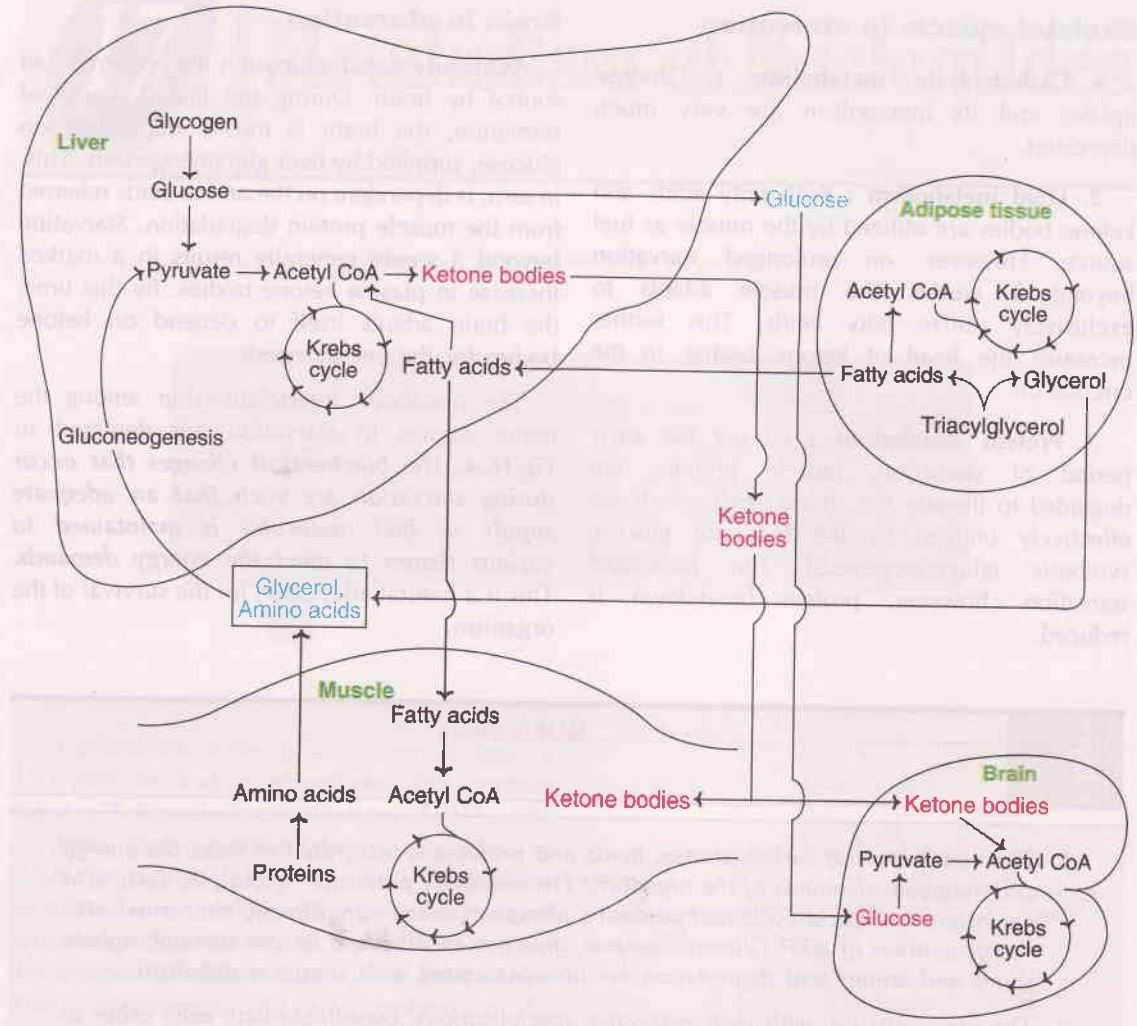


Fig. 16.4 : Metabolic interrelationship among major tissues during starvation.

2. **Lipid metabolism :** Fatty acid oxidation is increased with an elevated synthesis of ketone bodies. This is due to the fact that TCA (Krebs) cycle cannot cope up with the excess production of acetyl CoA, hence the latter is diverted for ketone body synthesis.

Ketone bodies (primarily β-hydroxybutyrate) effectively serve as fuel source for the peripheral tissues. The brain slowly adapts itself to use ketone bodies. Thus, after a 3-day fast, about $^1/_3$rd of the brain's fuel demands are met by ketone bodies, while, after 40 days' starvation, they countribute to about 70% of energy needs.

Adipose tissue in starvation

1. **Carbohydrate metabolism :** Glucose uptake and its metabolism are lowered.

2. **Lipid metabolism :** The degradation of triacylglycerol is elevated, leading to an increased release of fatty acids from the adipose tissue which serve as fuel source for various tissues (brain is an exception). The glycerol liberated in lipolysis serves as a precursor for glucose synthesis by liver. The synthesis of fatty acids and triacylglycerols is totally stopped in adipose tissue.

Skeletal muscle in starvation

1. **Carbohydrate metabolism** : Glucose uptake and its metabolism are very much depressed.

2. **Lipid metabolism :** Both fatty acids and ketone bodies are utilized by the muscle as fuel source. However, on prolonged starvation beyond 3 weeks, the muscle adapts to exclusively utilize fatty acids. This further increases the level of ketone bodies in the circulation.

3. **Protein metabolism** : 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.

Brain in starvation

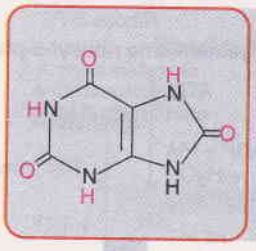
As already stated, glucose is the preferred fuel source by brain. During the first 2 weeks of starvation, the brain is mostly dependent on glucose, supplied by liver gluconeogenesis. This, in turn, is dependent on the amino acids released from the muscle protein degradation. Starvation beyond 3 weeks generally results in a marked increase in plasma ketone bodies. By this time, the brain adapts itself to depend on ketone bodies for the energy needs.

The metabolic interrelationship among the major organs in starvation are depicted in ***Fig.16.4***. The ***biochemical changes that occur during starvation are such that an adequate supply of fuel molecules is maintained to various tissues to meet the energy demands.*** This is a natural adaptation for the survival of the organism.

SUMMARY

1. *The metabolism of carbohydrates, lipids and proteins is integrated to meet the energy and metabolic demands of the organism. The metabolic pathways—glycolysis, fatty acid oxidation, citric acid cycle and oxidative phosphorylation—are directly concerned with the generation of ATP. Gluconeogenesis, glycogen metabolism, hexose monophosphate shunt and amino acid degradation are also associated with energy metabolism.*
2. *The organs/tissues, with their respective specializations, coordinate with each other to meet the metabolic demands of the organism as a whole. Liver is specialized to serve as the body's central metabolic clearing house. It processes and distributes the nutrients to different tissues for their utilization. Adipose tissue is primarily a storage organ of fat. The major bulk of the body protein is located in the muscle tissue.*
3. *Brain is a specialized organ which, in the normal situation, is exclusively dependent on the supply of glucose (120 g/day) for its fuel needs.*
4. *Starvation is a metabolic stress, as it imposes certain metabolic compulsions on the organism. The stored fat of adipose tissue and the muscle protein are degraded and utilized to meet the body's fuel demands. Brain gradually adapts itself to use ketone bodies (instead of glucose) for its energy requirements. Starvation is, thus, associated with metabolic reorganization for the survival of the organism.*

17 Metabolism of Nucleotides



The uric acid speaks :

"I am the end product of purines;
An increase in my production causes gout;
Inflammation of joints is the symptom,
And administration of allopurinol a relief.

Nucleotides consist of a nitrogenous base, a pentose and a phosphate. The pentose sugar is D-ribose in ribonucleotides of RNA while in deoxyribonucleotides (deoxynucleotides) of DNA, the sugar is 2-deoxy D-ribose. Nucleotides participate in almost all the biochemical processes, either directly or indirectly. They are the structural components of nucleic acids (DNA, RNA), coenzymes, and are involved in the regulation of several metabolic reactions.

BIOSYNTHESIS OF PURINE RIBONUCLEOTIDES

Many compounds contribute to the purine ring of the nucleotides (***Fig.17.1***).

1. N_1 of purine is derived from amino group of aspartate.

2. C_2 and C_8 arise from formate of N^{10}-formyl THF.

3. N_3 and N_9 are obtained from amide group of glutamine.

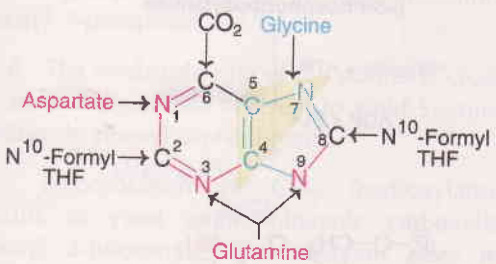
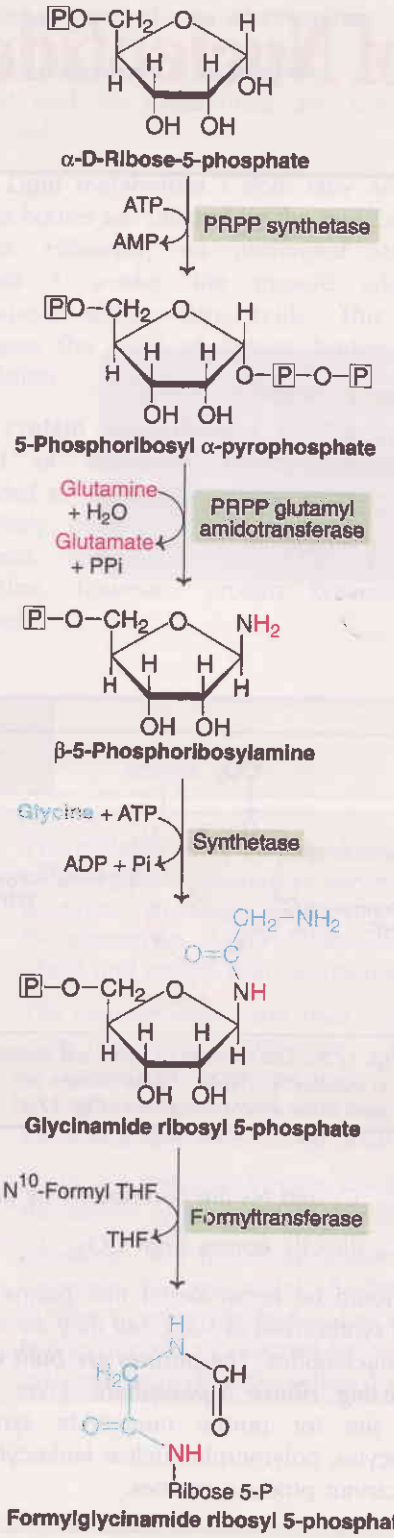


Fig. 17.1 : *The sources of individual atoms in purine ring.* ***(Note :*** *Same colours are used in the synthetic pathway* ***Fig. 17.2).***

4. C_4, C_5 and N_7 are contributed by glycine.

5. C_6 directly comes from CO_2.

It should be remembered that purine bases are not synthesized as such, but they are formed as ribonucleotides. ***The purines are built upon a pre-existing ribose 5-phosphate.*** Liver is the major site for purine nucleotide synthesis. Erythrocytes, polymorphonuclear leukocytes and brain cannot produce purines.



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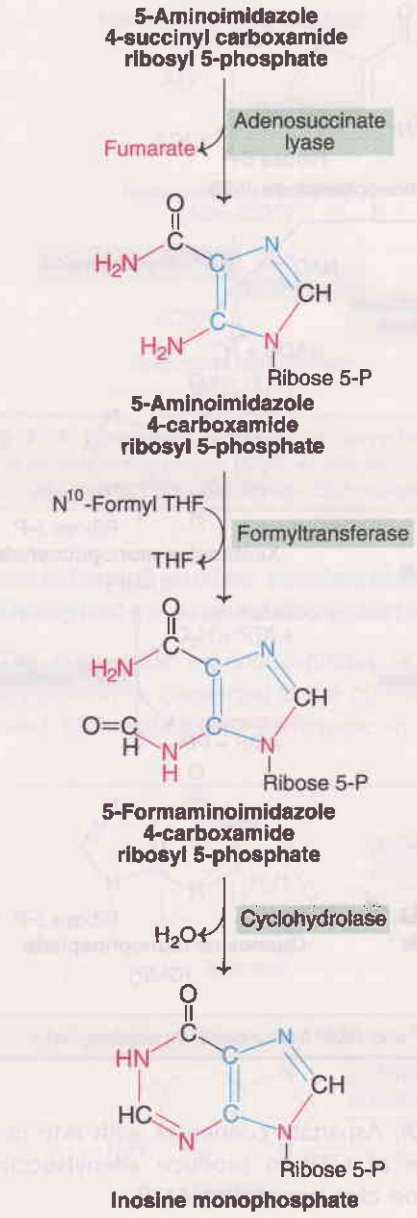


Fig. 17.2 : The metabolic pathway for the synthesis of inosine monophosphate, the parent purine nucleotide (PRPP–Phosphoribosyl pyrophosphate; PPi–Pyrophosphate).

The pathway for the synthesis of ***inosine monophosphate*** (IMP or inosinic acid), the 'parent' purine nucleotide is given in ***Fig.17.2***. The reactions are briefly described in the next column.

1. Ribose 5-phosphate, produced in the hexose monophosphate shunt of carbohydrate metabolism is the starting material for purine nucleotide synthesis. It reacts with ATP to form phosphoribosyl pyrophosphate (PRPP).

2. Glutamine transfers its amide nitrogen to PRPP to replace pyrophosphate and produce 5-phosphoribosylamine. The enzyme ***PRPP glutamyl amidotransferase*** is controlled by ***feedback inhibition*** of nucleotides (IMP, AMP and GMP). This reaction is the '***committed step***' in purine nucleotide biosynthesis.

3. Phosphoribosylamine reacts with glycine in the presence of ATP to form glycinamide ribosyl 5-phosphate or glycinamide ribotide (GAR).

4. N^{10}-Formyl tetrahydrofolate donates the formyl group and the product formed is formyl-glycinamide ribosyl 5-phosphate.

5. Glutamine transfers the second amido amino group to produce formylglycinamidine ribosyl 5-phosphate.

6. The imidazole ring of the purine is closed in an ATP dependent reaction to yield 5-amino-imidazole ribosyl 5-phosphate.

7. Incorporation of CO_2 (carboxylation) occurs to yield aminoimidazole carboxylate ribosyl 5-phosphate. This reaction ***does not require*** the vitamin ***biotin*** and/or ATP which is the case with most of the ***carboxylation*** reactions.

8. Aspartate condenses with the product in reaction 7 to form aminoimidazole 4-succinyl carboxamide ribosyl 5-phosphate.

9. Adenosuccinate lyase cleaves off fumarate and only the amino group of aspartate is retained to yield aminoimidazole 4-carboxamide ribosyl 5-phosphate.

10. N^{10}-Formyl tetrahydrofolate donates a one-carbon moiety to produce formamino-imidazole 4-carboxamide ribosyl 5-phosphate. With this reaction, all the carbon and nitrogen atoms of purine ring are contributed by the respective sources.

11. The final reaction catalysed by cyclohydrolase leads to ring closure with an elimination of water molecule. The product obtained is inosine monophosphate (IMP), the parent purine nucleotide from which other purine nucleotides can be synthesized.

Inhibitors of purine synthesis

Folic acid (THF) is essential for the synthesis of purine nucleotides (reactions 4 and 10). ***Sulfonamides*** are the structural analogs of para-aminobenzoic acid (PABA). These sulfa drugs can be used to ***inhibit the synthesis of folic acid by microorganisms.*** This indirectly reduces the synthesis of purines and, therefore, the nucleic acids (DNA and RNA). Sulfonamides have no influence on humans, since folic acid is not synthesized and is supplied through diet.

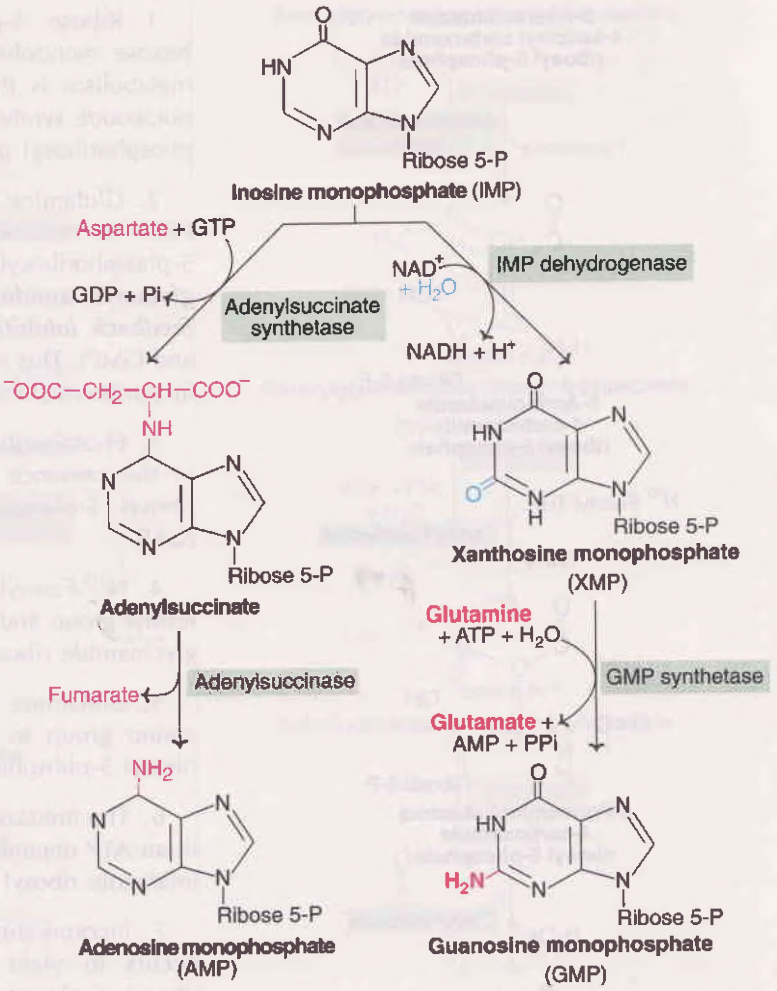


Fig. 17.3 : Synthesis of AMP and GMP from inosine monophosphate.

The structural ***analogs of folic acid*** (e.g. ***methotrexate***) are widely ***used to control cancer.*** They inhibit the synthesis of purine nucleotides (reaction 4 and 10) and, thus, nucleic acids. Both these reactions are concerned with the transfer of one-carbon moiety (formyl group). These inhibitors also affect the proliferation of normally growing cells. This causes many side-effects including anemia, baldness, scaly skin etc.

Synthesis of AMP and GMP from IMP

Inosine monophosphate is the immediate precursor for the formation of AMP and GMP (***Fig.17.3***). Aspartate condenses with IMP in the presence of GTP to produce adenylsuccinate which, on cleavage, forms AMP.

For the synthesis of GMP, IMP undergoes NAD^+ dependent dehydrogenation to form xanthosine monophosphate (XMP). Glutamine then transfers amide nitrogen to XMP to produce GMP.

6-Mercaptopurine is an inhibitor of the synthesis of AMP and GMP. It acts on the enzyme adenylsuccinase (of AMP pathway) and IMP dehydrogenase (of GMP pathway).

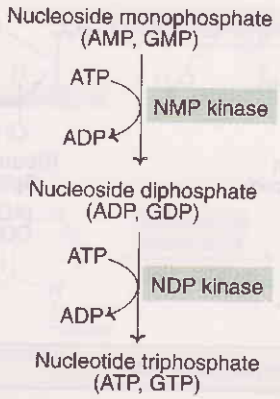


Fig. 17.4 : Conversion of nucleoside monophosphates to di- and triphosphates (NMP–Nucleoside monophosphate; NDP–Nucleoside diphosphate).

Formation of purine nucleoside diphosphates and triphosphates

The nucleoside monophosphates (AMP and GMP) have to be converted to the corresponding di- and triphosphates to participate in most of the metabolic reactions. This is achieved by the transfer of phosphate group from ATP, catalysed by nucleoside monophosphate (NMP) kinases and nucleoside diphosphate (NDP) kinases (***Fig 17.4***).

Salvage pathway for purines

The free purines (adenine, guanine and hypoxanthine) are formed in the normal turnover of nucleic acids (particularly RNA), and also obtained from the dietary sources. The ***purines*** can be ***directly converted to the corresponding nucleotides***, and this process is known as 'salvage pathway' (***Fig.17.5***).

Adenine phosphoribosyl transferase catalyses the formation of AMP from adenine. Hypoxanthine-guanine phosphoribosyl transferase (HGPRT) converts guanine and hypoxanthine, respectively, to GMP and IMP. Phosphoribosyl pyrophosphate (PRPP) is the donor of ribose 5-phosphate in the salvage pathway.

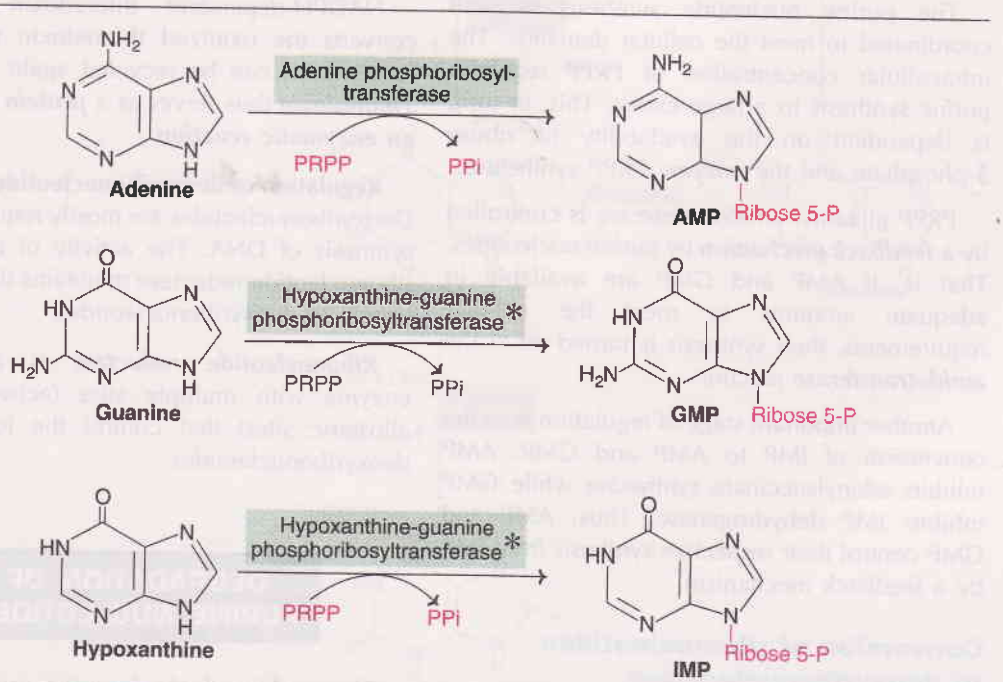


*Fig. 17.5 : Salvage pathways of purine nucleotide synthesis (PRPP–Phosphoribosyl pyrophosphate; PPi–Inorganic pyrophosphate; AMP–Adenosine monophosphate; GMP–Guanosine monophosphate; IMP–Inosine monophosphate; * Deficiency of HGPRT causes Lesch-Nyhan syndrome).*

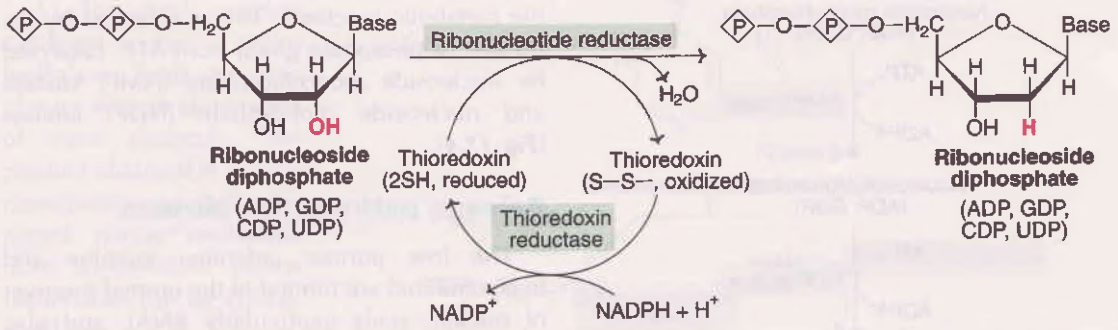


Fig. 17.6 : Formation of deoxyribonucleotides from ribonucleotides.

The salvage pathway is particularly important in certain tissues such as erythrocytes and brain where *de novo* (a new) synthesis of purine nucleotides is not operative.

A ***defect*** in the enzyme ***HGPRT*** causes ***Lesch-Nyhan syndrome*** (details given later).

Regulation of purine nucleotide biosynthesis

The purine nucleotide synthesis is well coordinated to meet the cellular demands. The intracellular concentration of ***PRPP*** regulates purine synthesis to a large extent. This, in turn, is dependent on the availability of ribose 5-phosphate and the enzyme PRPP synthetase.

PRPP glutamyl amidotransferase is controlled by a ***feedback mechanism*** by purine nucleotides. That is, if AMP and GMP are available in adequate amounts to meet the cellular requirements, their synthesis is turned off at the ***amidotransferase*** reaction.

Another important stage of regulation is in the conversion of IMP to AMP and GMP. AMP inhibits adenylsuccinate synthetase while GMP inhibits IMP dehydrogenase. Thus, AMP and GMP control their respective synthesis from IMP by a feedback mechanism.

Conversion of ribonucleotides to deoxyribonucleotides

The synthesis of purine and pyrimidine deoxyribonucleotides occurs from ribonucleotides by a reduction at the C_2 of ribose moiety (***Fig.17.6***). This reaction is catalysed by a multisubunit (two B_1 and two B_2 subunits) enzyme, ***ribonucleotide reductase***.

Supply of reducing equivalents : The enzyme ribonucleotide reductase itself provides the hydrogen atoms needed for reduction from its sulfhydryl groups. The reducing equivalents, in turn, are supplied by ***thioredoxin***, a monomeric protein with two cysteine residues.

NADPH-dependent thioredoxin reductase converts the oxidized thioredoxin to reduced form which can be recycled again and again. ***Thioredoxin*** thus serves as a ***protein cofactor in an enzymatic reaction***.

Regulation of deoxyribonucleotide synthesis : Deoxyribonucleotides are mostly required for the synthesis of DNA. The activity of the enzyme ribonucleotide reductase maintains the adequate supply of deoxyribonucleotides.

Ribonucleotide reductase is a complex enzyme with multiple sites (active site and allosteric sites) that control the formation of deoxyribonucleotides.

DEGRADATION OF PURINE NUCLEOTIDES

The ***end product*** of purine metabolism in humans is ***uric acid***. The sequence of reactions in purine nucleotide degradation is given in ***Fig.17.7***.

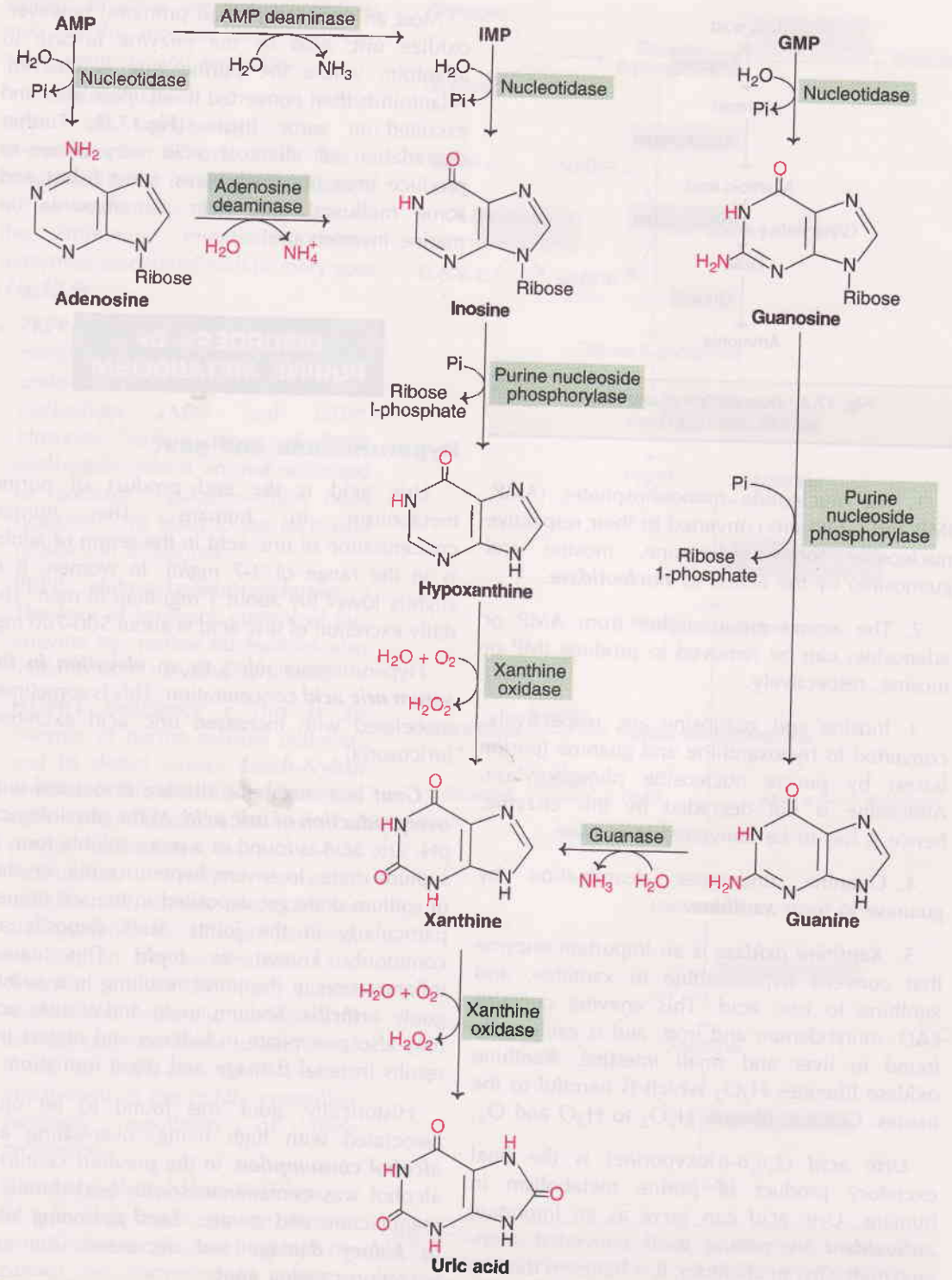


Fig. 17.7 : Degradation of purine nucleotides to uric acid (AMP–Adenosine monophosphate; IMP–Inosine monophosphate; GMP–Guanosine monophosphate).

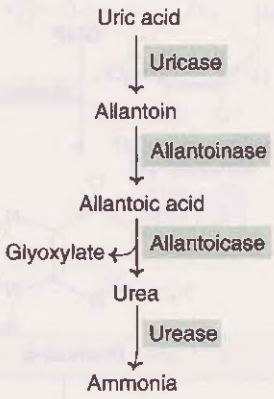


Fig. 17.8 : Degradation of uric acid in animals other than man.

1. The nucleotide monophosphates (AMP, IMP and GMP) are converted to their respective nucleoside forms (adenosine, inosine and guanosine) by the action of ***nucleotidase***.

2. The amino group, either from AMP or adenosine, can be removed to produce IMP or inosine, respectively.

3. Inosine and guanosine are, respectively, converted to hypoxanthine and guanine (purine bases) by purine nucleoside phosphorylase. Adenosine is not degraded by this enzyme, hence it has to be converted to inosine.

4. Guanine undergoes deamination by guanase to form ***xanthine***.

5. ***Xanthine oxidase*** is an important enzyme that converts hypoxanthine to xanthine, and xanthine to uric acid. This enzyme contains FAD, molybdenum and iron, and is exclusively found in liver and small intestine. Xanthine oxidase liberates H_2O_2 which is harmful to the tissues. Catalase cleaves H_2O_2 to H_2O and O_2.

Uric acid (2,6,8-trioxypurine) is the final excretory product of purine metabolism in humans. Uric acid can serve as an important ***antioxidant*** by getting itself converted (non-enzymatically) to allantoin. It is believed that the antioxidant role of ascorbic acid in primates is replaced by uric acid, since these animals have lost the ability to synthesize ascorbic acid.

Most animals (other than primates) however, oxidize uric acid by the enzyme uricase to allantoin, where the purine ring is cleaved. Allantoin is then converted to allantoic acid and excreted in some fishes (***Fig.17.8***). Further degradation of allantoic acid may occur to produce urea (in amphibians, most fishes and some molluscs) and, later, to ammonia (in marine invertebrates).

DISORDERS OF PURINE METABOLISM

Hyperuricemia and gout

Uric acid is the end product of purine metabolism in humans. The normal concentration of uric acid in the serum of adults is in the range of 3-7 mg/dl. In women, it is slightly lower (by about 1 mg) than in men. The daily excretion of uric acid is about 500-700 mg.

Hyperuricemia refers to an ***elevation in the serum uric acid*** concentration. This is sometimes associated with increased uric acid excretion (uricosuria).

Gout is a metabolic disease associated with ***overproduction of uric acid***. At the physiological pH, uric acid is found in a more soluble form as sodium urate. In severe hyperuricemia, crystals of sodium urate get deposited in the soft tissues, particularly in the joints. Such deposits are commonly known as ***tophi***. This causes inflammation in the joints resulting in a painful gouty ***arthritis***. Sodium urate and/or uric acid may also precipitate in kidneys and ureters that results in renal damage and stone formation.

Historically, gout was found to be often associated with high living, over-eating and ***alcohol consumption***. In the previous centuries, alcohol was contaminated with lead during its manufacture and storage. ***Lead poisoning*** leads to ***kidney damage*** and decreased uric acid excretion causing ***gout***.

The ***prevalence*** of gout is about ***3 per 1,000*** persons, mostly affecting males. Post-menopausal

women, however, are as susceptible as men for this disease. Gout is of two types—primary and secondary.

1. **Primary gout :** It is an inborn error of metabolism due to ***overproduction of uric acid***. This is mostly related to increased synthesis of purine nucleotides. The following are the important metabolic defects (enzymes) associated with primary gout (***Fig.17.9***)

- **PRPP synthetase :** In normal circumstances, PRPP synthetase is under feedback control by purine nucleotides (ADP and GDP). However, variant forms of PRPP synthetase—which are not subjected to feedback regulation—have been detected. This leads to the increased production of purines.
- **PRPP glutamylamidotransferase :** The lack of feedback control of this enzyme by purine nucleotides also leads to their elevated synthesis.
- **HGPRT deficiency :** This is an enzyme of purine salvage pathway, and its defect causes ***Lesch-Nyhan syndrome***. This disorder is associated with increased synthesis of purine nucleotides by a two-fold mechanism. Firstly, decreased utilization of purines (hypoxanthine and guanine) by salvage pathway, resulting in the accumulation and diversion of PRPP for purine nucleotides. Secondly, the defect in salvage pathway leads to decreased levels of IMP and GMP causing impairment in the tightly controlled feedback regulation of their production.
- **Glucose 6-phosphatase deficiency :** In type I glycogen storage disease (von Gierke's), glucose 6-phosphate cannot be converted to glucose due to the deficiency of glucose 6-phosphatase. This leads to the

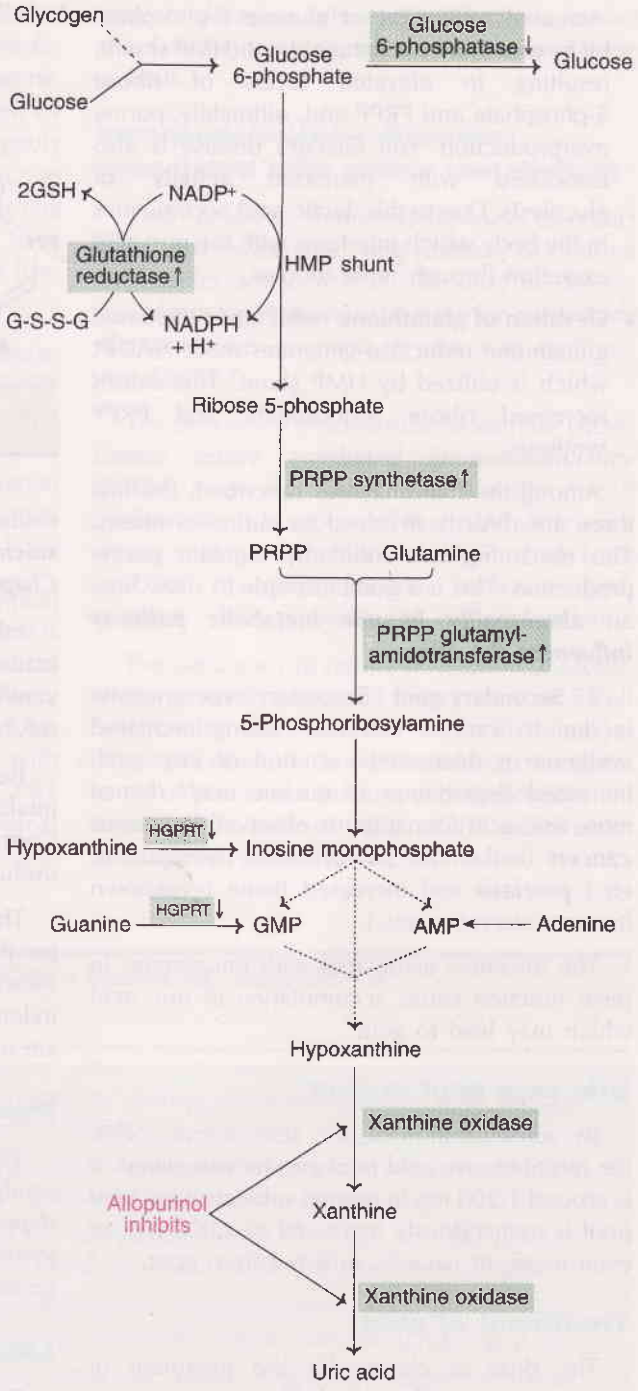


Fig. 17.9 : *Summary of possible enzyme alterations causing gout (↑–Increased enzyme activity; ↓–Decreased enzyme activity; GSH–Reduced glutathione; G-S-S-G–Oxidized glutathione; PRPP–Phosphoribosyl pyrophosphate; HGPRT–Hypoxanthine-guanine phosphoribosyltransferase).*

increased utilization of glucose 6-phosphate by hexose monophosphate shunt (HMP shunt), resulting in elevated levels of ribose 5-phosphate and PRPP and, ultimately, purine overproduction. von Gierke's disease is also associated with increased activity of glycolysis. Due to this, lactic acid accumulates in the body which interferes with the uric acid excretion through renal tubules.

- **Elevation of glutathione reductase :** Increased glutathione reductase generates more $NADP^+$ which is utilized by HMP shunt. This causes increased ribose 5-phosphate and PRPP synthesis.

Among the five enzymes described, the first three are directly involved in purine synthesis. The remaining two indirectly regulate purine production. This is a good example to show how an ***abnormality in one metabolic pathway influences the other.***

2. **Secondary gout :** Secondary hyperuricemia is due to various diseases causing increased synthesis or decreased excretion of uric acid. Increased degradation of nucleic acids (hence more uric acid formation) is observed in various ***cancers*** (leukemias, polycythemia, lymphomas, etc.) ***psoriasis*** and increased tissue breakdown (trauma, starvation etc.).

The disorders associated with impairment in renal function cause accumulation of uric acid which may lead to gout.

Uric acid pool in gout

By administration of uric acid isotope (N^{15}), the miscible uric acid pool can be calculated. It is around 1,200 mg in normal subjects. Uric acid pool is tremendously increased to 3,000 mg. or even more, in patients suffering from gout.

Treatment of gout

The drug of choice for the treatment of primary gout is ***allopurinol***. This is a structural analog of hypoxanthine that competitively inhibits the enzyme ***xanthine oxidase***. Further, allopurinol is oxidized to alloxanthine by xanthine oxidase (***Fig.17.10***). Alloxanthine, in turn, is a more effective inhibitor of xanthine oxidase. This type of inhibition is referred to as ***suicide inhibition*** (For more details, ***Refer Chapter 6***).

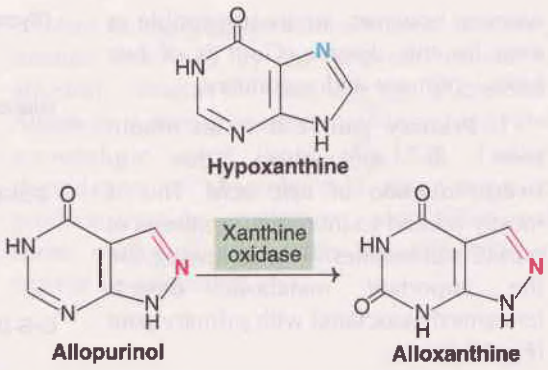


Fig. 17.10 : Structures of hypoxanthine and its structural analogs.

Inhibition of xanthine oxidase by allopurinol leads to the accumulation of hypoxanthine and xanthine. These two compounds are more soluble than uric acid, hence easily excreted.

Besides the drug therapy, restriction in dietary intake of purines and alcohol is advised. Consumption of plenty of water will also be useful.

The ***anti-inflammatory drug*** colchicine is used for the treatment of gouty arthritis. Other anti-inflammatory drugs—such as phenylbutazone, indomethacin, oxyphenbutazone, corticosteroids—are also useful.

Pseudogout

The clinical manifestations of pseudogout are similar to gout. But this disorder is caused by the ***deposition of calcium pyrophosphate crystals*** in joints. Further, serum uric acid concentration is normal in pseudogout.

Lesch-Nyhan syndrome

This disorder is due to the deficiency of ***hypoxanthine-guanine phosphoribosyltransferase*** (HGPRT), an enzyme of purine salvage pathway (***See Fig.17.5***). It was first described in 1964 by Michael Lesch (a medical student) and William L. Nyhan (his teacher).

Lesch-Nyhan syndrome is a ***sex-linked metabolic disorder*** since the structural gene for HGPRT is located on the X-chromosome. It ***affects*** only the ***males*** and is characterized by excessive uric acid production (often gouty arthritis), and ***neurological abnormalities*** such as mental retardation, aggressive behavior, learning disability etc. The patients of this disorder have an irresistible urge to bite their fingers and lips, often causing self-mutilation.

The overproduction of uric acid in Lesch-Nyhan syndrome is explained. HGPRT deficiency results in the accumulation of PRPP and decrease in GMP and IMP, ultimately leading to increased synthesis and degradation of purines (more details given under primary gout).

The biochemical basis for the neurological symptoms observed in Lesch-Nyhan syndrome is not clearly understood. This may be related to the dependence of brain on the salvage pathway for *de novo* synthesis of purine nucleotides. Uric acid is not toxic to the brain, since patients with severe hyperuricemia (not related to HGPRT deficiency) do not exhibit any neurological symptoms. Further, allopurinol treatment that helps to decrease uric acid production, has no affect on the neurological manifestations in these patients.

Immunodeficiency diseases associated with purine metabolism

Two different immunodeficiency disorders associated with the degradation of purine nucleosides are identified. The enzyme defects are ***adenosine deaminase*** and ***purine nucleoside phosphorylase,*** involved in uric acid synthesis (***See Fig.17.7***).

The deficiency of adenosine deaminase (ADA) ***causes severe combined immunodeficiency (SCID)*** involving T-cell and usually B-cell dysfunction. It is explained that ADA deficiency results in the accumulation of dATP which is an inhibitor of ribonucleotide reductase and, therefore, DNA synthesis and cell replication.

The deficiency of purine nucleotide phosphorylase is associated with impairment of T-cell function but has no effect on B-cell function. Uric acid synthesis is decreased and the tissue levels of purine nucleosides and nucleotides are higher. It is believed that dGTP inhibits the development of normal T-cells.

BIOMEDICAL / CLINICAL CONCEPTS

- *Folic acid is essential for the synthesis of purine nucleotides. Folic acid analogs (methotrexate) are employed to control cancer.*
- *The salvage pathway, involving the direct conversion of purines to corresponding nucleotides, is important in tissues—brain and erythrocytes.*
- *Gout is the disorder associated with the overproduction of uric acid, the end product of purine metabolism. Allopurinol is the drug of choice for the treatment of gout.*
- *Lesch-Nyhan syndrome is caused by a defect in the enzyme hypoxanthine-guanine phosphoribosyltransferase. The patients have an irresistible urge to bite their fingers and lips.*
- *A defect in the enzyme adenosine deaminase (ADA) results in severe combined immunodeficiency (SCID) involving both T-cell and B-cell dysfunction. A girl suffering from SCID was cured by transferring ADA gene (in 1990) and that was the first attempt for gene therapy in modern medicine.*
- *Orotic aciduria, a metabolic defect in pyrimidine biosynthesis, is characterized by anaemia and retarded growth, besides the excretion of orotic acid in urine.*

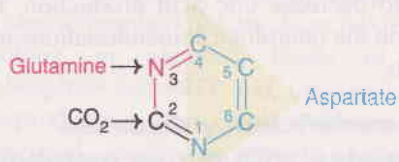


*Fig. 17.11 : Sources of individual atoms in pyrimidine ring (**Note** : Same colours are used in the synthetic pathway, **Fig. 17.12**).*

Hypouricemia

Decreased uric acid levels in the serum (< 2 mg/dl) represent hypouricemia. This is mostly associated with a rare genetic defect in the enzyme xanthine oxidase. It leads to the increased excretion of xanthine and hypoxanthine. Xanthinuria frequently causes the formation of xanthine stones in the urinary tract.

BIOSYNTHESIS OF PYRIMIDINE RIBONUCLEOTIDES

The synthesis of pyrimidines is a much simpler process compared to that of purines. *Aspartate, glutamine (amide group) and CO_2 contribute to atoms in the formation of* pyrimidine ring (***Fig.17.11***). Pyrimidine ring is first synthesized and then attached to ribose 5-phosphate. This is in contrast to purine nucleotide synthesis wherein purine ring is built upon a pre-existing ribose 5-phosphate. The pathway of pyrimidine synthesis is depicted in ***Fig.17.12***, and the salient features are described below.

Glutamine transfers its amido nitrogen to CO_2 to produce carbamoyl phosphate. This reaction is ATP-dependent and is catalysed by cytosomal enzyme carbamoyl phosphate synthetase II (CPS II).

CPS II is activated by ATP and PRPP and inhibited by UTP. Carbamoyl phosphate synthetase I (CPS I) is a mitochondrial enzyme which synthesizes carbamoyl phosphate from ammonia and CO_2 and, in turn urea (***Refer*** protein metabolism, ***Chapter 15***, for more details). Prokaryotes have only one carbamoyl phosphate synthetase which is responsible for the biosynthesis of arginine and pyrimidines.

Carbamoyl phosphate condenses with aspartate to form carbamoyl aspartate. This reaction is catalysed by aspartate transcarbamoylase. Dihydroorotase catalyses the pyrimidine ring closure with a loss of H_2O.

The three enzymes—CPS II, aspartate transcarbamoylase and dihydroorotase are the domains (functional units) of the same protein. This is a good example of a ***multifunctional enzyme.***

The next step in pyrimidine synthesis is an NAD^+ dependent dehydrogenation, leading to the formation of orotate.

Ribose 5-phosphate is now added to orotate to produce orotidine monophosphate (OMP). This reaction is catalysed by orotate phosphoribosyltransferase, an enzyme comparable with HGPRT in its function. OMP undergoes decarboxylation to uridine mono-phosphate (UMP).

Orotate phosphoribosyltransferase and OMP decarboxylase are ***domains*** of a single protein. A *defect in this **bifunctional enzyme** causes orotic aciduria (details given later).*

By an ATP-dependent kinase reaction, UMP is converted to UDP which serves as a precursor for the synthesis of dUMP, dTMP, UTP and CTP.

Ribonucleotide reductase converts UDP to dUDP by a thioredoxin-dependent reaction. Thymidylate synthetase catalyses the transfer of a methyl group from N^5, N^{10}-methylene tetrahydrofolate to produce deoxythymidine monophosphate (dTMP).

UDP undergoes an ATP-dependent kinase reaction to produce UTP. Cytidine triphosphate (CTP) is synthesized from UTP by amination. CTP synthetase is the enzyme and glutamine provides the nitrogen.

Regulation of pyrimidine synthesis

In bacteria, ***aspartate transcarbamoylase*** (ATCase) catalyses a ***committed step*** in

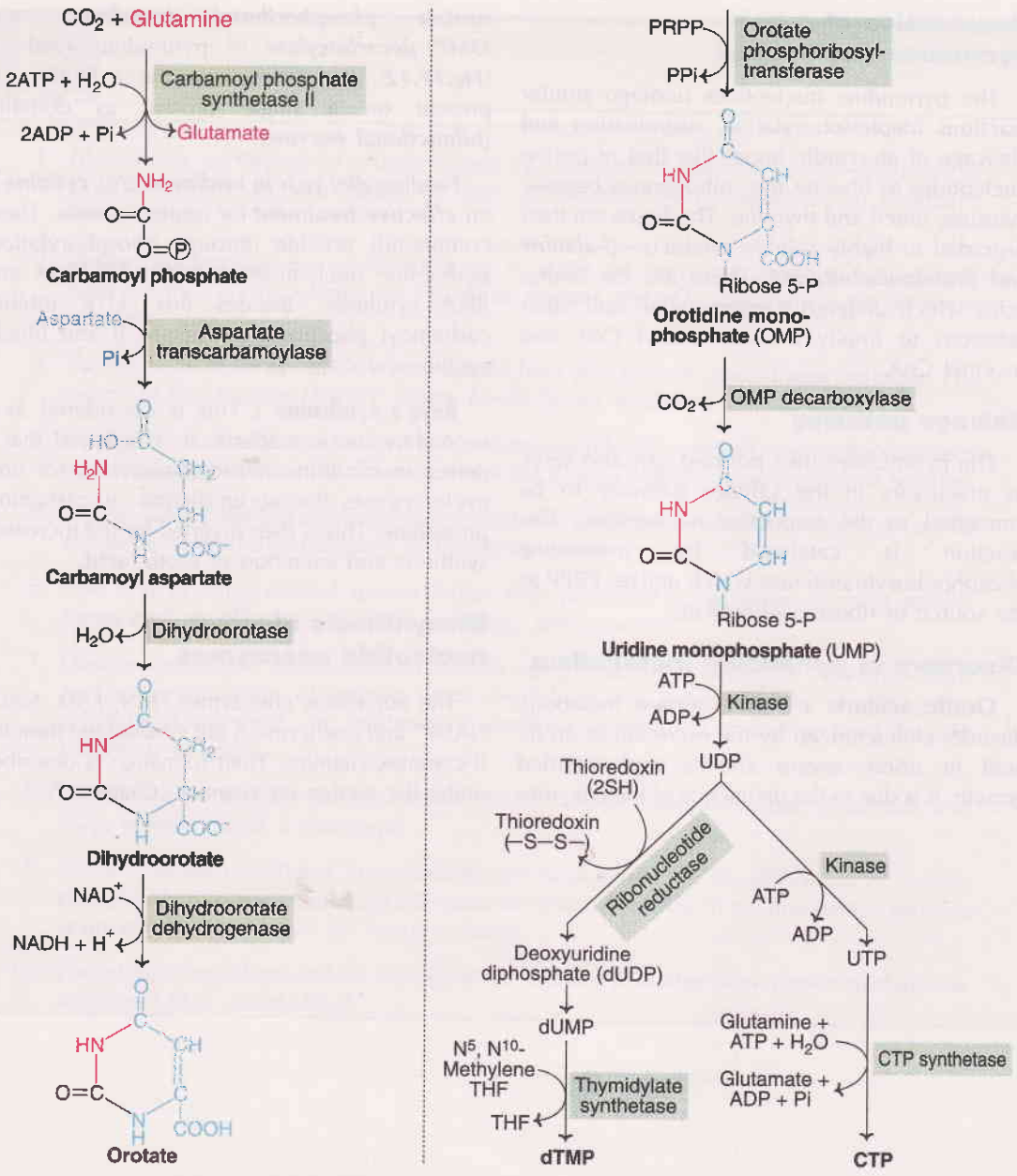


Fig. 17.12 contd. next column

Fig. 17.12 : Metabolic pathway for the synthesis of pyrimidine nucleotides.

pyrimidine biosynthesis. ATCase is a good example of an enzyme controlled by ***feedback mechanism*** by the end product CTP. In certain bacteria, UTP also inhibits ATCase. ATP, however, stimulates ATCase activity.

Carbamoyl phosphate synthetase II (CPS II) is the regulatory enzyme of pyrimidine synthesis in animals. It is activated by PRPP and ATP and inhibited by UDP and UTP. OMP decarboxylase, inhibited by UMP and CMP, also controls pyrimidine formation.

Degradation of pyrimidine nucleotides

The pyrimidine nucleotides undergo similar reactions (dephosphorylation, deamination and cleavage of glycosidic bond) like that of purine nucleotides to liberate the nitrogenous bases—cytosine, uracil and thymine. The bases are then degraded to highly soluble products—***β-alanine*** and ***β-aminoisobutyrate.*** These are the amino acids which undergo transamination and other reactions to finally produce acetyl CoA and succinyl CoA.

Salvage pathway

The pyrimidines (like purines) can also serve as precursors in the salvage pathway to be converted to the respective nucleotides. This reaction is catalysed by pyrimidine phosphoribosyltransferase which utilizes PRPP as the source of ribose 5-phosphate.

Disorders of pyrimidine metabolism

Orotic aciduria : This is a rare metabolic disorder characterized by the excretion of orotic acid in urine, severe anemia and retarded growth. It is due to the deficiency of the enzymes ***orotate phosphoribosyl transferase*** and ***OMP decarboxylase*** of pyrimidine synthesis (***Fig.17.12***). Both these enzyme activities are present on a single protein as domains (***bifunctional enzyme***).

Feeding ***diet rich in uridine*** and/or ***cytidine*** is an ***effective treatment*** for orotic aciduria. These compounds provide (through phosphorylation) pyrimidine nucleotides required for DNA and RNA synthesis. Besides this, UTP inhibits carbamoyl phosphate synthetase II and blocks synthesis of orotic acid.

Reye's syndrome : This is considered as a secondary orotic aciduria. It is believed that a defect in ornithine transcarbamoylase (of urea cycle) causes the accumulation of carbamoyl phosphate. This is then diverted for the increased synthesis and excretion of orotic acid.

Biosynthesis of nucleotide coenzymes

The nucleotide coenzymes FMN, FAD, NAD^+ $NADP^+$ and coenzyme A are synthesized from the B-complex vitamins. Their formation is described under the section on vitamins (***Chapter 7***).

SUMMARY

1. *Nucleotides participate in a wide variety of reactions in the living cells—synthesis of DNA and RNA; as constituents of many coenzymes; in the regulation of metabolic reactions etc.*
2. *Purine nucleotides are synthesized in a series of reactions starting from ribose 5-phosphate. Glycine, glutamine, aspartate, formate and CO_2 contribute to the synthesis of purine ring.*
3. *Purine nucleotides can also be synthesized from free purines by a salvage pathway. The defect in the enzyme HGPRT causes Lesch-Nyhan syndrome.*
4. *Deoxyribonucleotides are formed from ribonucleotides by a reduction process catalysed by ribonucleotide reductase. Thioredoxin is the protein cofactor required for this reaction.*
5. *Purine nucleotides are degraded to uric acid, the excretory product in humans. Uric acid serves as a natural antioxidant in the living system.*
6. *Uric acid in many animal species (other than primates) is converted to more soluble forms such as allantoin, allantoic acid etc., and excreted.*
7. *Gout is a metabolic disease associated with overproduction of uric acid. This often leads to the accumulation of sodium urate crystals in the joints, causing painful gouty arthritis. Allopurinol, an inhibitor of xanthine oxidase, is the drug used for the treatment of gout.*
8. *Pyrimidine nucleotides are synthesized from the precursors aspartate, glutamine and CO_2, besides ribose 5-phosphate.*
9. *Orotic aciduria is a defect in pyrimidine synthesis caused by the deficiency of orotate phosphoribosyltransferase and OMP decarboxylase. Diet rich in uridine and/or cytidine is an effective treatment for orotic aciduria.*
10. *Pyrimidines are degraded to amino acids, namely β-alanine and β-aminoisobutyrate which are then metabolized.*

SELF-ASSESSMENT EXERCISES

I. Essay questions

1. Describe the catabolism of purine nucleotides and the associated metabolic disorders.
2. Write an account of the biosynthesis of inosine monophosphate.
3. Discuss the synthesis and degradation of pyrimidines.
4. Describe the role of PRPP in purine and pyrimidine synthesis.
5. Write an account of salvage pathway in purine nucleotide synthesis. Add a note on Lesch-Nyhan syndrome.

II. Short notes

(a) Gout, (b) PRPP, (c) Synthesis of deoxyribonucleotides, (d) Functions of nucleotides, (e) Immunodeficiency diseases in purine metabolism, (f) Orotic aciduria, (g) Carbamoyl phosphate synthetase II, (h) HGPRT, (I) Degradation of uric acid in different animals, (j) Regulation of purine synthesis (k) Inhibitors of purine synthesis.

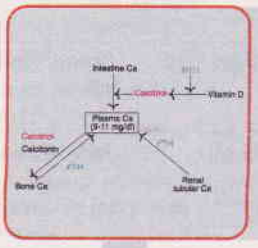
III. Fill in the blanks

1. The amino acids required for the synthesis of purines and pyrimidines are _____.
2. The enzyme xanthine oxidase is inhibited by _____.
3. Tophi are mostly made up of _____.
4. Hypouricemia is due to the deficiency of the enzyme _____.
5. The disorder in which the patients have an irresistible urge to bite their fingers and lips is _____.
6. The cofactor required by the enzyme ribonucleotide reductase is _____.
7. The 'parent' nucleotide synthesized in the biosynthesis of purines is _____.
8. Xanthine oxidase converts allopurinol to _____.
9. The amino acid that contributes to the synthesis of more than half of the pyrimidine ring _____.
10. The regulatory enzyme in the pyrimidine biosynthesis in animals is _____.

IV. Multiple choice questions

11. Name the enzyme associated with hyperuricemia
    (a) PRPP synthetase (b) HGPRT (c) Glucose 6-phosphatase (d) All of them.
12. An enzyme of purine metabolism associated with immunodeficiency disease
    (a) Adenosine deaminase (b) Xanthine oxidase (c) PRPP synthetase (d) HGPRT.
13. Orotic aciduria can be treated by a diet rich in
    (a) Adenine (b) Guanine (c) Uridine (d) Any one of them.
14. The end product of purine metabolism in humans is
    (a) Xanthine (b) Uric acid (c) Urea (d) Allantoin.
15. The nitrogen atoms in the purine ring are obtained from
    (a) Glycine (b) Glutamine (c) Aspartate (d) All of them.

18 Mineral Metabolism



The mineral, calcium speaks :

"I am the most abundant mineral;
Calcify and strengthen bones, teeth.....
Coagulate blood and contract muscle;
Regulated by calcitriol, PTH and calcitonin."

The mineral (inorganic) elements constitute only a small proportion of the body weight. There is a wide variation in their body content. For instance, calcium constitutes about 2% of body weight while cobalt about 0.00004%.

General functions

Minerals perform several vital functions which are absolutely ***essential for the very existence of the organism***. These include calcification of bone, blood coagulation, neuromuscular irritability, acid-base equilibrium, fluid balance and osmotic regulation.

Certain minerals are integral components of biologically important compounds such as hemoglobin (Fe), thyroxine (I), insulin (Zn) and vitamin B_{12} (Co). Sulfur is present in thiamine, biotin, lipoic acid and coenzyme A. Several minerals participate as cofactors for enzymes in metabolism (e.g. Mg, Mn, Cu, Zn, K). Some elements are essential constituents of certain enzymes (e.g. Co, Mo, Se).

Classification

The minerals are classified as principal elements and trace elements.

The seven **principal elements** (***macro-minerals***) constitute 60-80% of the body's inorganic material. These are ***calcium***, ***phosphorus***, ***magnesium***, ***sodium***, ***potassium***, ***chloride*** and ***sulfur***.

The principal elements are required in amounts greater than 100 mg/day.

The (***microminerals)*** are required in amounts less than 100 mg/day. They are subdivided into three categories

1. **Essential trace elements :** Iron, copper, iodine, manganese, zinc, molybdenum, cobalt, fluorine, selenium and chromium.

2. **Possibly essential trace elements :** Nickel, vanadium, cadmium and barium.

3. **Non-essential trace elements :** Aluminium, lead, mercury, boron, silver, bismuth etc.

Table 18.1 A summary of the major characteristics of principal elements (macroelements)

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, chondroitin sulfate).	Almost unknown	—	Sulfur containing amino acids

A summary of the major characteristics of principal elements and trace elements is respectively given in ***Tables 18.1*** and ***18.2***. The individual elements are described next.

CALCIUM

Calcium is the ***most abundant among the minerals in the body***. The total content of calcium in an adult man is about 1 to 1.5 kg. As much as 99% of it is present in the bones and teeth. A small fraction (1%) of the calcium, found outside the skeletal tissue, performs a wide variety of functions.

Biochemical functions

1. **Development of bones and teeth :** Calcium, along with phosphate, is required for the formation (***of hydroxyapatite***) and physical strength of skeletal tissue. Bone is regarded as a mineralized connective tissue. Bones which are in a dynamic state serve as reservoir of Ca. Osteoblasts are responsible for bone formation while osteoclasts result in demineralization.

2. **Muscle contraction :** Ca^{2+} interacts with troponin C to trigger muscle contraction. Calcium also activates ATPase, increases the interaction between actin and myosin.

3. **Blood coagulation :** Several reactions in the cascade of blood clotting process are dependent on Ca^{2+}(factor IV).

4. **Nerve transmission :** Ca^{2+} is necessary for the transmission of nerve impulse.

5. **Membrane integrity and permeability :** Ca^{2+} influences the membrane structure and transport of water and several ions across it.

Table 18.2 A summary of the major characteristics of trace elements (microelements)

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, goiter, 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

6. **Activation of enzymes :** Ca^{2+} is needed for the direct activation of enzymes such as lipase (pancreatic), ATPase and succinate dehydrogenase.

7. **Calmodulin mediated action of Ca^{2+} :** Calmodulin (mol. wt. 17,000) is a calcium binding regulatory protein. Ca-calmodulin complex activates certain enzymes e.g. adenylate cyclase, Ca^{2+} dependent protein kinases.

8. **Calcium as intracellular messenger :** Certain hormones exert their action through the mediation of Ca^{2+} (instead of cAMP). Calcium is regarded as a ***second messenger*** for such hormonal action e.g. epinephrine in liver glycogenolysis. Calcium serves as a third messenger for some hormones e.g. antidiuretic hormone (ADH) acts through cAMP, and then Ca^{2+}.

9. **Release of hormones :** The release of certain hormones (insulin, PTH, calcitonin) from the endocrine glands is facilitated by Ca^{2+}.

10. **Secretory processes :** Ca^{2+} regulates microfilament and microtubule mediated

processes such as endocytosis, exocytosis and cell motility.

11. **Contact inhibition :** Calcium is believed to be involved in cell to cell contact and adhesion of cells in a tissue (Refer p. 692 also). The cell to cell communication may also require Ca^{2+}.

12. **Action on heart :** Ca^{2+} acts on myocardium and prolongs systole.

Dietary requirements

Adult men and women	—	800 mg/day
Women during pregnancy, lactation and post-menopause	—	1.5 g/day
Children (1-18 yrs.)	—	0.8–1.2 g/day
Infants (<1 year)	—	300–500 mg/day

Sources

Best sources	—	Milk and milk products
Good sources	—	Beans, leafy vegetables, fish, cabbage, egg yolk.

Absorption

The absorption of calcium mostly occurs in the duodenum by an energy dependent active process. It is influenced by several factors.

Factors promoting Ca absorption

1. Vitamin D (through its active form calcitriol) induces the synthesis of calcium binding protein in the intestinal epithelial cells and promotes Ca absorption.

2. Parathyroid hormone enhances Ca absorption through the increased synthesis of calcitriol.

3. Acidity (low pH) is more favourable for Ca absorption.

4. Lactose promotes calcium uptake by intestinal cells.

5. The amino acids lysine and arginine facilitate Ca absorption.

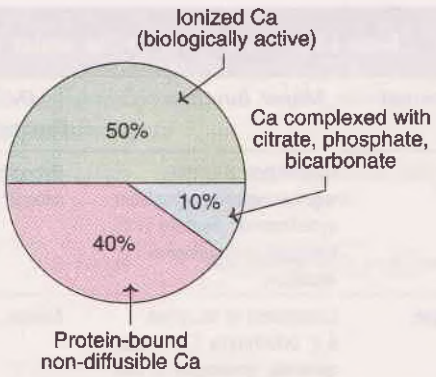


Fig. 18.1 : Different forms of circulating calcium.

Factors inhibiting Ca absorption

1. Phytates and oxalates form insoluble salts and interfere with Ca absorption.

2. High content of dietary phosphate results in the formation of insoluble calcium phosphate and prevents Ca uptake. The dietary ratio of Ca and P—between 1 : 2 and 2 : 1—is ideal for optimum Ca absorption by intestinal cells.

3. The free fatty acids react with Ca to form insoluble calcium soaps. This is particularly observed when the fat absorption is impaired.

4. Alkaline condition (high pH) is unfavourable for Ca absorption.

5. High content of dietary fiber interferes with Ca absorption.

Plasma calcium

Most of the blood Ca is present in the plasma since the blood cells contain very little of it. The normal concentration of plasma or serum Ca is ***9-11 mg/dl*** (***4.5-5.5 mEq/l***). About half of this (5 mg/dl) is in the ionized form which is functionally the most active (***Fig.18.1***). At least 1 mg/dl serum Ca is found in association with citrate and/or phosphate. About 40% of serum Ca (4-5mg/dl) is bound to proteins, mostly albumin and, to a lesser extent, globulin. Ionized and citrate (or phosphate) bound Ca is diffusible from blood to the tissues while protein bound Ca is non-diffusible. In the usual laboratory determination of serum Ca, all the three fractions are measured together.

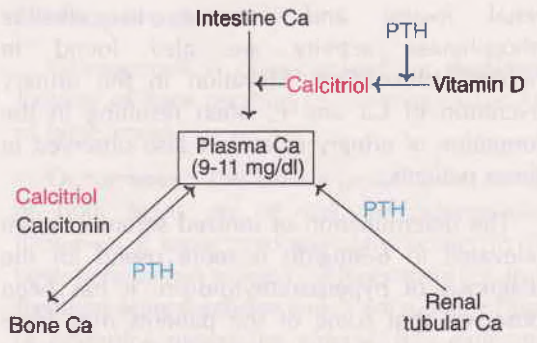


Fig. 18.2 : Overview of calcium homeostasis (PTH–Parathyroid hormone).

FACTORS REGULATING PLASMA Ca LEVEL

As already stated, calcium is almost exclusively present in blood plasma (or serum). The hormones—***calcitriol***, ***parathyroid hormone*** (PTH) and ***calcitonin*** are the major factors that regulate the plasma calcium ***(homeostasis of Ca; Fig.18.2)*** within a narrow range (9-11 mg/dl).

Calcitriol

The physiologically active form of vitamin D is a hormone, namely calcitriol or 1,25-dihydroxy-cholecalciferol (1,25 DHCC). The synthesis of calcitriol and its wide range of biochemical actions are described under Vitamins (***Chapter 7***).

Calcitriol 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 (the active vitamin D). Furthermore, calcitriol ***stimulates calcium uptake by osteoblasts of bone and promotes calcification*** or mineralization (deposition of calcium phosphate) and remodelling.

Parathyroid hormone

Parathyroid hormone (PTH) is secreted by two pairs of parathyroid glands that are closely associated with thyroid glands. Parathyroid hormone (mol. wt. 95,000) is a single chain polypeptide, containing 84 amino acids. It is *originally synthesized as preproPTH which is* degraded to proPTH and, finally, to active PTH. The rate of formation (by degradation of proPTH) and the secretion of PTH are promoted by low Ca^{2+} concentration. Thus, the release of PTH from parathyroid glands is under the negative feedback regulation of serum Ca^{2+}.

Mechanism of action of PTH : PTH binds to a membrane receptor protein on the target cell and activates adenylate cyclase to liberate cAMP. This, in turn, increases intracellular calcium that promotes the phosphorylation of proteins (by kinases) which, finally brings about the biological actions. PTH has 3 independent tissues—bone, kidneys and intestine—to exert its action. The ***prime function of PTH is to elevate serum calcium level.***

Action on the bone : PTH causes decalcification or ***demineralization of bone***, a process carried out by ***osteoclasts***. This is brought out by PTH stimulated increased activity of the enzymes pyrophosphatase and collagenase. These enzymes result in bone resorption. Demineralization ultimately leads to an increase in the blood Ca level. The action of PTH on bone is quantitatively very significant to maintain Ca homeostasis. It must, however, be noted that this is being done at the expense of loss of Ca from bone, particularly in dietary Ca deficiency.

Action on the kidney : PTH ***increases the Ca reabsorption*** by kidney tubules. This is the most rapid action of PTH to elevate blood Ca levels. However, quantitatively, this is less important compared to the action of PTH on bone. PTH promotes the production of calcitriol (1,25 DHCC) in the kidney by stimulating 1-hydroxylation of 25-hydroxycholecalciferol.

Action on the intestine : The action of PTH on the intestine is indirect. It increases the intestinal absorption of Ca by promoting the synthesis of calcitriol.

Calcitonin

Calcitonin is a peptide containing 32 amino acids. It 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. Further, calcitonin decreases bone resorption and increases the excretion of Ca into urine. CT, therefore, has a ***decreasing influence on blood calcium***.

Importance of Ca : P ratio

The ratio of plasma Ca : P is important for calcification of bones. The product of Ca × P (in mg/dl) in children is around 50 and in adults around 40. This product is less than 30 in rickets.

Excretion of calcium

Calcium is excreted partly through the kidneys and mostly through the intestine. The renal threshold for serum Ca is 10 mg/dl. Calcium gets excreted into urine beyond this concentration. Ingestion of excess protein 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.

Excretion of Ca into the feces is a continuous process and this is increased in vitamin D deficiency.

Calcium in the teeth

The teeth calcium is not subjected to regulation as observed for bone calcium. Thus the adult teeth, once formed, ***do not undergo decalcification*** to meet the body needs of calcium. However, proper calcification of teeth is important in the growing children.

DISEASE STATES

The blood Ca level is maintained within a narrow range by the homeostatic control, most predominantly by PTH. Hence abnormalities in Ca metabolism are mainly associated with alterations in PTH.

Hypercalcemia

Elevation in serum Ca level (normal 9–11 mg/dl) is hypercalcemia. Hypercalcemia is associated with ***hyperparathyroidism*** caused by increased activity of parathyroid glands. Decrease in serum phosphate (due to increased renal losses) and increase in alkaline phosphatase activity are also found in hyperparathyroidism. Elevation in the urinary excretion of Ca and P, often resulting in the formation of urinary calculi, is also observed in these patients.

The determination of ionized serum calcium (elevated to 6-9mg/dl) is more useful for the diagnosis of hyperparathyroidism. It has been observed that some of the patients may have normal levels of total calcium in the serum but differ with regard to ionized calcium.

The symptoms of hypercalcemia include lethargy, muscle weakness, loss of appetite, constipation, nausea, increased myocardial contractility and susceptibility to fractures.

Hypocalcemia

Hypocalcemia is a more serious and life threatening condition. It is characterized by a fall in the serum Ca to below 7 mg/dl, causing ***tetany***. The symptoms of tetany include neuromuscular irritability, spasms and convulsions.

Hypocalcemia is mostly due to ***hypoparathyroidism***. This may happen after an accidental surgical removal of parathyroid glands or due to an autoimmune disease.

Rickets

Rickets is a disorder of defective calcification of bones. This may be due to a ***low levels of vitamin D*** in the body or due to a dietary deficiency of Ca and P—or both. The concentration of serum Ca and P may be low or normal. An ***increase*** in the activity of ***alkaline phosphatase*** is a characteristic feature of rickets.

Renal rickets

Renal rickets is associated with damage to renal tissue, causing impairment in the synthesis of calcitriol. It does not respond to vitamin D in ordinary doses, therefore, some workers regard this as ***vitamin D resistant rickets***. Renal rickets can be treated by administration of calcitriol.

Osteoporosis

Osteoporosis is characterized by ***demineralization of bone*** resulting in the progressive loss of bone mass.

Occurrence : The elderly people (over 60 yr.) of both sexes are at risk for osteoporosis. However, it more predominantly occurs in the post-menopausal women. Osteoporosis results in ***frequent bone fractures*** which are a major cause of disability among the elderly. It is estimated that more than 50% of the fractures in USA are due to this disorder. Osteoporosis may be regarded as a silent thief.

Etiology : The etiology of osteoporosis is largely unknown, but it is believed that several causative factors may contribute to it. The ability to produce ***calcitriol*** from vitamin D is ***decreased*** with age, particularly in the postmenopausal women. Immobilized or sedentary individuals tend to decrease bone mass while those on regular exercise tend to increase bone mass. ***Deficiency of sex hormones*** (in women) has been implicated in the development of osteoporosis.

Treatment : ***Estrogen*** administration along with ***calcium*** supplementation (in combination with vitamin D) to postmenopausal women reduces the risk of fractures. Higher dietary intake of Ca (about 1.5 g/day) is recommended for elderly people.

Osteopetrosis (marble bone disease)

Osteopetrosis is characterized by ***increased bone density***. This is primarily due to inability to resorb bone. This disorder is mostly ***observed in*** association with ***renal tubular acidosis*** (due to a defect in the enzyme carbonic anhydrase) and cerebral calcification.

PHOSPHORUS

An adult body contains about 1 kg phosphate and it is found in every cell of the body. Most of it (about 80%) occurs in combination with Ca in the bones and teeth. About 10% of body P is found in muscles and blood in association with proteins, carbohydrates and lipids. The remaining 10% is widely distributed in various chemical compounds.

Biochemical functions

1. Phosphorus is essential for the development of ***bones and teeth***.

2. It plays a central role for the formation and utilization of high-energy phosphate compounds e.g. ATP, GTP, creatine phosphate etc.

3. Phosphorus is required for the formation of phospholipids, phosphoproteins and nucleic acids (DNA and RNA).

4. It is an essential component of several nucleotide coenzymes e.g. NAD^+, $NADP^+$, pyridoxal phosphate, ADP, AMP.

5. Several proteins and enzymes are activated by phosphorylation.

6. Phosphate buffer system is important for the maintenance of pH in the blood (around 7.4) as well as in the cells.

Dietary requirements

The recommended dietary allowance (RDA) of phosphate is based on the intake of calcium. The ratio of ***Ca : P of 1 : 1 is recommended*** (i.e. 800 mg/day) for an adult. For infants, however, the ratio is around 2 : 1, which is based on the ratio found in human milk. Calcium and phosphate are distributed in the majority of natural foods in 1 : 1 ratio. Therefore, adequate intake of Ca generally takes care of the P requirement also.

Sources

Milk, cereals, leafy vegetables, meat, eggs.

Absorption

Phosphate absorption occurs from jejunum

1. Calcitriol promotes phosphate uptake along with calcium.

2. Absorption of phosphorus and calcium is optimum when the dietary Ca : P is between 1 : 2 and 2 : 1.

3. Acidity favours while phytate decreases phosphate uptake by intestinal cells.

Serum phosphate

The phosphate level of the whole blood is around 40 mg/dl while serum contains about 3-4 mg/dl. This is because the RBC and WBC have very high content of phosphate.

The serum phosphate may exist as free ions (40%) or in a complex form (50%) with cations such as Ca^{2+}, Mg^{2+}, Na^{+}, K^{+}. About 10% of serum phosphate is bound to proteins. It is interesting to note that the ***fasting serum phosphate*** levels are ***higher*** than the post-prandial. This is attributed to the fact that following the ingestion of carbohydrate (glucose), the phosphate from the serum is drawn by the cells for metabolism (phosphorylation reactions).

Excretion

About 500 mg phosphate is excreted in urine per day. The renal threshold is 2 mg/dl. The reabsorption of phosphate by renal tubules is inhibited by PTH.

Disease states

1. Serum phosphate level is increased in hypoparathyroidism and decreased in hyperparathyroidism.

2. In severe renal diseases, serum phosphate content is elevated causing acidosis.

3. Vitamin D deficient rickets is characterized by decreased serum phosphate (1–2 mg/dl).

4. Renal rickets is associated with low serum phosphate levels and increased alkaline phosphatase activity.

5. In diabetes mellitus, serum content of organic phosphate is lower while that of inorganic phosphate is higher.

MAGNESIUM

The adult body contains about 20 g magnesium, 70% of which is found in bones in combination with calcium and phosphorus. The remaining 30% occurs in the soft tissues and body fluids.

Biochemical functions

1. Magnesium is required for the ***formation of bones and teeth***.

2. Mg^{2+} serves as a cofactor for several enzymes requiring ATP e.g. hexokinase, glucokinase, phosphofructokinase, adenylate cyclase.

3. Mg^{2+} is necessary for proper neuromuscular function. Low Mg^{2+} levels lead to neuromuscular irritability.

Dietary requirements

Adult man — 350 mg/day
Adult woman — 300 mg/day

Sources

Cereals, nuts, beans, vegetables (cabbage, cauliflower), meat, milk, fruits.

Absorption

Magnesium is absorbed by the intestinal cells through a specific carrier system. About 50% of the dietary Mg is normally absorbed. Consumption of large amounts of calcium, phosphate and alcohol diminishes Mg absorption. PTH increases Mg absorption.

Serum Mg

Normal serum concentration of Mg is 2–3 mg/dl. It is present in the ionized form (60%), in combination with other ions (10%) and bound to proteins (30%).

Disease states

1. Magnesium deficiency causes neuromuscular irritation, weakness and convulsions.

These symptoms are similar to that observed in tetany (Ca deficiency) which are relieved only by Mg. Malnutrition, alcoholism and cirrhosis of liver may lead to Mg deficiency.

2. Low levels of Mg may be observed in uremia, rickets and abnormal pregnancy.

SODIUM

Sodium is the ***chief cation*** of the ***extracellular fluid***. About 50% of body sodium is present in the bones, 40% in the extracellular fluid and the remaining (10%) in the soft tissues.

Biochemical functions

1. In association with chloride and bicarbonate, sodium ***regulates*** the body's ***acid-base balance.***

2. Sodium is required for the maintenance of osmotic pressure and fluid balance.

3. It is necessary for the normal muscle irritability and cell permeability.

4. Sodium is involved in the intestinal absorption of glucose, galactose and amino acids.

5. It is necessary for initiating and maintaining heart beat.

Dietary requirements

For normal individuals, the requirement of sodium is about 5-10 g/day which is mainly consumed as NaCl. For persons with a family history of hypertension, the daily NaCl intake should be less than 5 g. For patients of hypertension, around 1 g/day is recommended. It may be noted that 10 g of NaCl contains 4 g of sodium. The daily consumption of Na is generally higher than required due to its flavour.

Sources

The common salt (NaCl) used in the cooking medium is the major source of sodium. The ingested foods also contribute to sodium. The good sources of sodium include bread, whole grains, leafy vegetables, nuts, eggs and milk.

Absorption

Sodium is readily absorbed in the gastrointestinal tract and, therefore, very little of it (< 2%) is normally found in feces. However, in diarrhea, large quantities of sodium is lost in feces.

Plasma sodium

In the plasma (serum), the normal concentration of sodium is ***135-145 mEq/l.*** Sodium is an ***extracellular cation,*** therefore, the blood cells contain much less (35 mEq/l). The mineralocorticoids, secreted by adrenal cortex, influence sodium metabolism. A decrease in plasma sodium and an increase in its urinary excretion are observed in adrenocortical insufficiency.

Excretion

Kidney is the major route of sodium excretion from the body. As much as 800 g Na/day is filtered by the glomeruli, 99% of this is reabsorbed by the renal tubules by an active process. This is controlled by ***aldosterone.*** Extreme sweating also causes considerable amount of sodium loss from the body. There is, however, individual variation in sodium loss through sweat.

Disease states

1. **Hyponatremia :** This is a condition in which the serum sodium level falls below the normal. Hyponatremia may occur due to diarrhea, vomiting, chronic renal diseases, adrenocortical insufficiency ***(Addison's disease).*** Administration of salt free fluids to patients may also cause hyponatremia. This is due to overhydration. Decreased serum sodium concentration is also observed in edema which occurs in cirrhosis or congestive heart failure.

The manifestations of hyponatremia include reduced blood pressure and circulatory failure.

2. **Hypernatremia :** This condition is characterized by an elevation in the serum sodium level. The symptoms include increase in

blood volume and blood pressure. It may occur due to hyperactivity of adrenal cortex ***(Cushing's syndrome)***, prolonged administration of cortisone, ACTH and/or sex hormones. Loss of water from the body causing dehydration, as it occurs in diabetes insipidus, results in hypernatremia. Rapid administration of sodium salts also increases serum sodium concentration. It may be noted that in pregnancy, steroid and placental hormones cause sodium and water retention in the body, leading to edema.

In ***edema***, along with ***water***, ***sodium*** concentration in the body is also ***elevated.*** Administration of diuretic drugs increases the urinary output of water along with sodium. In the patients of ***hypertension*** and congestive cardiac failure salt ***(Na^+) restriction*** is advocated.

POTASSIUM

Potassium is the principal ***intracellular cation.*** It is equally important in the extracellular fluid for specific functions.

Biochemical functions

1. Potassium maintains ***intracellular osmotic pressure***.

2. It is required for the regulation of acid-base balance and water balance in the cells.

3. The enzyme pyruvate kinase (of glycolysis) is dependent on K^+ for optimal activity.

4. Potassium is required for the transmission of nerve impulse.

5. Adequate intracellular concentration K^+ is necessary for proper biosynthesis of proteins by ribosomes.

6. Extracellular K^+ influences cardiac muscle activity.

Dietary requirements

About 3-4 g/day.

Sources

Banana, orange, pineapple, potato, beans, chicken, and liver. Tender coconut water is a rich source of potassium.

Absorption

The absorption of K^+ from the gastrointestinal tract is very efficient (90%) and very little is lost through feces. However, in subjects with diarrhea, a good proportion of K^+ is lost in the feces.

Plasma potassium

The plasma (serum) concentration of potassium is ***3.4-5.0 mEq/l.*** The whole blood contains much higher level of K^+ (50 mEq/l), since it is predominantly an intracellular cation. Care should, therefore, be taken to avoid hemolysis of RBC for the estimation of serum K^+.

Excretion

Potassium is mainly excreted through urine. The maintenance of body acid-base balance influences K^+ excretion. Aldosterone increases excretion of potassium.

Disease states

Serum potassium concentration is maintained within a narrow range. Either high or low concentrations are dangerous since potassium effects the contractility of heart muscle.

Hypokalemia : Decrease in the concentration of serum potassium is observed due to overactivity of adrenal cortex ***(Cushing's syndrome)***, prolonged cortisone therapy, intravenous administration of K^+-free fluids, treatment of diabetic coma with insulin, prolonged diarrhea and vomiting.

The symptoms of hypokalemia include irritability, muscular weakness, tachycardia, cardiomegaly and cardiac arrest. Changes in the ECG are observed (flattened waves with inverted T wave).

Hyperkalemia : Increase in the concentration of serum potassium is observed in renal failure,

adrenocortical insufficiency ***(Addison's disease)***, diabetic coma, severe dehydration, intravenous administration of fluids with excessive potassium salts.

The manifestations of hyperkalemia include depression of central nervous system, mental confusion, numbness, bradycardia with reduced heart sounds and, finally, cardiac arrest. Changes in ECG are also observed (elevated T wave).

CHLORINE

Chlorine is a constituent of sodium chloride. Hence, the metabolism of chlorine and sodium are intimately related.

Biochemical functions

1. Chloride is involved in the regulation of ***acid-base equilibrium***, fluid balance and osmotic pressure. These functions are carried out by the interaction of chloride with Na^+ and K^+.

2. Chloride is necessary for the formation of HCl in the gastric juice.

3. Chloride shift involves the active participation of Cl^-.

4. The enzyme salivary amylase is activated by chloride.

Dietary requirements

The daily requirement of chloride as NaCl is 5-10 g. Adequate intake of sodium will satisfy the chloride requirement of the body.

Sources

Common salt as cooking medium, whole grains, leafy vegetables, eggs and milk.

Absorption

In normal circumstances, chloride is almost totally absorbed in the gastrointestinal tract.

Plasma chloride

The normal plasma concentration of chloride is 95-105 mEq/l. Cerebrospinal fluid (CSF) contains higher level of Cl^- (125 mEq/l). This is due to the fact that protein content is low in CSF and, therefore, Cl^- is higher in order to maintain Donnan membrane equilibrium.

Excretion

There exists a parallel relationship between excretion of chloride and sodium. The renal threshold for Cl^- is about 110 mEq/l.

Disease states

1. **Hypochloremia :** A reduction in the serum Cl^- level may occur due to vomiting, diarrhea, respiratory alkalosis, Addison's disease and excessive sweating.

2. **Hyperchloremia :** An increase in serum Cl^- concentration may be due to dehydration, respiratory acidosis and Cushing's syndrome.

SULFUR

Sulfur of the body is mostly present in the organic form. Methionine, cysteine and cystine are the three sulfur-containing amino acids present in the proteins. Generally, proteins contain about 1% sulfur by weight.

Biochemical functions

1. Sulfur-containing amino acids are very essential for the structural conformation and biological functions of proteins (enzymes, hormones, structural proteins etc.). The disulfide linkages (−S−S−) and sulfhydryl groups (−SH) are largely responsible for this.

2. The vitamins thiamine, biotin, lipoic acid, and coenzyme A of pantothenic acid contain sulfur.

3. Heparin, chondroitin sulfate, glutathione, taurocholic acid are some other important sulfur-containing compounds.

4. Phosphoadenosine phosphosulfate (PAPS) is the active sulfate utilized for several reactions e.g. synthesis of glycosaminoglycans, detoxification mechanism.

5. The sulfur-containing amino acid methionine (as S–adenosylmethionine) is actively involved in transmethylation reactions.

Dietary requirements and sources

There is no specific dietary requirement for sulfur. Adequate intake of sulfur-containing essential amino acid methionine will meet the body needs. Food proteins rich in methionine and cysteine are the sources of sulfur.

Excretion

The sulfur from different compounds is oxidized in the liver to sulfate and excreted in urine. The urine contains inorganic sulfate (80%), organic or conjugated or ethereal sulfate (10%) and unoxidized sulfur (10%). The unoxidized sulfur is in the form of sulfur-containing amino acids, thiocyanates etc.

IRON

The total content of iron in an adult body is 3-5 g. About 70% of this occurs in the erythrocytes of blood as a constituent of hemoglobin. At least 5% of body iron is present in myoglobin of muscle. Heme is the most predominant iron-containing substance. It is a constituent of several proteins/enzymes ***(hemoproteins)***—hemoglobin, myoglobin, cytochromes, xanthine oxidase, catalase, tryptophan pyrrolase, peroxidase. Certain other proteins contain ***non-heme iron*** e.g. transferrin, ferritin, hemosiderin.

Biochemical functions

1. Iron mainly exerts its functions through the compounds in which it is present. Hemoglobin and myoglobin are required for the transport of O_2 and CO_2.

2. Cytochromes and certain non-heme proteins are necessary for electron transport chain and oxidative phosphorylation.

3. Peroxidase, the lysosomal enzyme, is required for phagocytosis and killing of bacteria by neutrophils.

4. Iron is associated with effective immunocompetence of the body.

Dietary requirements

Adult man	— 10 mg/day
Menstruating woman	— 18 mg/day
Pregnant and lactating woman	— 40 mg/day

Sources

Rich sources	—	Organ meats (liver, heart, kidney).
Good sources	—	Leafy vegetables, pulses, cereals, fish, apples, dried fruits, molasses.
Poor sources	—	Milk, wheat, polished rice.

Absorption, transport and storage

Iron is mainly absorbed in the stomach and duodenum. In normal people, ***about 10%*** of dietary iron is usually ***absorbed***. However, in iron deficient (anemic) individuals and growing children, a much higher proportion of dietary iron is absorbed to meet the increased body demands.

Iron is mostly found in the foods in ferric form (Fe^{3+}), bound to proteins or organic acids. In the acid medium provided by gastric HCl, the Fe^{3+} is released from foods. Reducing substances such as ascorbic acid (vitamin C) and cysteine convert ferric iron (Fe^{3+}) to ferrous form (Fe^{2+}). Iron in the ***ferrous form is soluble and readily absorbed.***

Factors affecting Fe absorption

1. Acidity, ascorbic acid and cysteine promote iron absorption.

2. In iron deficiency anemia, Fe absorption is increased to 2-10 times that of normal.

3. Small peptides and amino acids favour iron uptake.

4. Phytate (found in cereals) and oxalate (found in leafy vegetables) interfere with Fe absorption.

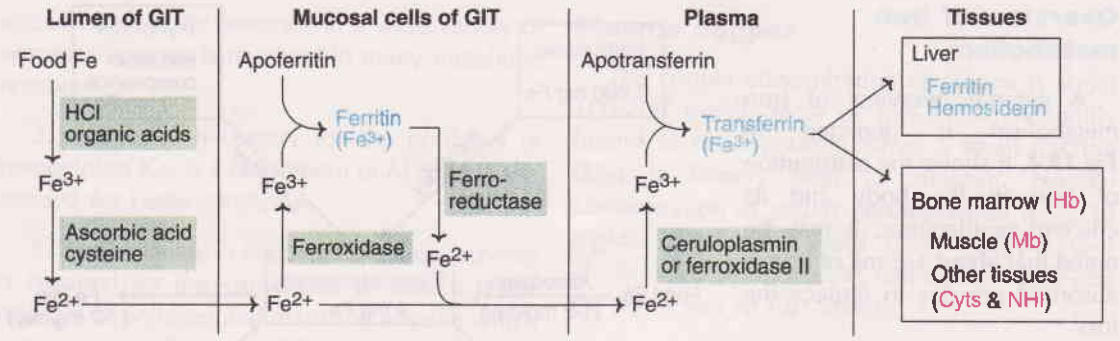


***Fig. 18.3** : Iron absorption and transport (GIT–Gastrointestinal tract; Hb–Hemoglobin; Mb–Myoglobin; Cyts–Cytochromes; NHI–Non-heme iron).*

5. A diet with high phosphate content decreases Fe absorption while low phosphate promotes.

6. Impaired absorption of iron is observed in malabsorption syndromes such as steatorrhea.

7. In patients with partial or total surgical removal of stomach and/or intestine, iron absorption is severely impaired.

Iron in the mucosal cells : The iron (Fe^{2+}) entering the mucosal cells by absorption is oxidized to ferric form (Fe^{3+}) by the enzyme ferroxidase. Fe^{3+} then combines with apoferritin to form ferritin which is the temporary storage form of iron. From the mucosal cells, iron may enter the blood stream (which mainly depends on the body needs) or lost when the cells are desquamated.

Transport of Fe in the plasma : The iron liberated from the ferritin of mucosal cells enters the plasma in ferrous state. Here, it is oxidized to ferric form by a copper-containing protein, ceruloplasmin which possesses ferroxidase activity. Another cuproprotein ferroxidase II also helps for the conversion of Fe^{2+} to Fe^{3+}.

Ferric iron then binds with a specific iron-binding protein, namely ***transferrin*** or siderophilin (a glycoprotein with mol. wt. 90,000). Each transferrin molecule can bind with *two atoms of ferric iron (Fe^{3+}). The plasma* transferrin (concentration 250 mg/dl) can bind with 400 mg of iron/dl plasma. This is known as ***total iron binding capacity (TIBC)*** *of plasma.*

Storage of iron : Iron is stored in liver, spleen and bone marrow in the form of ***ferritin***. In the mucosal cells, ferritin is the temporary storage form of iron. A molecule of apoferritin (mol. wt. 500,000) can combine with 4,000 atoms of iron. The maximum iron content of ferritin on weight basis is around 25%.

Hemosiderin is another iron storage protein which can hold about 35% of iron by weight. Hemosiderin accumulates in the body (spleen, liver) when the supply of iron is in excess of body demands.

Iron is a one-way substance

Iron metabolism is unique as it operates in a closed system. It is very efficiently ***utilized and reutilized by the body***. Further, iron losses from the body are minimal (< 1 mg/day) which may occur through bile, sweat, hair loss etc. Iron is ***not excreted into urine.*** Thus, iron differs from the vitamins or other organic and inorganic substances which are either inactivated or excreted during the course of metabolic function. Hence, iron is appropriately regarded as a one-way substance.

Iron entry into the body is controlled at the absorption level, depending on the body needs. *Thus the periodical blood loss in menstruating* women increases its requirements. Increased iron demands are also observed in pregnancy, *lactation, and in growing children.*

Overview of iron metabolism

A general overview of iron metabolism is depicted in ***Fig.18.4***. It shows the distribution of iron in the body and its efficient reutilization. It may be noted that about 1-2 mg of iron is absorbed per day to replace the loss.

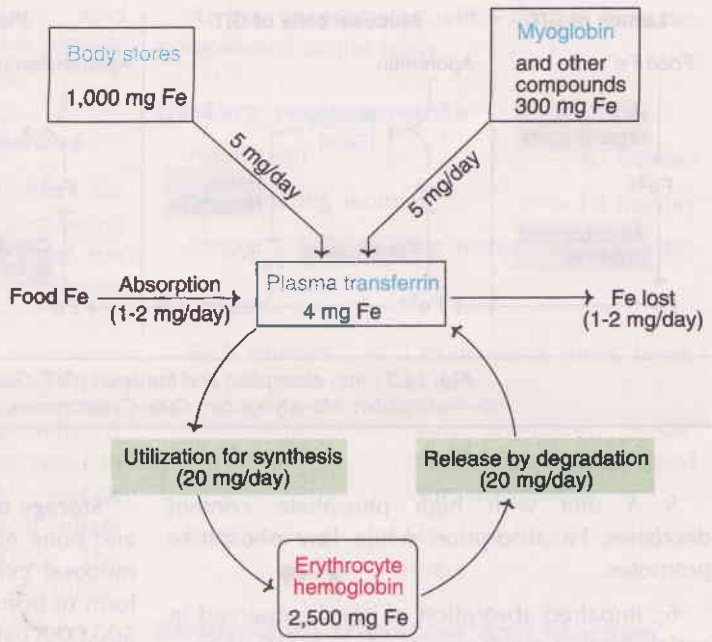


Fig. 18.4 : A general overview of iron metabolism.

Disease states

1. **Iron deficiency anemia :** This is the most prevalent nutritional disorder worldover, including the well developed countries (e.g. USA). Several factors may contribute to iron deficiency anemia. These include inadequate intake or defective absorption of iron, chronic blood loss, repeated pregnancies and hookworm infections.

Strict vegetarians are more prone for iron deficieny anemia. This is due to the presence of inhibitors of iron absorption in the vegetarian foods, besides the relatively low content of iron.

Iron deficiency anemia mostly occurs in growing children, adolescent girls, pregnant and lactating women. It is characterized by ***microcytic hypochromic anemia*** with reduced blood hemoglobin levels (<12 g/dl). The other manifestations include apathy (dull and inactive), sluggish metabolic activities, retarded growth and loss of appetite.

2. **Hemosiderosis :** This is a less common disorder and is due to ***excessive iron in the body***. It is commonly observed in subjects receiving repeated blood transfusions over the years, e.g. patients of hemolytic anemia, hemophilia. As already stated, iron is a one-way compound, once it enters the body, it cannot escape. Excessive iron is deposited as ferritin and hemosiderin.

Hemosiderosis is commonly observed among the Bantu tribe in South Africa. This is attributed to a high intake of iron from their staple diet corn and their habit of cooking foods in iron pots.

3. **Hemochromatosis :** This is a rare disease in which ***iron is directly deposited in the tissues*** (liver, spleen, pancreas and skin). Hemosiderosis is sometimes accompanied by hemochromatosis. Bronzed-pigmentation of the skin, cirrhosis of liver, pancreatic fibrosis are the manifestations of this disorder. Hemochromatosis causes a condition known as ***bronze diabetes***.

COPPER

The body contains about 100 mg copper distributed in different organs. It is involved in several important functions.

Biochemical functions

1. Copper is an essential constituent of several enzymes. These include cytochrome oxidase, catalase, tyrosinase, superoxide dismutase, monoamine oxidase, ascorbic acid oxidase, ALA synthase, phenol oxidase and

uricase. Due to its presence in a wide variety of enzymes, copper is involved in many metabolic reactions.

2. Copper is necessary for the synthesis of hemoglobin (Cu is a constituent of ALA synthase, needed for heme synthesis).

3. Lysyl oxidase (a copper-containing enzyme) is required for the conversion of certain lysine residues of collagen and elastin to allysine which are necessary for cross-linking these structural proteins.

4. Ceruloplasmin serves as ferroxidase and is involved in the conversion of iron from Fe^{2+} to Fe^{3+} in which form iron (transferrin) is transported in plasma.

5. Copper is necessary for the synthesis of melanin and phospholipids.

6. Development of bone and nervous system (myelin) requires Cu.

7. Certain copper-containing non-enzymatic proteins have been identified, although their functions are not clearly known. These include hepatocuprein (storage form in liver), cerebrocuprein (in brain) and hemocuprein (in RBC).

8. Hemocyanin, a copper protein complex in invertebrates, functions like hemoglobin for O_2 transport.

Dietary requirements

Adults — 2-3 mg/day

Infants and children — 0.5-2 mg/day

Sources

Liver, kidney, meat, egg yolk, cereals, nuts and green leafy vegetables. Milk is a poor source.

Absorption

About 10% of dietary copper is absorbed, mainly in the duodenum. ***Metallothionein*** is a transport protein that facilitates copper absorption. Phytate, zinc and molybdenum decrease copper uptake.

Plasma copper

The copper concentration of plasma is about 100-200 mg/dl. Most of this (95%) is tightly bound to ceruloplasmin while a small fraction (5%) is loosely held to albumin. Normal concentration of serum ***ceruloplasmin*** is 25-50 mg/dl. It contains about 0.34% copper (6-8 atoms of Cu per molecule, half in Cu^{2+} state and the other half in Cu^{+} state).

Disease states

1. **Copper deficiency :** Severe deficiency of copper causes demineralization of bones, demyelination of neural tissue, anemia, fragility of arteries, myocardial fibrosis, hypopigmentation of skin, greying of hair.

2. **Menke's disease :** This disorder is due to a defect in the intestinal absorption of copper. It is possible that copper may be trapped by metallothionein in the intestinal cells. The symptoms of Menke's disease include decreased copper in plasma and urine, anemia and depigmentation of hair.

3. **Wilson's disease (hepatolenticular degeneration) :** It is a rare disorder of abnormal copper metabolism and is characterized by the following manifestations.

- Copper is deposited in abnormal amounts in liver and lenticular nucleus of brain. This may lead to hepatic cirrhosis and brain necrosis.
- Low levels of copper and ceruloplasmin in plasma with increased excretion of copper in urine.
- Copper deposition in kidney causes renal damage. This leads to increased excretion of amino acids, glucose, peptides and hemoglobin in urine.
- Intestinal absorption of copper is very high, about 4-6 times higher than normal.

Probable causes of Wilson's disease : The following explanations are offered to understand the etiology of this disease.

1. A failure to synthesize ceruloplasmin or an impairment in the binding capacity of copper to

this protein or both. As a result of this, copper is free in the plasma which easily enters the tissues (liver, brain, kidney), binds with the proteins and gets deposited. The albumin bound copper is either normal or increased.

2. Some workers suggest that a reduced intestinal excretion of copper may be responsible for the occurrence of Wilson's disease.

Treatment : Administration of penicillamine, a naturally occurring copper chelating agent, is used for the treatment of Wilson's disease.

IODINE

The total body contains about 20 mg iodine, most of it (80%) being present in the ***thyroid gland***. Muscle, salivary glands and ovaries also contain some amount of iodine.

Biochemical functions

The only known function of iodine is its requirement for the synthesis of thyroid hormones namely, ***thyroxine*** (T_4) and ***triiodothyronine*** (T_3). These hormones are involved in several biochemical functions (***Chapter 19***). Functionally, T_3 is more active than T_4.

Dietary requirements

Adults	—	100-150 µg/day
Pregnant women	—	200 µg/day

Sources

Seafoods, drinking water, vegetables, fruits (grown on seaboard). High altitudes are deficient in iodine content in water as well as soil. Plant and animal foods of these areas, therefore, contain lesser amount of iodine. In these regions, iodine is added to drinking water or to table salt.

Absorption, storage and excretion

Iodine as iodide is mainly absorbed from the small intestine. Normally, about 30% of dietary iodine is taken up by the intestinal cells. Iodine absorption also occurs through skin and lungs.

About 80% of body's iodine is stored in the organic form as ***iodothyroglobulin*** (a glycoprotein) in the thyroid gland. This protein contains thyroxine, diiodotyrosine and triiodothyronine in different proportions.

Excretion of iodine mostly occurs through kidney. It is also excreted through saliva, bile, skin, and milk (in lactating women).

Plasma iodine

The normal concentration of plasma iodine is 4-10 mg/dl. Most of this is present as ***protein bound iodine (PBI)*** and represents the iodine contained in the circulating thyroid hormones. PBI level decreases in hypothyroidism and increases in hyperthyroidism. RBC do not contain iodine.

Disease states

The disorders of iodine metabolism—***simple goiter*** and ***toxic goiter***—are discussed in detail under thyroid hormones (***Chapter 19***).

MANGANESE

The total body content of manganese is about 15 mg. The liver and kidney are rich in Mn. Within the cells, Mn is mainly found in the nuclei in association with nucleic acids.

Biochemical functions

1. Mn serves as a ***cofactor*** for several ***enzymes***. These include arginase, pyruvate carboxylase, isocitrate dehydrogenase, superoxide dismutase (mitochondrial) and peptidase.

2. Mn is required for the formation of bone, proper reproduction and normal functioning of nervous system.

3. Mn is necessary for the synthesis of mucopolysaccharides and glycoproteins.

4. Hemoglobin synthesis involves Mn.

5. Mn inhibits lipid peroxidation.

6. Mn is necessary for cholesterol biosynthesis.

Dietary requirements

The exact requirement of Mn is not known. About 2-9 mg/day is recommended for an adult.

Sources

Cereals, nuts, leafy vegetables and fruits. Tea is a rich source of Mn.

Absorption

About 3-4% of dietary Mn is normally absorbed in the small intestine. Iron inhibits Mn absorption.

Serum Mn

Manganese in the serum is bound to a specific carrier protein—***transmagnanin*** (a β-globulin). The normal blood contains about 5-20 mg/dl.

Disease states

Mn deficiency in animals causes

1. Retarded growth, bone deformities and, in severe deficiency, sterility.

2. Accumulation of fat in liver.

3. Increased activity of serum alkaline phosphatase, and

4. Diminished activity of β-cells of pancreas (low insulin).

ZINC

The total content of zinc in an adult body is about 2 g. ***Prostate gland*** is very ***rich*** in Zn (100 mg/g). Zinc is mainly an intracellular element.

Biochemical functions

1. Zn is an essential component of several enzymes e.g. carbonic anhydrase, alcohol dehydrogenase, alkaline phosphatase, carboxypeptidase, superoxide dismutase (cytosolic).

2. Zinc may be regarded as an ***antioxidant*** since the enzyme superoxide dismutase (Zn containing) protects the body against free radical damage.

3. The storage and secretion of insulin from the β-cells of pancreas require Zn.

4. Zn is necessary to maintain the normal levels of vitamin A in serum. Zn promotes the synthesis of retinol binding protein.

5. It is required for wound healing. Zn enhances cell growth and division, besides stabilizing biomembranes.

6. ***Gusten,*** a zinc containing protein of the saliva, is important for taste sensation.

7. Zn is essential for proper reproduction.

Dietary requirements

Zinc requirement for an adult is 10-15 mg/day. It is increased (by about 50%) in pregnancy and lactation.

Sources

Meat, fish, eggs, milk, beans, nuts.

Absorption

Zinc is absorbed mainly in the duodenum. Zn from the animal sources is better absorbed than the vegetable sources. Zn absorption appears to be dependent on a transport protein—***metallothionein***. Phytate, calcium, copper and iron interfere while small peptides and amino acids promote Zn absorption.

Serum Zn

The concentration of Zn in serum is about 100 mg/dl. Erythrocytes contain higher content of Zn (1.5 mg/dl) which is found in association with the enzyme carbonic anhydrase.

Disease states

1. Zinc deficiency is associated with growth retardation, poor wound healing, anemia, loss of appetite, loss of taste sensation, impaired spermatogenesis etc. It is reported that Zn deficiency in pregnant animals causes congenital malformations of the fetus. Deficiency of Zn may result in depression, dementia and other psychiatric disorders. The neuropsychiatric manifestations of chronic alcoholism may be partly due to zinc deficiency.

Acrodermatitis enteropathica is a rare inherited metabolic disease of zinc deficiency caused by a defect in the absorption of Zn from the intestine.

2. Zinc toxicity is often observed in welders due to inhalation of zinc oxide fumes. The manifestations of Zn toxicity include nausea, gastric ulcer, pancreatitis, anemia and excessive salivation.

MOLYBDENUM

Molybdenum is a constituent of the enzymes ***xanthine oxidase***, aldehyde oxidase and sulfite oxidase. Nitrite reductase (containing Mo) is a plant enzyme, required for nitrogen fixation.

The requirements of Mo are not clearly known. However, it is widely distributed in the natural foods. Dietary Mo is effectively (60%-70%) absorbed by the small intestine.

Some workers have reported that Mo decreases the mobilization and utilization of copper in the body.

Molybdenosis is a rare disorder caused by excessive consumption of Mo. Its manifestations include impairment in growth, diarrhea and anemia. Intestinal absorption of copper is diminished.

COBALT

Cobalt is only important as a constituent of ***vitamin B_{12}***. Cobalt content of vitamin B_{12} is about 4% by weight. The functions of cobalt are the same as that of vitamin B_{12} (***Chapter 7***). Administration of cobalt stimulates the production of the hormone erythropoietin, which promotes erythropoiesis.

Prolonged administration of cobalt is toxic as it results in polycythemia (increased RBC in blood).

FLUORINE

Fluoride is mostly found in bones and teeth. The beneficial effects of fluoride in trace amounts are overshadowed by its harmful effects caused by excess consumption.

Biochemical functions

1. Fluoride ***prevents*** the development of ***dental caries***. It forms a protective layer of acid resistant fluoroapatite with hydroxyapatite of the enamel and prevents the tooth decay by bacterial acids. Further, fluoride inhibits the bacterial enzymes and reduces the production of acids.

2. Fluoride is necessary for the proper development of bones.

3. It inhibits the activities of certain enzymes. Sodium fluoride inhibits enolase (of glycolysis) while fluoroacetate inhibits aconitase (of citric acid cycle).

Dietary requirements and sources

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

Disease states

1. **Dental caries :** It is clearly established that drinking water containing less than 0.5 ppm of fluoride is associated with the development of dental caries in children.

2. **Fluorosis :** Excessive intake of fluoride is harmful to the body. An intake above 2 ppm (particularly > 5 ppm) in children causes mottling of enamel and discoloration of teeth. The teeth are weak and become rough with characteristic brown or yellow patches on their surface. These manifestations are collectively referred to as dental fluorosis.

An intake of fluoride above 20 ppm is toxic and causes pathological changes in the bones. Hypercalcification, increasing the density of the bones of limbs, pelvis and spine, is a characteristic feature. Even the ligaments of spine

and collagen of bones get calcified. Neurological disturbances are also commonly observed. The manifestations described here constitute ***skeletal fluorosis***. In the advanced stages, the individuals are crippled and cannot perform their daily routine work due to stiff joints. This condition of advanced fluorosis is referred to as ***genu valgum***.

The fluoride content of water in some parts of Andhra Pradesh, Punjab and Karnataka is quite high. Fluorosis is prevalent in these regions, causing concern to government and health officials.

3. **Fluoridation of water and use of fluoride tooth-pastes :** In order to prevent the dental caries in children, some advanced countries like USA have started fluoridation of water. Further, the consumer markets till recently were flooded with fluoride toothpastes. There is some rethinking on these aspects due to the toxic effects of excess fluoride.

SELENIUM

Selenium was originally identified as an element that causes toxicity to animals (alkali disease) in some parts of USA, containing large amounts of Se in the soil. Later work, however, has shown that Se in smaller amounts is biologically important.

Biochemical functions

1. Selenium, along ***with vitamin E***, prevents the development of hepatic necrosis and muscular dystrophy.

BIOMEDICAL / CLINICAL CONCEPTS

- *Serum calcium level is increased (normal 9–11 mg/dl) in hyperparathyroidism. This condition is also associated with elevated urinary excretion of Ca and P, often leading to stone formation.*
- *Tetany, caused by a drastic reduction in serum Ca, is characterized by neuromuscular irritability and convulsions.*
- *Rickets is due to defective calcification of bones. This may be caused by deficiency of Ca and P or vitamin D or both.*
- *Osteoporosis is the bone disorder of the elderly, characterized by demineralization resulting in a progressive loss of bone mass. It is the major cause of bone fractures and disability in the old people.*
- *Decreased levels of serum Na (hyponatremia) is observed in diarrhea and vomiting, besides Addison's disease, while increased serum Na (hypernatremia) is found in Cushing's syndrome.*
- *Iron deficiency anemia is the most prevalent nutritional disorder worldover. It is most commonly observed in pregnant and lactating women.*
- *Wilson's disease is due to an abnormal copper metabolism. It is characterized by abnormal deposition of copper in liver and brain, besides the low levels of plasma copper and ceruloplasmin.*
- *Endemic goitre, due to dietary iodine deficiency, is very common. Consumption of iodized salt is advocated to overcome this problem.*
- *Fluorosis is caused by an excessive intake of fluoride. The manifestations include mottling of enamel and discoloration of teeth. In the advanced stages, hypercalcification of limb bones and ligaments of spine get calcified, ultimately crippling the individual.*

2. Se is involved in maintaining structural integrity of biological membranes.

3. Se as ***selenocysteine*** is an essential component of the enzyme glutathione peroxidase. This enzyme protects the cells against the damage caused by H_2O_2. It appears from recent studies that selenocysteine is directly incorporated during protein biosynthesis. Therefore, selenocysteine is considered as a separate (21st) amino acid.

4. Se prevents lipid peroxidation and protects the cells against the free radicals, including superoxide (O_2^-).

5. Se protects animals from carcinogenic chemicals. However, the precise role of Se in humans with regard to cancer prevention is not clearly identified.

6. Se binds with certain heavy metals (Hg, Cd) and protects the body from their toxic effects.

7. A selenium containing enzyme ***5'-deiodinase*** converts thyroxine (T4) to triiodothyronine in the thyroid gland.

8. ***Thioredoxin reductase***, involved in purine nucleotide metabolism, is also a selenoprotein.

Requirements and sources

A daily intake of 50-200 mg of Se has been recommended for adults. The good sources of Se are organ meats (liver, kidney) and sea foods.

Disease states

Deficiency : Se deficiency in animals leads to muscular dystrophy, pancreatic fibrosis and reproductive disorders. In humans, ***Keshan disease***, an endemic cardiomyopathy (in China) is attributed to the deficiency of Se. Epidemiological studies reveal that low serum Se levels are associated with increased risk of cardiovascular disease, and various cancers.

Toxicity

Selenosis is the toxicity due to very excessive intake of Se. The manifestations of selenosis include weight loss, emotional disturbances, diarrhea, hair loss and garlic odor in breath. The compound ***dimethyl selenide*** is responsible for the ***garlic odor***.

CHROMIUM

The total human body contains about 6 mg chromium. The Cr content of blood is about 20 mg/dl. Cr performs several biochemical functions.

1. In association with insulin, Cr promotes the utilization of glucose. Cr is a component of a protein namely ***chromodulin*** which facilitates the binding of insulin to cell receptor sites.

2. Cr lowers the total serum cholesterol level.

3. It is involved in lipoprotein metabolism. Cr decreases serum low density lipoproteins (LDL) and increases high density lipoproteins (HDL) and, thus, promotes health.

4. It is believed that Cr participates in the transport of amino acids into the cells (heart and liver).

The dietary requirement of Cr is not known. It is estimated that an adult man consumes about 10 to 100 mg/day. The good sources of Cr include brewer's yeast, grains, cereals, cheese and meat.

Chromium deficiency causes disturbances in carbohydrate, lipid and protein metabolisms. Excessive intake of Cr results in toxicity, leading to liver and kidney damage.

SUMMARY

1. *The minerals or inorganic elements are required for normal growth and maintenance of the body. They are classified as principal elements and trace elements. There are seven principal elements—Ca, P, Mg, Na, K, Cl and S. The trace elements include Fe, Cu, I, Zn, Mn, Mo, Co, F, Se and Cr.*

2. *Calcium is required for the development of bones and teeth, muscle contraction, blood coagulation, nerve transmission etc. Absorption of Ca from the duodenum is promoted by vitamin D, PTH and acidity while it is inhibited by phytate, oxalate, free fatty acids and fiber. The normal level of serum Ca (9-11 mg/dl) is controlled by an interplay of PTH, calcitriol and calcitonin.*

3. *Serum Ca level is elevated in hyperparathyroidism and diminished in hypoparathyroidism. Hypocalcemia causes tetany, the symptoms of which include neuromuscular irritability, spasm and convulsions.*

4. *Phosphorus, besides being essential for the development of bones and teeth, is a constituent of high energy phosphate compounds (ATP, GTP) and nucleotide coenzymes (NAD^+, $NADP^+$).*

5. *Sodium, potassium and chlorine are involved in the regulation of acid-base equilibrium, fluid balance and osmotic pressure in the body. Sodium is the principal extracellular cation (serum level 135-145 mEq/l), while potassium is the chief intracellular cation (serum level 3.5-5.0 mEq/l).*

6. *Iron is mainly required for O_2 transport and cellular respiration. Absorption of iron is promoted by ascorbic acid, cysteine, acidity and small peptides while it is inhibited by phytate, oxalate and high phosphate.*

7. *Iron (Fe^{3+}) is transported in the plasma in a bound form to transferrin. It is stored as ferritin in liver, spleen and bone marrow. Iron deficiency anemia causes microcytic hypochromic anemia. Excessive consumption of iron results in hemosiderosis which is due to the tissue deposition of hemosiderin.*

8. *Copper is an essential constituent of several enzymes (e.g catalase, cytochrome oxidase, tyrosinase). Ceruloplasmin is a copper containing protein required for the transport of iron (Fe^{3+}) in the plasma. Wilson's disease is an abnormality in copper metabolism, characterized by the deposition of copper in liver, brain and kidney.*

9. *Iodine is important as a component of thyroid hormones (T_4 and T_3) while cobalt is a constituent of vitamin B_{12}. Zinc is necessary for the storage and secretion of insulin and maintenance of normal vitamin A levels in serum, besides being a component of several enzymes (e.g. carbonic anhydrase, alcohol dehydrogenase).*

10. *Fluorine in trace amounts (<2 ppm) prevents dental caries while its higher intake leads to fluorosis. Selenium is assigned an antioxidant role as it protects the cells from free radicals. Chromium promotes the utilization of glucose and reduces serum cholesterol.*

SELF-ASSESSMENT EXERCISES

I. Essay questions

1. Write briefly on the trace elements and their metabolism in the body.
2. Discuss the biochemical functions, dietary requirements, sources and absorption of calcium.
3. Write an essay on the iron metabolism in the body.
4. Describe the metabolism of copper, zinc and manganese.
5. Write on the biochemical importance and disease states of fluorine and selenium.

II. Short notes

(a) Homeostasis of calcium, (b) Osteoporosis, (c) Phosphorus, (d) Sodium and chlorine, (e) Potassium, (f) Factors affecting Fe absorption, (g) Hemosiderosis, (h) Wilson's disease, (i) Iodine, (j) Magnesium.

III. Fill in the blanks

1. The normal concentration of serum calcium _____.
2. The vitamin derived hormone that regulates calcium homeostasis _____.
3. The inorganic element found in the structure of majority of high-energy compounds _____.
4. Several kinase enzymes require the mineral cofactor _____.
5. The principal cation of extracellular fluid _____.
6. The normal concentration of serum potassium _____.
7. Iron is transported in the plasma in a bound form to a protein _____.
8. The copper containing protein involved for the conversion of ferrous iron (Fe^{2+}) to ferric iron (Fe^{3+}) in the plasma _____.
9. The zinc containing protein in the saliva involved in taste sensation _____.
10. The element involved in the protection of cells against the damage of H_2O_2 and other free radicals _____.

IV. Multiple choice questions

11. The following substance(s) is(are) involved in the regulation of plasma calcium level

    (a) Calcitriol (b) Parathyroid hormone (c) Calcitonin (d) All of them.

12. The following is a sulfur containing essential amino acid

    (a) Methionine (b) Cysteine (c) Cystine (d) All of them.

13. Iron in the mucosal cells binds with the protein

    (a) Transferrin (b) Ferritin (c) Ceruloplasmin (d) Hemosiderin.

14. The following element is involved in wound healing

    (a) Calcium (b) Sodium (c) Zinc (d) Magnesium.

15. Pick up element that prevents the development of dental caries

    (a) Fluorine (b) Calcium (c) Phosphorus (d) Sodium.