Chapter-8

Metabolism (Study of cycle/pathways without chemical structures)

(BIOCHEMISTRY & CLINICAL PATHOLOGY)

Metabolism (Study of cycle/pathways without chemical structures)

Unit-1

- **Metabolism of Carbohydrates: Glycolysis, TCA cycle and glycogen metabolism, regulation of blood glucose level. Diseases related to abnormal metabolism of Carbohydrates.**

Unit-2

- **Metabolism of lipids:** Lipolysis, β-oxidation of Fatty acid (Palmitic acid) ketogenesis and ketolysis. Diseases related to abnormal metabolism of lipids such as Ketoacidosis, Fatty liver, Hypercholesterolemia

Unit-3

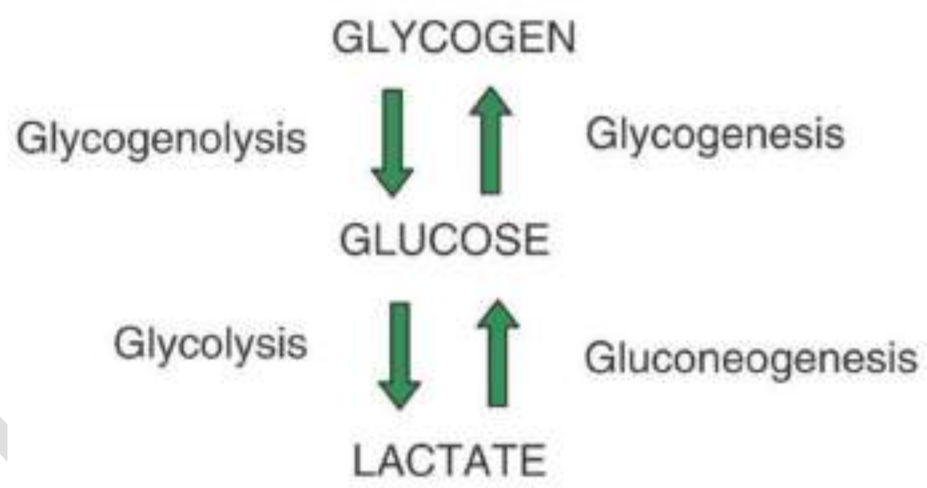
- **Metabolism of Amino acids (Proteins):** General reactions of amino acids and its significance– Transamination, deamination, Urea cycle and decarboxylation. Diseases related to abnormal metabolism of amino acids, Disorders of ammonia metabolism, phenylketonuria, alkaptonuria and Jaundice.
- **Biological oxidation:** Electron transport chain and Oxidative phosphorylation

Unit-1

Metabolism of carbohydrates.

Introduction—The major pathways of carbohydrate metabolism either begin or end with glucose. An understanding of the pathways and their regulation is necessary because of the important role played by glucose in the body. Glucose is the major form in which carbohydrate absorbed from the intestinal tract is presented to cells of the body. Glucose is the only fuel used to any significant extent by a few specialized cells and the major fuel used by the brain. Indeed, glucose is so important to these specialized cells and the brain that several of the major tissues of the body work together to ensure a continuous supply of this essential substrate.

Relationship of glucose to the major pathways of carbohydrate metabolism.

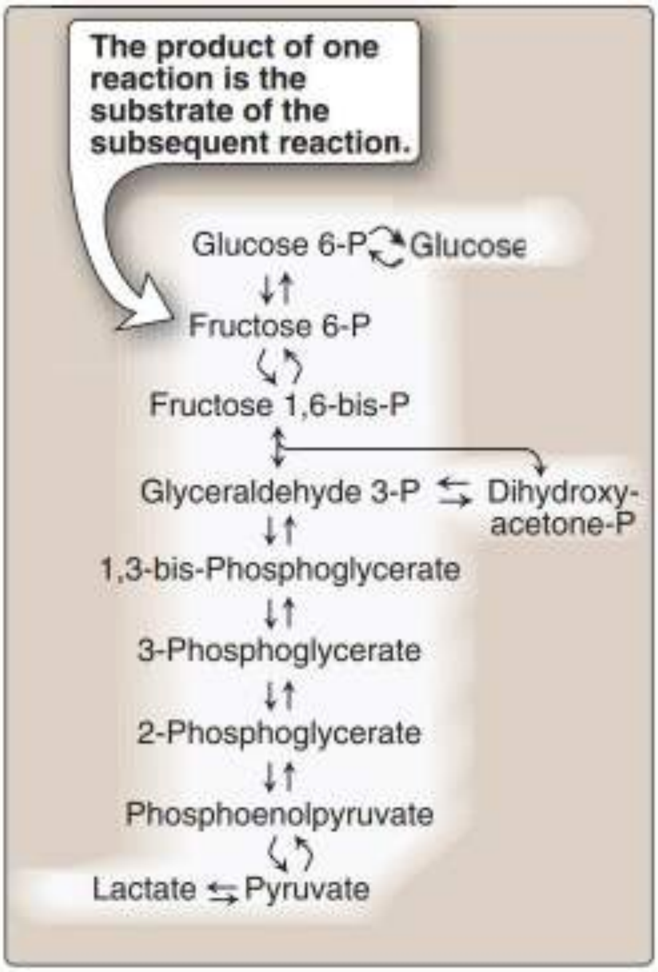


Glycolysis

- Glycolysis, a pathway used by all cells of the body to extract part of the chemical energy inherent in the glucose molecule. This pathway also converts glucose to pyruvate and sets the stage for complete oxidation of glucose to CO_2 and H_2O.

- In contrast to glycolysis, which produces ATP, gluconeogenesis requires ATP and is therefore an energy requiring process.

Metabolic pathway of glycolysis.



Reactions of glycolysis.

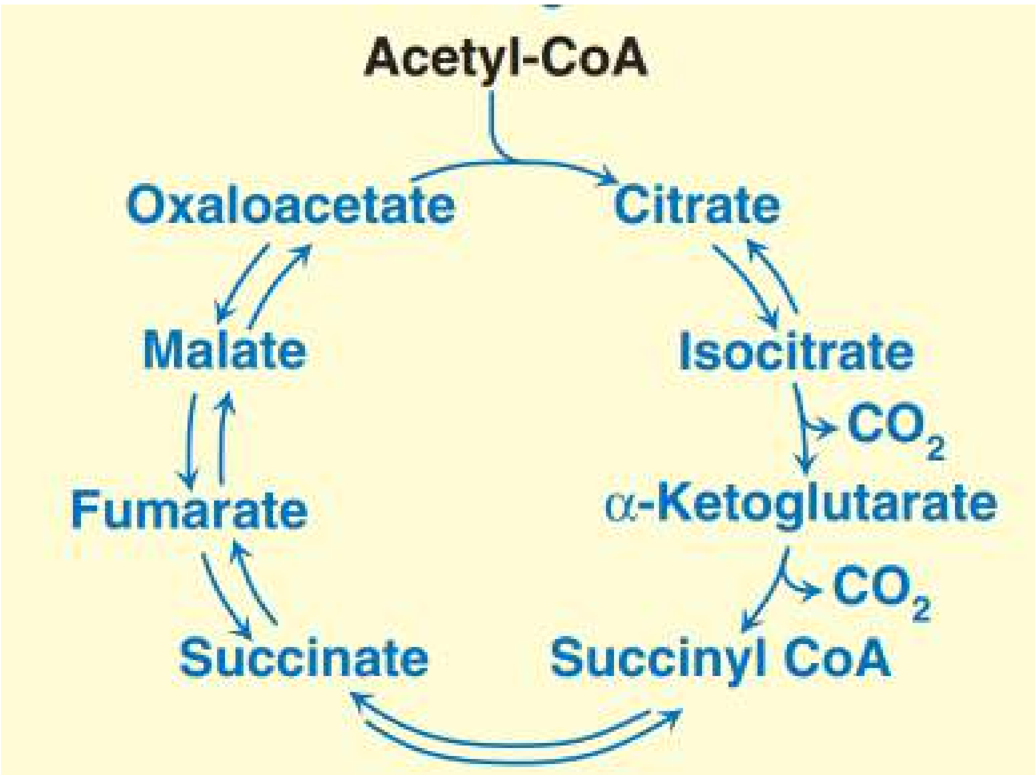
The conversion of glucose to pyruvate occurs in two stages. The first five reactions of glycolysis correspond to an energy investment phase in which the phosphorylated forms of intermediates are synthesized at the expense of ATP. The subsequent reactions of glycolysis constitute an energy generation phase in which a net of two molecules of ATP are formed by substrate-level phosphorylation per glucose molecule metabolized.

1. **Phosphorylation of glucose—** Phosphorylated sugar molecules do not readily penetrate cell membranes, because there are no specific transmembrane carriers for these compounds, and because they are too polar to diffuse through the lipid core of membranes. Mammals have several isozymes of the enzyme hexokinase that catalyse the phosphorylation of glucose to glucose 6-phosphate.
2. **Isomerization of glucose 6-phosphate—**The isomerization of glucose 6-phosphate to fructose 6-phosphate is catalysed by phosphoglucose isomerase.
3. **Phosphorylation of fructose 6-phosphate—** The irreversible phosphorylation reaction catalysed by phosphor fructokinase-1 (PFK-1) is the most important control point and the rate-limiting and committed step of glycolysis.
4. **Cleavage of fructose 1,6-bisphosphate—**Aldolase cleaves fructose 1,6-bisphosphate to dihydroxy acetone phosphate and glyceraldehyde 3-phosphate.
5. **Isomerization of dihydroxyacetone phosphate—**Triose phosphate isomerase interconverts dihydroxyacetone phosphate and glyceraldehyde 3-phosphate (see Figure 8.16). Dihydroxy -acetone phosphate must be isomerized to glyceraldehyde 3-phosphate for further metabolism by the glycolytic pathway. This isomerization results in the net production of two molecules of glyceraldehyde 3-phosphate from the cleavage products of fructose 1,6-bisphosphate
6. **Oxidation of glyceraldehyde 3-phosphate—**The conversion of glyceraldehyde 3-phosphate to 1,3-bisphosphoglycerate by glyceraldehyde 3-phosphate dehydrogenase is the first oxidation-reduction reaction of glycolysis.
7. **Synthesis of 3-phosphoglycerate producing ATP—**When 1,3-BPG is converted to 3-phosphoglycerate, the high-energy phosphate group of 1,3-BPG is used to synthesize ATP from ADP. This reaction is catalysed by phosphoglycerate kinase.
8. **Shift of the phosphate group from carbon 3 to carbon 2—**The shift of the phosphate group from carbon 3 to carbon 2 of phosphoglycerate-by-phosphoglycerate mutase is freely reversible
9. **Dehydration of 2-phosphoglycerate—** The dehydration of 2-phosphoglycerate by enolase redistributes the energy within the 2-phosphoglycerate molecule, resulting in the formation of phosphoenolpyruvate (PEP), which contains a high energy enol phosphate

10. **Formation of pyruvate producing ATP—**The conversion of PEP to pyruvate is catalysed by pyruvate kinase, the third irreversible reaction of glycolysis. The equilibrium of the pyruvate kinase reaction favours the formation of ATP
11. **Reduction of pyruvate to lactate—**Lactate, formed by the action of lactate dehydrogenase, is the final product of anaerobic glycolysis in eukaryotic cells.
12. **Energy yield from glycolysis—**Despite the production of some ATP during glycolysis, the end products, pyruvate or lactate, still contain most of the energy originally contained in glucose. The TCA cycle is required to release that energy completely
    - Anaerobic glycolysis— Two molecules of ATP are generated for each molecule of glucose converted to two molecules of lactate. There is no net production or consumption of NADH
    - Aerobic glycolysis— The direct consumption and formation of ATP is the same as in anaerobic glycolysis—that is, a net gain of two ATP per molecule of glucose. Two molecules of NADH are also produced per molecule of glucose. Ongoing aerobic glycolysis requires the oxidation of most of this NADH by the electron transport chain, producing approximately three ATP for each NADH molecule entering the chain.

TCA cycle

- The tricarboxylic acid cycle (TCA cycle, also called the Krebs cycle or the citric acid cycle) plays several roles in metabolism. It is the final pathway where the oxidative metabolism of carbohydrates, amino acids, and fatty acids converge, their carbon skeletons being converted to CO_2. This oxidation provides energy for the production of the majority of ATP in most animals, including humans.
- The cycle occurs totally in the mitochondria and is, therefore, in close proximity to the reactions of electron transport which oxidize the reduced coenzymes produced by the cycle.
- The TCA cycle is an aerobic pathway, because O_2 is required as the final electron acceptor. Reactions such as the catabolism of some amino acids generates intermediates of the cycle and are called anaplerotic reactions.



Reaction of TCA cycle.

1. **Oxidative decarboxylation of pyruvate—**Pyruvate, the end product of aerobic glycolysis, must be transported into the mitochondrion before it can enter the TCA cycle. This is accomplished by a specific pyruvate transporter that helps pyruvate cross the inner mitochondrial membrane. Once in the matrix, pyruvate is converted to acetyl CoA by the pyruvate dehydrogenase complex, which is a multienzyme complex. Strictly speaking, the pyruvate dehydrogenase complex is not part of the TCA cycle proper, but is a major source of acetyl CoA—the two-carbon substrate for the cycle.
2. **Synthesis of citrate from acetyl CoA and oxaloacetate—**The condensation of acetyl CoA and oxaloacetate to form citrate (a tricarboxylic acid) is catalysed by citrate synthase. This aldol condensation has an equilibrium far in the direction of citrate synthesis
3. **Isomerization of citrate—** Citrate is isomerized to isocitrate by aconitase, an Fe-S protein.
4. **Oxidation and decarboxylation of isocitrate—**Isocitrate dehydrogenase catalyses the irreversible oxidative decarboxylation of isocitrate yielding

the first of three NADH molecules produced by the cycle, and the first release of CO2

5. **Oxidative decarboxylation of α-ketoglutarate—**The conversion of α-ketoglutarate to succinyl CoA is catalysed by the α-ketoglutarate dehydrogenase complex, a multimolecular aggregate of three enzymes. The mechanism of this oxidative decarboxylation is very similar to that used for the conversion of pyruvate to acetyl CoA by the PDH complex. The reaction releases the second CO2 and produces the second NADH of the cycle. The coenzymes required are thiamine pyrophosphate, lipoic acid, FAD, NAD+, and CoA.
6. **Cleavage of succinyl CoA—** Succinate thiokinase (also called succinyl CoA synthetase—named for the reverse reaction) cleaves the high-energy thioester bond of succinyl. This reaction is coupled to phosphorylation of guanosine diphosphate (GDP) to guanosine triphosphate (GTP). GTP and ATP are energetically interconvertible by the nucleoside diphosphate kinase reaction
7. **Oxidation of succinate—**Succinate is oxidized to fumarate by succinate dehydrogenase, as FAD (its coenzyme) is reduced to FADH2. Succinate dehydrogenase is the only enzyme of the TCA cycle that is embedded in the inner mitochondrial membrane.
8. **Hydration of fumarate—**Fumarate is hydrated to malate in a freely reversible reaction catalysed by fumarase (also called fumarate hydratase).
9. **Oxidation of malate—**Malate is oxidized to oxaloacetate by malate dehydrogenase. This reaction produces the third and final NADH of the cycle.

Glycogen metabolism.

- Glycogen metabolism refers to the process by which glycogen is synthesized, stored, and broken down in the body. Glycogen is a complex carbohydrate made up of glucose molecules that is primarily stored in the liver and muscle tissue.
- The synthesis of glycogen, also known as glycogenesis, occurs when glucose molecules are joined together through a process called glycosylation. This process is catalysed by the enzyme glycogen synthase and requires the presence of a primer molecule called glycogenin.

- Glycogen is broken down through a process called glycogenolysis, which occurs when the body needs energy. The breakdown of glycogen is catalysed by the enzyme glycogen phosphorylase, which cleaves off individual glucose molecules from the glycogen chain. These glucose molecules are then converted into glucose-6-phosphate and can be further metabolized to produce energy.
- Control of glycogen metabolism—The regulation of glycogen metabolism is tightly controlled by hormones such as insulin and glucagon. Insulin promotes the synthesis of glycogen, while glucagon stimulates the breakdown of glycogen to release glucose into the bloodstream. The regulation of glycogen metabolism is critical for maintaining blood glucose levels and providing energy to the body.

Regulation of blood glucose level

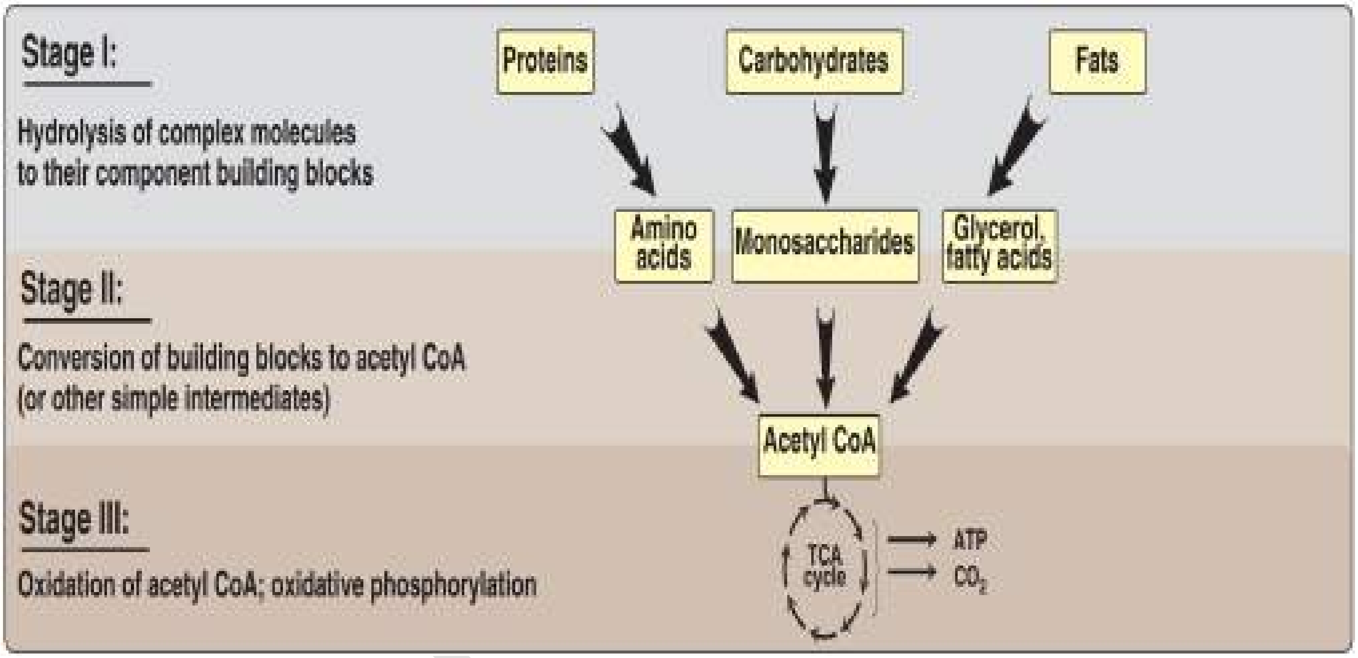
The regulation of blood glucose level is a complex process involving various hormones and physiological mechanisms. The main hormones involved in this process are insulin, glucagon, and epinephrine.

- **Insulin—**Insulin is a hormone produced by the pancreas that helps regulate glucose levels in the blood. It stimulates the uptake of glucose from the blood into cells, where it can be used for energy or stored as glycogen. Insulin also promotes the conversion of excess glucose into fat for storage.
- **Glucagon**—Glucagon, another hormone produced by the pancreas, has the opposite effect of insulin. It stimulates the liver to break down glycogen into glucose and release it into the bloodstream, thereby increasing blood glucose levels.
- **Epinephrine—**Epinephrine, also known as adrenaline, is a hormone produced by the adrenal glands in response to stress or exercise. It increases blood glucose levels by stimulating the liver to release glucose and by reducing the uptake of glucose by muscles.

Diseases related to abnormal metabolism of Carbohydrates.

- Glucose metabolism is defective in two very common metabolic diseases, obesity and diabetes, which contribute in development of a number of major medical problems, including
- Atherosclerosis,
- Hypertension,
- Small vessel disease,
- Kidney disease, and blindness etc.

NOTE—Three stages of catabolism of substrate.



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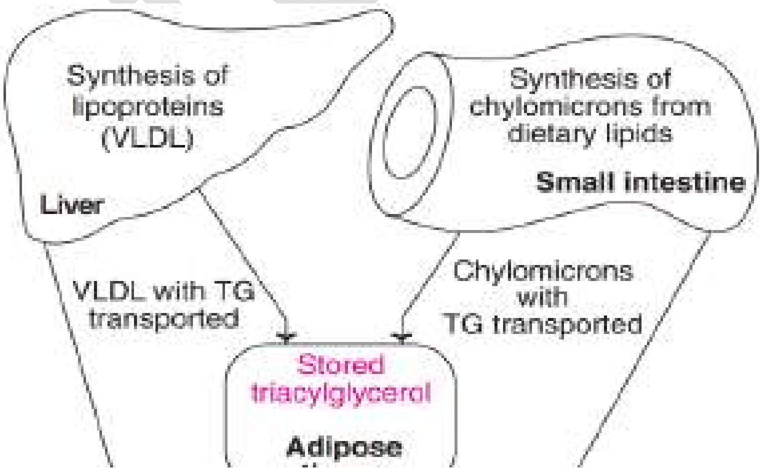
Unit-2

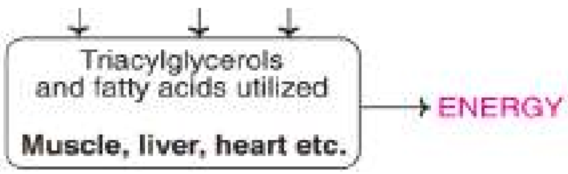
Metabolism of lipid.

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 are stored in the adipose tissue and serve as energy reserve of the body. 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. Fat also acts as an insulating material for maintaining the body temperature of animals.

Transport of lipid— 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.

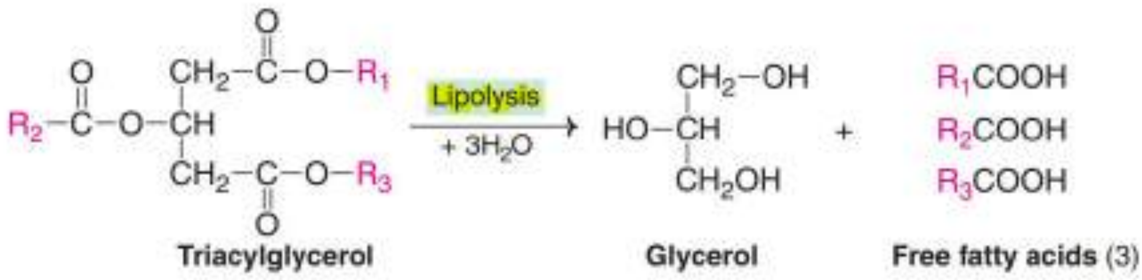


Overview of lipid metabolism



Lipolysis

- 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.
- 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



Complete hydrolysis (lipolysis) of triacylglycerol.

adenylate cyclase and, thus, increase lipolysis.
- **Fate of glycerol—** The adipose tissue lacks the enzyme glycerol kinase, hence glycerol produced in lipolysis cannot be phosphorylated 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.
- **Fate of free fatty acids—**The fatty acids released 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

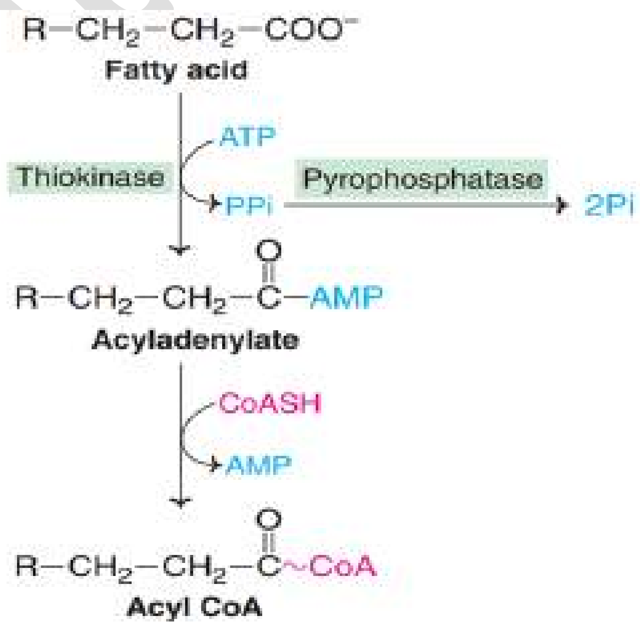
acids. Certain tissues, however, cannot oxidize fatty acids, e.g., brain, erythrocytes.

β-oxidation of Fatty acid (Palmitic acid).

Introduction—This process is known as beta-oxidation, because the oxidation and splitting of two carbon units occur at the beta-carbon atom. The oxidation of the hydrocarbon chain occurs by a sequential cleavage of two carbon atoms. 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. The β-oxidation of fatty acids involves three stages.

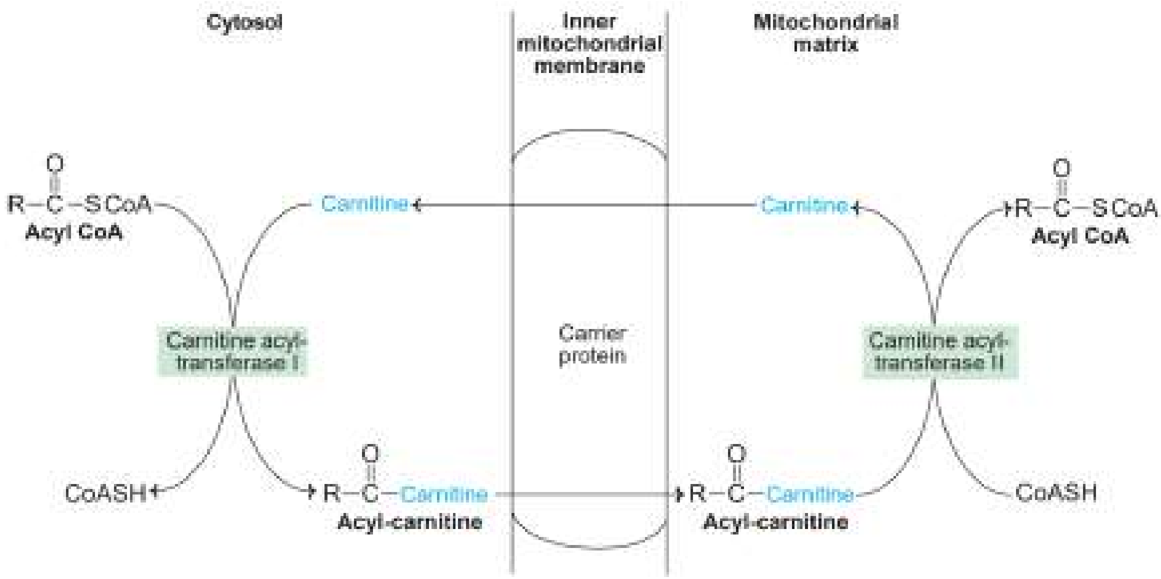
- Activation of fatty acids occurring in the cytosol.
- Transport of fatty acids into mitochondria.
- β-Oxidation proper in the mitochondrial matrix.

1. **Activation of fatty acids—** Fatty acids are activated to acyl CoA by thiokinase or acyl CoA synthetases. The reaction occurs in two steps and requires ATP, coenzyme A and Mg2+. Fatty acid reacts with ATP to form acyladenylate which then combines with coenzyme A to produce acyl CoA. 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.



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

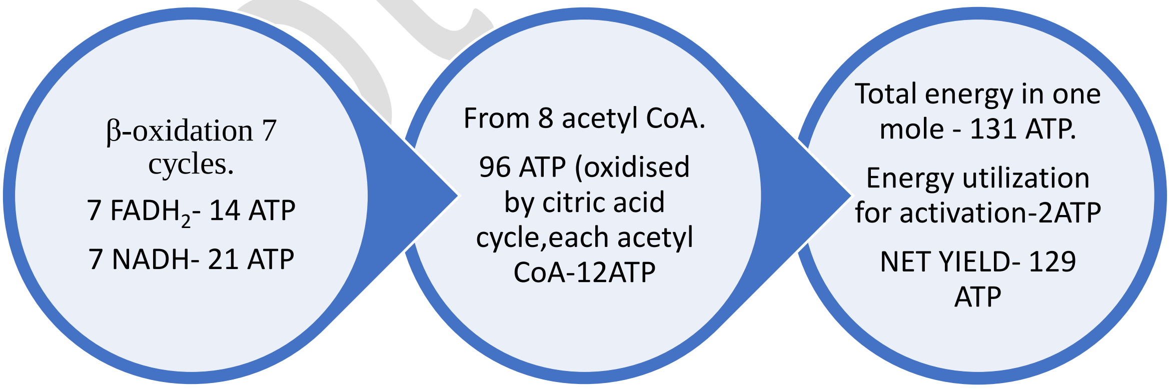
2. **Transport of fatty acids into mitochondria—** The inner mitochondrial membrane is impermeable to fatty acids. A specialized carnitine carrier system (carnitine shuttle) operates to transport activated fatty acids from cytosol to the mitochondria. This occurs in four steps.
   - Acyl group of acyl CoA is transferred to carnitine (β-hydroxy ϒ-trimethyl aminobutyrate), catalysed by carnitine acyltransferase I (present on the outer surface of inner mitochondrial membrane).
   - The acyl-carnitine is transported across the membrane to mitochondrial matrix by a specific carrier protein.
   - Carnitine acyl transferase II (found on the inner surface of inner mitochondrial membrane) converts acyl-carnitine to acyl CoA.
   - The carnitine released returns to cytosol for reuse



Carnitine shuttle for transport of activated fatty acid (acyl CoA) into mitochondria

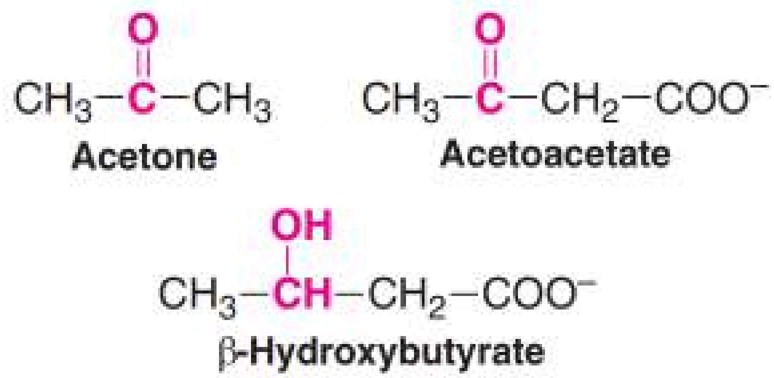
3. **β-Oxidation proper in the mitochondrial matrix—** Each cycle of β-oxidation, liberating a two-carbon unit-acetyl CoA, occurs in a sequence of four reactions.
    1. **Oxidation—**Acyl CoA undergoes dehydrogenation by an FAD-dependent flavoenzyme, acyl CoA dehydrogenase. A double bond is formed between 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.

Energy of palmitic acid oxidation—



Ketogenesis

- The synthesis of ketone bodies occurs in the liver. The enzymes for ketone body synthesis are located in the mitochondrial matrix. Ketone bodies are water-soluble and energy yielding. Acetyl CoA, formed by oxidation of fatty acids, pyruvate or some amino acids, is the precursor for ketone bodies.
- The three main types of ketone bodies produced are acetone, acetoacetate, and beta-hydroxybutyrate. Ketone bodies can be used by the brain and other tissues as an alternative energy source when glucose is scarce, and they are also involved in regulating blood glucose levels and reducing inflammation.
- However, excessive production of ketone bodies can lead to a condition known as ketoacidosis, which is a potentially life-threatening metabolic state characterized by high levels of ketone



bodies in the blood.

Ketogenesis occurs through the following reactions.

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 E-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 dehydrogenase to β-hydroxybutyrate.

Regulation of ketogenesis—

The ketone body formation (particularly overproduction) occurs primarily due to nonavailability 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.

Ketolysis

- Ketolysis is the metabolic process by which ketone bodies are broken down and converted into energy in the body's cells. This process occurs primarily in the mitochondria of cells, where the ketone bodies are broken down into acetyl-CoA, which can then enter the citric acid cycle to produce ATP, the energy currency of cells.
- This process is important for individuals who rely on ketone bodies as their primary source of energy, such as those on a ketogenic diet or during periods of prolonged fasting.
- The rate of Ketolysis is influenced by several factors, including the availability of ketone bodies and the metabolic state of the cells.
- In some metabolic disorders, such as diabetes, there can be a disruption in the balance between ketone production and utilization, leading to an accumulation of ketone bodies in the blood and potentially causing ketoacidosis.

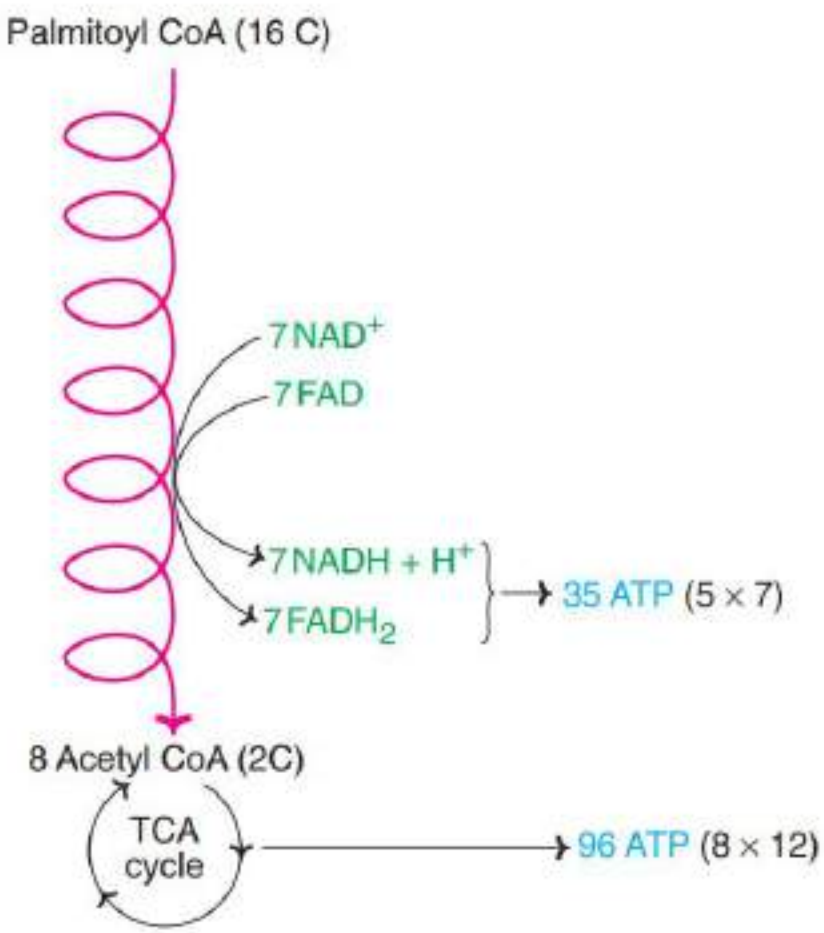
Diseases related to abnormal metabolism of lipids.

1. **Ketoacidosis—** Increase in concentration of both acetoacetate and β-hydroxybutyrate (strong acids) in blood would cause acidosis. The carboxyl group has a pKa around 4. Therefore, the ketone bodies in the blood dissociate and release H+ ions which lower the PH. Diabetic ketoacidosis is dangerous—may result in coma, and even death, if not treated. Ketosis due to starvation is not usually accompanied by ketoacidosis
2. **Hypercholesterolemia**—Increase in plasma cholesterol (> 200 mg/dl) concentration is known as hypercholesterolemia and is observed in many disorders
   - Diabetes mellitus— Due to increased cholesterol synthesis since the availability of acetyl CoA is increased.
   - Hypothyroidism (myxoedema)— This is believed to be due to decrease in the HDL receptors on hepatocytes.
   - Obstructive jaundice— Due to an obstruction in the excretion of cholesterol through bile.
   - 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.
3. **Fatty liver—** The normal concentration of lipid 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.

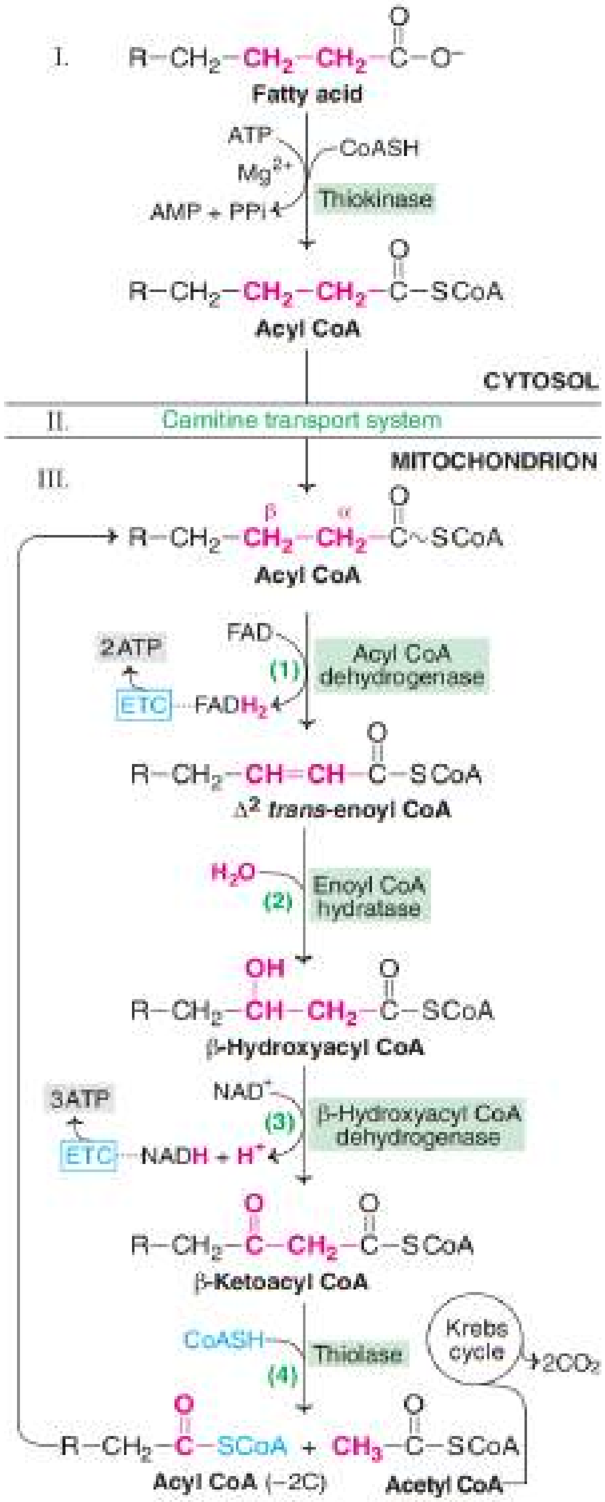
   In the normal liver, Kupffer cells contain lipids in the form of droplets. In fatty liver, droplets of triacylglycerols are found in the entire cytoplasm of hepatic cells. This causes impairment in metabolic functions of liver. Fatty liver is associated with fibrotic changes and cirrhosis, Fatty liver may occur due to two main causes

   - Increased synthesis of triacylglycerols
   - Impairment in lipoprotein synthesis.

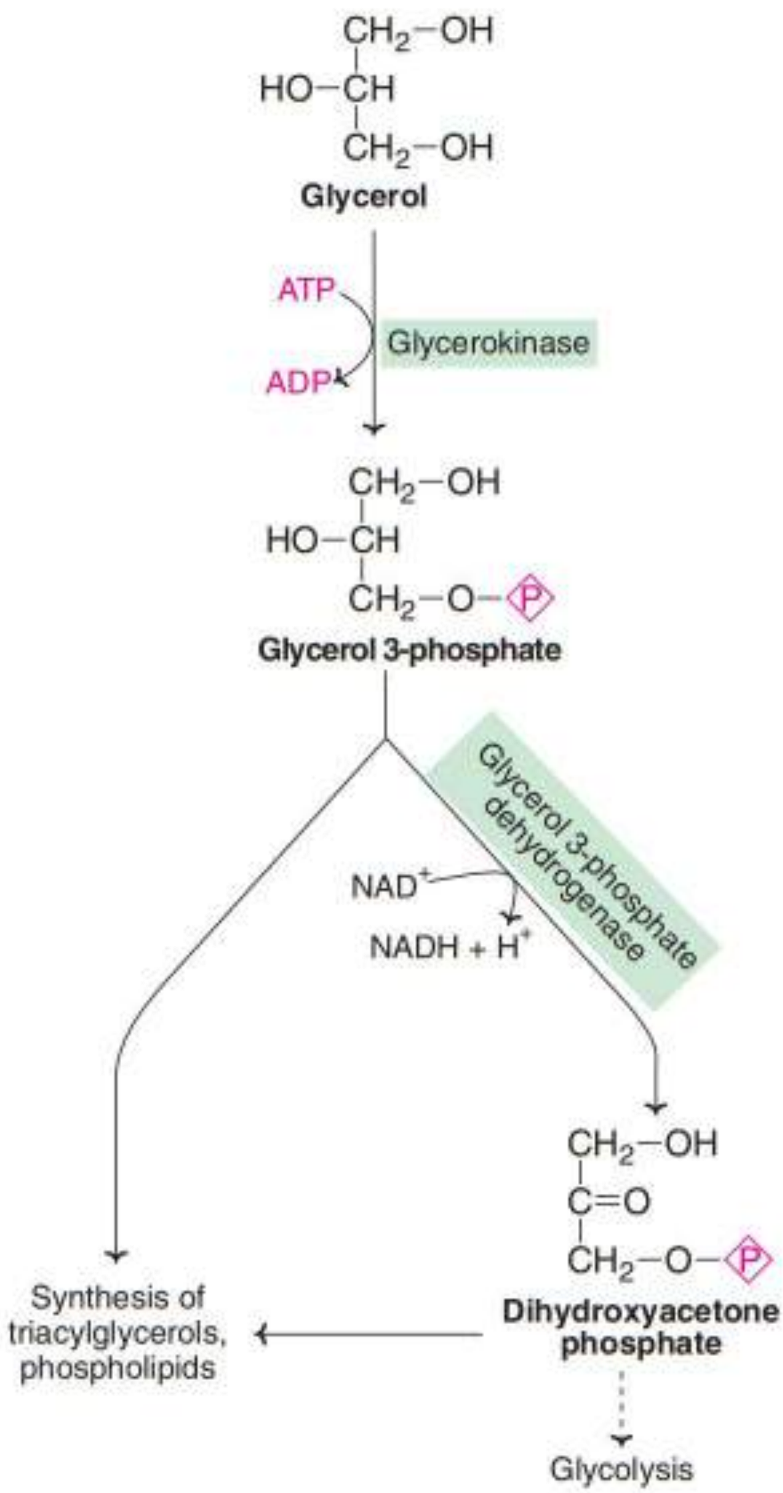
NOTE:



oxidation of palmitic acid.



β-Oxidation of fatty acids



Fate of glycerol

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Metabolism of amino acids.

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.

Amino acid—Proteins are nitrogen-containing macromolecules consisting of L-D-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).

General reactions of amino acids and its significance.

Amino acid pool—An adult has about 100 g of free amino acids which represent the amino acid pool of the body. 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. 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.

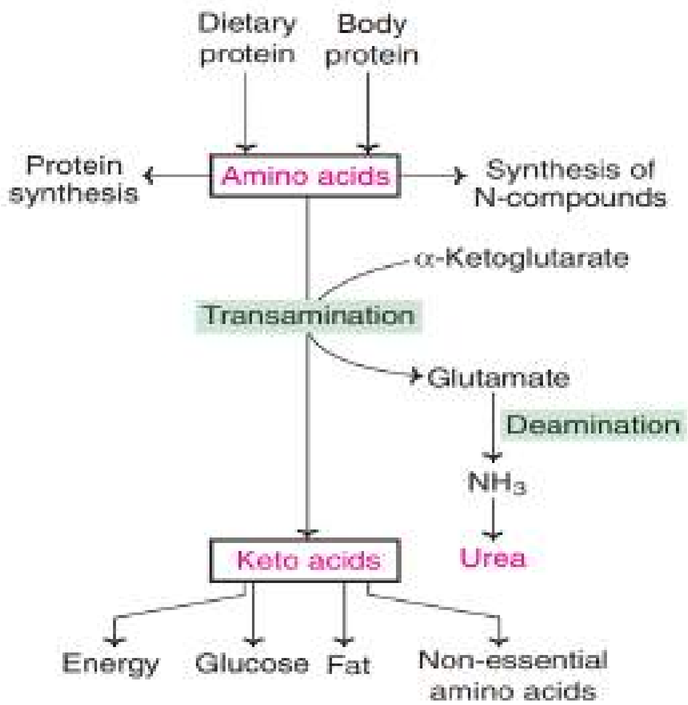
Sources of amino acid pool— Three major sources of amino acid pool.

- 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.

- 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 must, therefore, be supplied daily in the diet to maintain nitrogen balance. The purpose of dietary protein is to supply amino acids for the synthesis of proteins and other nitrogen compounds.
- 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.

General metabolism and significance.

- 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.
- 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.
- Generally, about 10-15% of body energy requirements are met from the amino acids.
- The amino acids are converted to carbohydrates and fats. This becomes predominant when the protein consumption is in excess of the body



General reaction/metabolism.

requirements.

Transamination.

The transfer of an amino (NH2) 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 /aminotransferases.

Silent features of Transamination—

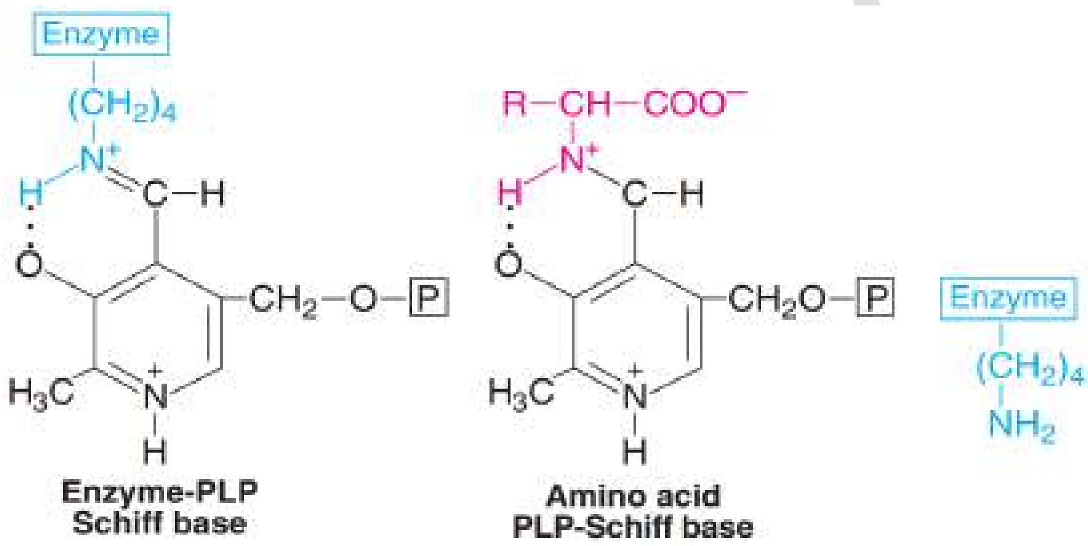
- All transaminases require pyridoxal phosphate (PLP), a coenzyme derived from vitamin B6. It is **reversible** and no free NH3 liberated, only the transfer of amino group occurs. Transamination diverts the excess amino acids towards energy generation.
- Specific transaminases exist for each pair of amino and keto acids. However, only two— namely, aspartate transaminase and alanine transaminase—make a significant contribution for transamination.
- 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 and anabolism of amino acids.
- 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 NH3 for urea synthesis.
- All amino acids except lysine, threonine, proline and hydroxyproline participate in transamination. Serum transaminases are important for diagnostic and prognostic purposes.

Mechanism of transamination— it occurs in two stages.

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 B6. The aldehyde group of PLP is linked with H-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 noncovalent forces. **Snell and Braustein** proposed a Ping Pong Bi Bi mechanism involving a series of intermediates (aldimines and ketimines) in transamination reaction.

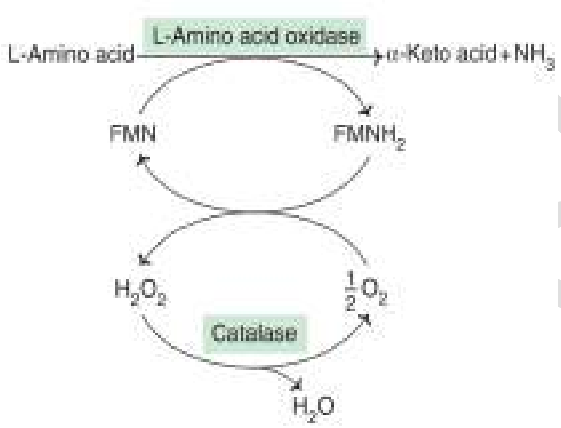


Deamination.

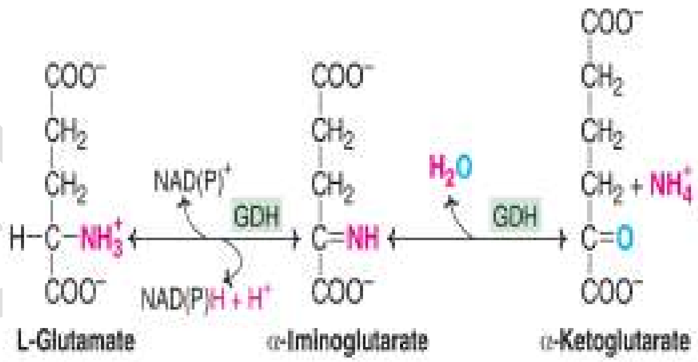
The removal of amino group from the amino acids as NH3 is deamination. Deamination results in the liberation of ammonia for urea synthesis (transamination involves only the shuffling of amino groups). It may be either oxidative or non-oxidative.

1. **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 NH3 for urea synthesis and D-keto acids for a variety of reactions, including energy generation.

- **Oxidation of glutamate-by-glutamate dehydrogenase—**In the transamination process glutamate is form. Now, 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.
- **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 D-keto acids and NH3. In this reaction, oxygen is



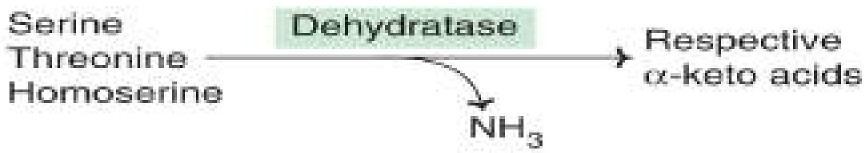
Oxidative deamination of amino acids.



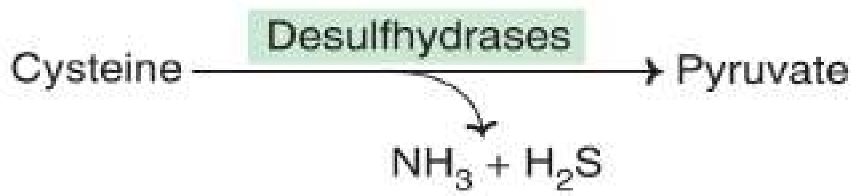
Oxidation of glutamate-by-glutamate dehydrogenase (GDH)

reduced to H_2O_2, which is later decomposed by catalase.

2. **Non-oxidative deamination—** Some of the amino acids can be deaminated to liberate NH3 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).

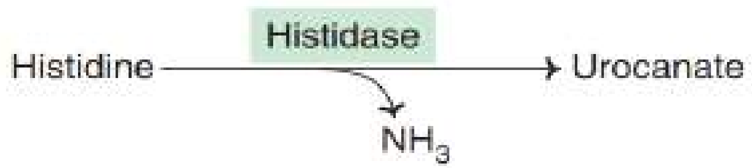


b. **Amino acid desulfhydrases—** The sulphur amino acids, namely cysteine and homocysteine, undergo deamination coupled with



desulfhydration to give keto acids.

c. **Deamination of histidine—**The enzyme histidase acts on histidine to liberate NH3 by a non-oxidative deamination process.



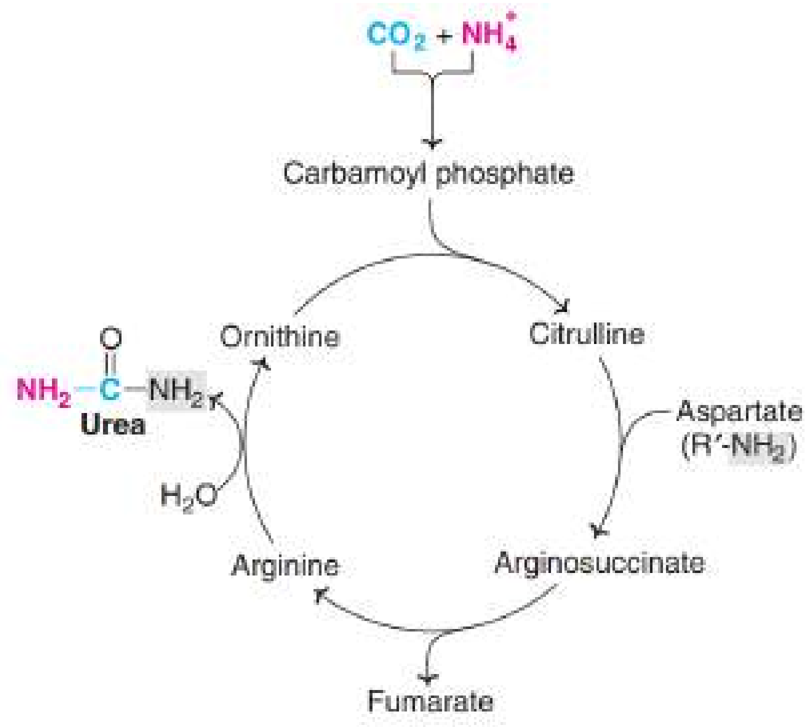
Urea cycle.

- 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. Ammonium ions are very important to maintain acid-base balance of the body.
- The production of NH3 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.

Introduction about urea cycle—

- Urea is the end product of protein metabolism (amino acid metabolism). The nitrogen of amino acids, converted to ammonia, 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.



Outline of urea cycle

Steps of urea cycle—

1. Synthesis of carbamoyl phosphate— Carbamoyl phosphate synthase I (CPS I) of mitochondria catalyses the condensation of $NH4^{+}$ ions with CO_2 to form carbamoyl phosphate. This step consumes two ATP and is irreversible, and rate-limiting.
2. Formation of citrulline— Citrulline is synthesized from carbamoyl phosphate and ornithine by ornithine transcarbamoylase. Ornithine is regenerated and used in urea cycle. Ornithine and citrulline are basic amino acids. Citrulline produced in this reaction is transported to cytosol by a transporter system.
3. Synthesis of arginosuccinate— Arginosuccinate 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).

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 Co2+ and Mn2+. 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.

Regulation of urea cycle.

- Regulation of the urea cycle is depending on concentration of ammonium ions in the liver. When the concentration of ammonium ions is high, the urea cycle is activated to convert ammonia to urea and vice-versa.
- Another important regulator of the urea cycle is the availability of substrates. The urea cycle requires several substrates, including ammonia, bicarbonate, and ornithine.
- The activity of the urea cycle is also regulated by hormones, including insulin, glucagon, and cortisol. Insulin and glucagon, for example, can modulate the expression of urea cycle enzymes, while cortisol can increase the activity of the enzyme that converts arginine to ornithine.

Decarboxylation.

- Decarboxylation of an amino acid is a chemical reaction in which the carboxyl group (-COOH) of an amino acid is removed, leading to the formation of an amine group (-NH_2) and the release of carbon dioxide (CO2). This reaction is catalysed by enzymes called decarboxylases.
- In general, the decarboxylation of amino acids or their derivatives results in the formation of amines.
- The decarboxylation of amino acids plays an important role in many biological processes. For example, the amino acid histidine is

decarboxylated to form histamine, which is involved in regulating many physiological processes, including digestion and immune response. Similarly, the amino acid glutamic acid is decarboxylated to form the neurotransmitter gamma-aminobutyric acid (GABA), which is involved in the regulation of neuronal activity.

Disorders of ammonia metabolism.

- **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 hepatic coma and mental retardation. The acquired hyperammonemia may be due to hepatitis, alcoholism etc. where the urea synthesis becomes defective, hence NH3 accumulates.

Diseases related to abnormal metabolism of amino acids.

Amino aciduria— The term amino aciduria is generally used to indicate the urinary excretion of amino acids. It is frequently associated with defects in amino acid metabolism. Most of the amino acidurias manifest in mental retardation

Phenylketonuria

- Phenylketonuria (PKU) is the most common metabolic disorder in amino acid metabolism. It is due to the deficiency of the hepatic enzyme, phenylalanine hydroxylase, caused by an autosomal recessive gene.
- 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 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.
- Clinical condition considered as- mental retardation, hypopigmentation.

Alkaptonuria (Black urine disease).

- Alkaptonuria is a rare genetic disorder characterized by the inability of the body to break down certain amino acids, specifically phenylalanine and tyrosine, which leads to the accumulation of a pigment called homogentisic acid in the connective tissues, cartilage, and bones.
- The defective enzyme in alkaptonuria is homogentisate oxidase in tyrosine metabolism. 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.
- Homogentisate gets oxidized by polyphenol oxidase to benzoquinone acetate which undergoes polymerization to produce a pigment called alkapton. Alkapton deposition occurs in connective tissue, bones and various organs (nose, ear etc.) resulting in a condition known as **ochronosis.**
- **Symptoms** of alkaptonuria typically appear in early childhood and include dark urine that turns brown upon standing, as well as joint pain and stiffness, especially in the spine and large joints such as the hips and knees.

Jaundice.

- Amino acid metabolism disorders can lead to jaundice if there is a disruption in the pathway that converts bilirubin into a form that can be eliminated from the body. For example, a genetic disorder called **Crigler-Najjar syndrome** can lead to jaundice because it impairs the ability of the liver to convert bilirubin into a form that can be excreted in the bile. This can cause bilirubin to build up in the blood, leading to jaundice.
- Similarly, other genetic disorders such as **Gilbert's syndrome** can cause jaundice due to problems with bilirubin metabolism. In Gilbert's syndrome, there is a deficiency of an enzyme called UDP-glucuronosyltransferase, which is responsible for conjugating bilirubin so

that it can be eliminated from the body. Without this enzyme, bilirubin builds up in the blood and can lead to jaundice.

Biological oxidation: Electron transport chain and Oxidative phosphorylation

Biological oxidation

Prior to the understanding the biological oxidation we need to know that some important concept and terminologies.

Bioenergetics—Bioenergetics or biochemical thermodynamics deals with the study of energy changes (transfer and utilization) in biochemical reactions. The reactions are broadly classified as exergonic (energy releasing) and endergonic (energy consuming). Bioenergetics is concerned with the initial and final states of energy component of the reactants and not the mechanism of chemical reactions.

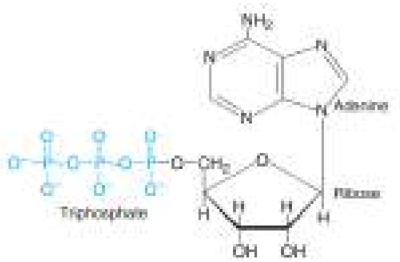
Free energy.

- The energy actually available to do work (utilizable) is known as free energy. Changes in the free energy (ΔG) are valuable in predicting the feasibility of chemical reactions. If free energy change (ΔG) is represented by a negative sign, there is a loss of free energy. The reaction is said to be exergonic, and proceeds spontaneously. On the other hand, a positive ΔG indicates that energy must be supplied to the reactants. The reaction cannot proceed spontaneously and is endergonic in character.
- Enthalpy (ΔH) is a measure of the change in heat content of the reactants, compared to products
- Entropy (ΔS) represents a change in the randomness or disorder of reactants and products. Entropy attains a maximum as the reaction approaches equilibrium.
- The relation between the changes of free energy (ΔG), enthalpy (ΔH) and entropy (ΔS) are expressed as.

$$\Delta G = \Delta H - T\Delta S.$$

High energy compound—

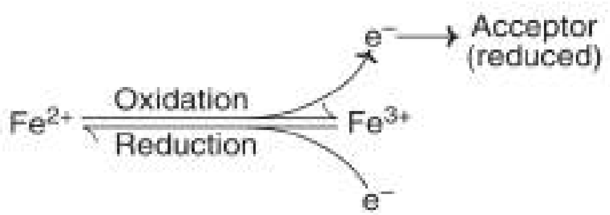
- Pyrophosphates e.g., ATP.
- Acyl phosphates e.g., 1,3-bisphosphoglycerate.
- Enol phosphates e.g., phosphoenolpyruvate.
- Thioesters e.g., acetyl CoA.
- Phosphagens e.g., phosphocreatine.



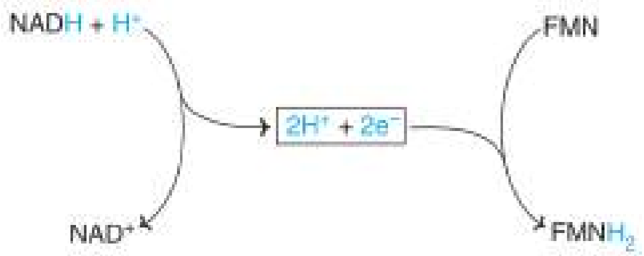
Structure of ATP

Biological oxidation

- Oxidation is defined as the loss of electrons and reduction as the gain of electrons. This may be illustrated by the interconversion of ferrous ion ($Fe2^{+}$) to ferric ion ($Fe3^{+}$).



- The electron lost in the oxidation is accepted by an acceptor which is said to be reduced. Thus, the oxidation-reduction is a tightly coupled process.
- The general principle of oxidation-reduction is applicable to biological systems also. The oxidation of NADH to NAD^{+} coupled with the reduction of FMN to FMNH2 is illustrated.

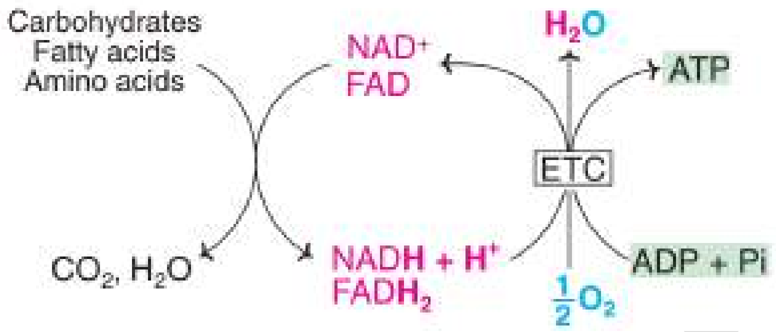


- In the above illustration, there are two redox pairs NADH/NAD+ and FMN/FMNH2. The redox pairs differ in their tendency to lose or gain electrons.

Redox potential.

- The oxidation-reduction potential or, simply, redox potential, is a quantitative measure of the tendency of a redox pair to lose or gain electrons. The redox pairs are assigned specific standard redox potential (E_0 volts) at pH 7.0 and 25°C.
- The more negative redox potential represents a greater tendency (of reductant) to lose electrons. On the other hand, a more positive redox potential indicates a greater tendency (of oxidant) to accept electrons. The electrons flow from a redox pair with more negative E0 to another redox pair with more positive E_0.

- The redox potential (E_0) is directly related to the change in the free energy ($\Delta G°$)



Overview on biological oxidation.

Electron transport chain.

The energy-rich carbohydrates (particularly glucose), fatty acids and amino acids undergo a series of metabolic reactions and, finally, get oxidized to CO_2 and H_2O. The reducing equivalents from various metabolic intermediates are transferred to coenzymes NAD^+ and FAD to produce, respectively, NADH and FADH2. The latter two reduced coenzymes pass through the electron transport chain (ETC) or respiratory chain and, finally, reduce oxygen to water. The passage of electrons through the ETC is associated with the loss of free energy. A part of this free energy is utilized to generate ATP from ADP and Pi

Mitochondrial organisation.

- The mitochondria are the centres for metabolic oxidative reactions to generate reduced coenzymes (NADH and FADH2) which, in turn, are utilized in ETC to liberate energy in the form of ATP. For this reason, mitochondrion is appropriately regarded as the power house of the cell.
- The mitochondrion consists of five distinct parts. These are the **outer membrane, the inner membrane, the intermembrane space, the cristae and the matrix.**

Organisation of respiratory chain.

- The inner mitochondrial membrane can be disrupted into five distinct respiratory or enzyme complexes, denoted as complex **I, II, III, IV and V**.
- The complexes I-IV are carriers of electrons while complex V is responsible for ATP synthesis.
- Besides these enzyme complexes, there are certain mobile electron carriers in the respiratory chain. These include NADH, coenzyme Q, cytochrome C and oxygen.
- The enzyme complexes (I-IV) and the mobile carriers are collectively involved in the transport of electrons which, ultimately, combine with oxygen to produce water. The largest proportion of the oxygen supplied to the body is utilized by the mitochondria for the operation of electron transport chain.

Components and reactions of the electron transport chain.

There are five distinct carriers that participate in the electron transport chain (ETC). These carriers are sequentially arranged and are responsible for the transfer of electrons from a given substrate to ultimately combine with proton and oxygen to form water.

A. **Nicotinamide nucleotides**— NAD^+ and $NADP^+$ derived from the vitamin niacin are the two coenzymes in this, NAD^+ is more actively involved in the ETC. NAD^+ is reduced to $NADH + H^+$ by dehydrogenases with the removal of two hydrogen atoms from the substrate (AH_2). The substrates include glyceraldehyde-3 phosphate, pyruvate, isocitrate, D-ketoglutarate and malate. $AH_2 + NAD^+ \rightleftharpoons A + NADH + H^+$.
B. **Flavoproteins**— The enzyme NADH dehydrogenase (NADH-coenzyme Q reductase) is a flavoprotein with FMN as the prosthetic group. The coenzyme FMN accepts two electrons and a proton to form FMNH2. NADH dehydrogenase is a complex enzyme closely associated with non-heme iron proteins (NHI) or iron-sulphur proteins (FeS).

$$NADH + H^+ + FMN \rightarrow NAD^+ + FMNH2$$

Succinate dehydrogenase (succinate-coenzyme Q reductase) is an enzyme found in the inner mitochondrial membrane. It is also a flavoprotein with FAD as the coenzyme. This can accept two hydrogen atoms ($2H^+ + 2e^-$) from succinate.

$$Succinate + FAD \rightarrow Fumarate + FADH_2.$$

C. **Iron-sulphur proteins**— The iron-sulphur (FeS) proteins exist in the oxidized ($Fe3^{+}$) or reduced ($Fe2^{+}$) state. About half a dozen FeS proteins connected with respiratory chain have been identified. However, the mechanism of action of iron-sulphur proteins in the ETC is not clearly understood. One FeS participates in the transfer of electrons from FMN to coenzyme Q. Other FeS proteins associated with cytochrome b and cytochrome c1 participate in the transport of electrons.

D. **Coenzyme Q**— Coenzyme Q is also known as ubiquinone since it is ubiquitous in living system. It is a quinone derivative with a variable isoprenoid side chain. The mammalian tissues possess a quinone with 10 isoprenoid units which is known as coenzyme Q_{10} (CoQ_{10}). Coenzyme Q is a lipophilic electron carrier. It can accept electrons from FMNH2 produced in the ETC by NADH dehydrogenase or FADH2 produced outside ETC (e.g. succinate dehydrogenase, acyl CoA dehydrogenase).

E. **Cytochromes**— The cytochromes are conjugated proteins containing heme group. The latter consists of a porphyrin ring with iron atom. The iron of heme in cytochromes is alternately oxidized ($Fe3^{+}$) and reduced ($Fe2^{+}$), which is essential for the transport of electrons in the ETC. Three cytochromes were initially discovered from the mammalian mitochondria. They were designated as cytochrome a, b and c depending on the type of heme present and the respective absorption spectrum. Additional cytochromes such as c1, b1, b2, a3 etc. were discovered later.

Oxidative phosphorylation.

The transport of electrons through the ETC is linked with the release of free energy. The process of synthesizing ATP from ADP and Pi coupled with the electron transport chain is known as oxidative phosphorylation. The complex V of the inner mitochondrial membrane is the site of oxidative phosphorylation.

P : O Ratio

- The P : O ratio refers to the number of inorganic phosphate molecules utilized for ATP generation for every atom of oxygen consumed. More appropriately, P : O ratio represents the number of molecules of ATP synthesized per pair of electrons carried through ETC.

- The mitochondrial oxidation of NADH with a classical P : O ratio of 3. Further, a P : O ratio of 2 has been assigned to the oxidation of FADH2.

Sites of oxidative phosphorylation in ETC.

There are three sites in the ETC that are exergonic to result in the synthesis of 3 ATP molecules.

1. **Oxidation of FMNH2 by coenzyme Q.**
2. **Oxidation of cytochrome b by cytochrome c1.**
3. **Cytochrome oxidase reaction.**

Mechanism of oxidative phosphorylation— Important hypothesis regarding the phosphorylation.

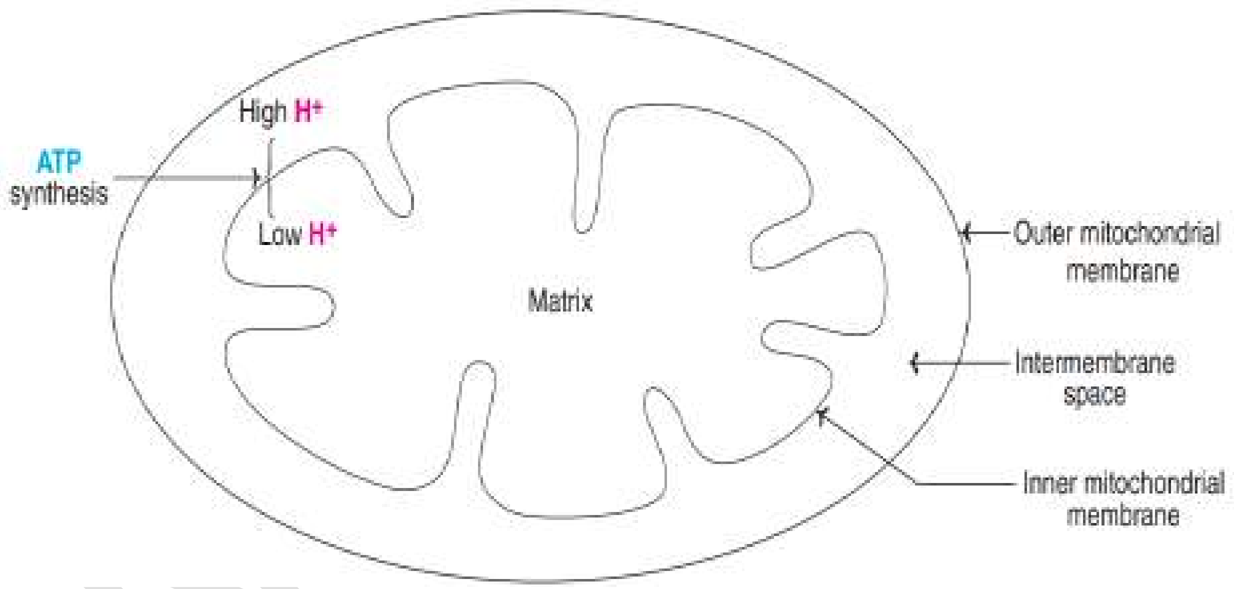
- **Chemical coupling hypothesis**— This hypothesis was put forth by Edward Slater (1953). According to chemical coupling hypothesis, during the course of electron transfer in respiratory chain, a series of phosphorylated high-energy intermediates are first produced which are utilized for the synthesis of ATP. These reactions are believed to be analogous to the substrate level phosphorylation that occurs in glycolysis or citric acid cycle. However, this hypothesis lacks experimental evidence, since all attempts, so far, to isolate any one of the high-energy intermediates have not been successful.

- **Chemiosmotic hypothesis**— This mechanism, originally proposed by Peter Mitchell (1961), is now widely accepted. It explains how the transport of electrons through the respiratory chain is effectively utilized to produce ATP from ADP + Pi. The concept of chemiosmotic hypothesis based on the positive and negative charges gradient **(Proton gradient) and enzymatic induction.**

Proton gradient— The inner mitochondrial membrane, as such, is impermeable to protons (H^+) and hydroxyl ions (OH^-). The transport of electrons through ETC is coupled with the translocation of protons (H+) across the inner mitochondrial membrane (coupling membrane) from the matrix to the intermembrane space.

The pumping of protons results in an electrochemical or proton gradient. This is due to the accumulation of more H^+ ions (low pH) on the outer side of the inner mitochondrial membrane than the inner side. The proton gradient developed due to the electron flow in the respiratory chain is sufficient to result in the synthesis of ATP from ADP and Pi.

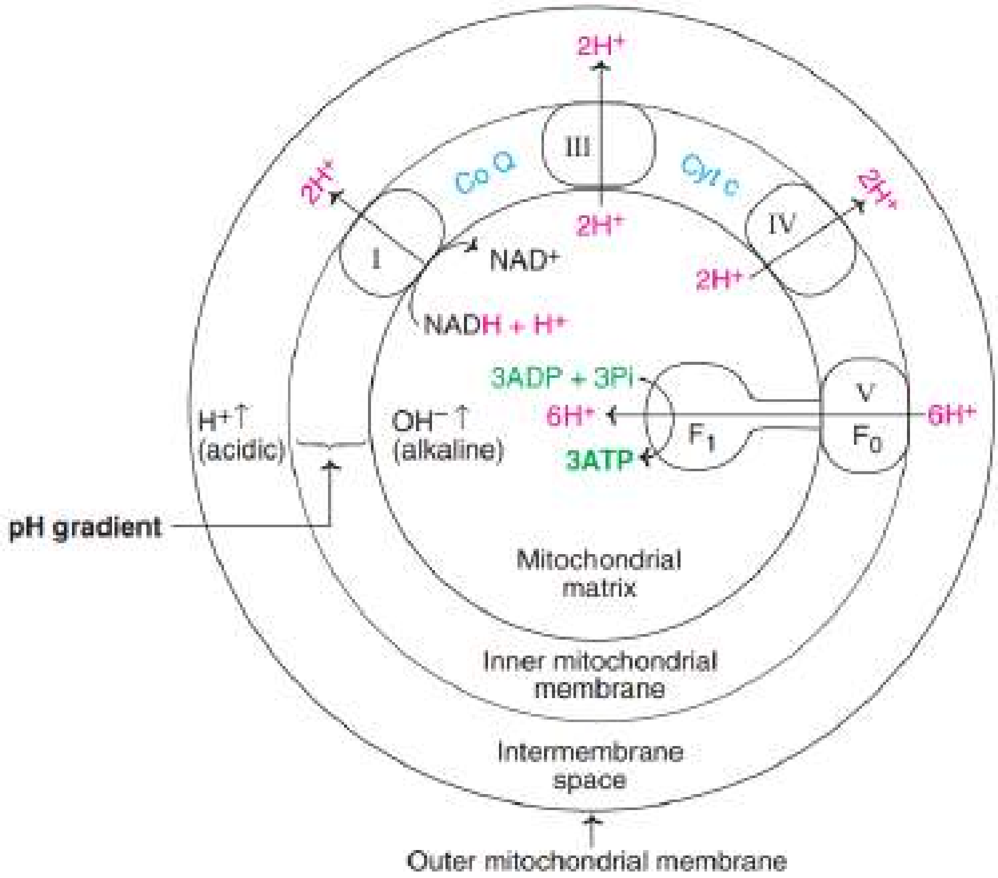
Enzyme system for ATP synthesis— ATP synthase, present in the **complex V**, utilizes the proton gradient for the synthesis of ATP. This enzyme is also known as ATPase since it can hydrolyse ATP to ADP and Pi. ATP synthase is a complex enzyme and consists of two functional subunits, namely F1 and F0. Its structure is comparable with 'lollipops'. The protons that accumulate on the intermembrane space re-enter the mitochondrial matrix leading to the synthesis of ATP.



Outline of chemiosmotic hypothesis for oxidative phosphorylation

Rotary motor model for ATP generation.

Paul Boyer in 1964 proposed (Nobel Prize, 1997) that a conformational change in the mitochondrial membrane proteins leads to the synthesis of ATP. The original Boyer hypothesis, now considered as rotary motor/engine driving model or binding change model, is widely accepted for the generation ATP.



Diagrammatic representation of chemiosmotic hypothesis for oxidative phosphorylation (I, III, IV and V–Respiratory chain complexes; F_0, F_1 –Protein subunits for phosphorylation).