

UNIT 14

CARBOHYDRATE METABOLISM-I

Structure

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14.1 INTRODUCTION

In the previous unit, you have learnt that carbohydrates serve as immediate source of energy in our body. In this unit you would learn how it is accomplished by carbohydrate metabolism. Carbohydrate metabolism involves various biochemical reactions for the formation (anabolism), breakdown (catabolism) and interconversion of carbohydrates in a living organism.

In the present unit we shall begin with glycolysis, universal pathway sugar catabolism. Glycolysis is central pathway of glucose catabolism. It is initial route of oxidative catabolism in both anaerobic and aerobic systems. It results in incomplete oxidation of glucose which yields pyruvate. Complete breakdown of pyruvate takes place under aerobic conditions by first converting it to acetylCoA followed by an entry to the Kreb's cycle. We shall discuss the various reactions involved in this pathway. Glucose once oxidized needs to be replenished. Although, humans can't synthesize glucose from CO₂ and water, but there are reactions which replenish its levels from other sources by a process called gluconeogenesis. This specialized pathway which forms glucose from non carbohydrate sources during prolonged starvation will be explained in this unit. Finally, the pentose phosphate pathway (PPP) and its role will be discussed.

Objectives

After studying this unit, you should be able to:

- ❖ explain how monosaccharides are catabolised to produce energy in the form of ATP through glycolysis and Kreb's cycle,
- ❖ describe the synthesis of glucose from non-carbohydrate precursors, and
- ❖ draw metabolic pathways of glucose metabolism which replenish reduced NADP, ribose-5-phosphate and erythrose-4-phosphate required for the synthesis of fatty acids, nucleic acids and aromatic amino acids respectively.

14.2 GLYCOLYSIS

These phases of glycolytic pathway are for the purpose of easy understanding. Inside the cell, all these phases occur in continuity. In fact, product of one reaction serves as the substrate for the next. reaction in pathway.

Glycolysis (glykos- sweet; lysis- splitting) is a sequence of reactions which converts glucose and related hexoses into two molecules of pyruvate with net production of two ATP molecules. It is the most important pathway in energy metabolism, present in both aerobic and anaerobic organisms. The cycle is completed in ten steps with the help of enzymes present in the cytoplasm. None of the reactions are oxygen dependent. In evolutionary terms, it is regarded as a primitive pathway.

HIGHLIGHTS OF GLYCOLYSIS

- Glycolysis is also called EMP pathway as elucidated by the scientists namely Embden, Meyerhof and Parnas.
- It is the major pathway for the production of ATP in tissues lacking mitochondria, e.g., RBCs, and cornea of the eye.
- Glycolysis can occur in the absence of oxygen (anaerobic). In such conditions lactate, instead of pyruvate, is the major end product.
- Many of the intermediates of this pathway provide branch point to other pathways, e.g., dihydroxyacetone phosphate is a source of glycerol that combines with fatty acids to form triglycerides.

- All the intermediates of the pathway are phosphorylated and are water soluble.
- Only 3 reactions out of ten are irreversible in this pathway.

This pathway can be divided into three distinct phases:

- A. Energy investment phase
- B. Splitting phase
- C. Energy generation phase

A. Energy investment phase: This phase involves reactions which require input of energy in the form of ATP. This energy is initially consumed by the cell to activate the glucose and form high energy intermediates in the form of phosphorylated products. The situation is similar to our day to day life experience as we invest small amounts of money to get better returns later. This phase involves following reactions:

1. Glucose is irreversibly phosphorylated to Glucose-6-phosphate by **hexokinase** or **glucokinase** in the presence of Mg^{2+} ions. One molecule of ATP is utilized in this step. **Glucokinase** (having low affinity for glucose) present in liver catalyses phosphorylation of only glucose. Similarly, **hexokinase** (having high affinity for glucose) present in almost all the tissues catalyses phosphorylation of various hexoses (fructose, glucose, mannose etc).
2. Glucose-6-phosphate undergoes isomerization to produce fructose-6-phosphate in the presence of **phosphohexoseisomerase** and Mg^{2+} .
3. Fructose-6-phosphate is irreversibly phosphorylated by **phosphofructokinase** into Fructose-1,6-bisphosphate. In this reaction one molecule of ATP is consumed. This reaction is the regulatory step in glycolysis.

B. Splitting phase: This phase leads to splitting of high energy 6 carbon intermediate into two 3 carbon products by involving the following reactions:

1. Enzyme **aldolase** catalyses the splitting of fructose-1,6-bisphosphate (6-C) into glyceraldehyde 3-phosphate (3-C) and dihydroxyacetone phosphate (3-C).
2. The enzyme **phosphotrioseisomerase** catalyses the interconversion of glyceraldehyde 3-phosphate and dihydroxyacetone phosphate. Thus, two molecules of glyceraldehydes-3-phosphate are produced from one molecule of glucose.

C. Energy generation phase: This phase includes reactions which finally return the principal energy initially consumed by cell with interest and involves the following reactions:

Glycolysis is the initial stage of glucose metabolism. It occurs in the cytosol. It does not involve oxygen. It produces two moles of ATP. Its end product is pyruvate.

1. Enzyme **Glyceraldehyde-3-phosphate dehydrogenase** converts glyceraldehyde-3-phosphate into 1, 3-bisphosphoglycerate. In this reaction, $\text{NADH} + \text{H}^+$ are produced and in turn it is oxidized via electron transport chain to synthesize ATP molecules.
2. **Phosphoglycerate kinase** catalyzes the reversible conversion of 1,3-bisphosphoglycerate into 3-phosphoglycerate. In this process, substrate level phosphorylation occurs as ATP is synthesized directly from the substrate without involving ETC.
3. 3-Phosphoglycerate is isomerized into 2-phosphoglycerate by **phosphoglycerate mutase**.
4. Enzyme **enolase** catalyses the conversion of 2-phosphoglycerate into phosphoenol pyruvate. This reaction requires Mg^{2+} or Mn^{2+} and is inhibited by fluoride.
5. The enzyme **pyruvate kinase** catalyses irreversible production of pyruvate from phosphoenol pyruvate. This reaction is also an example of substrate level phosphorylation where ATP is produced without the involvement of ETC. Complete sequence of reactions in glycolysis is given in Fig. 14.1.

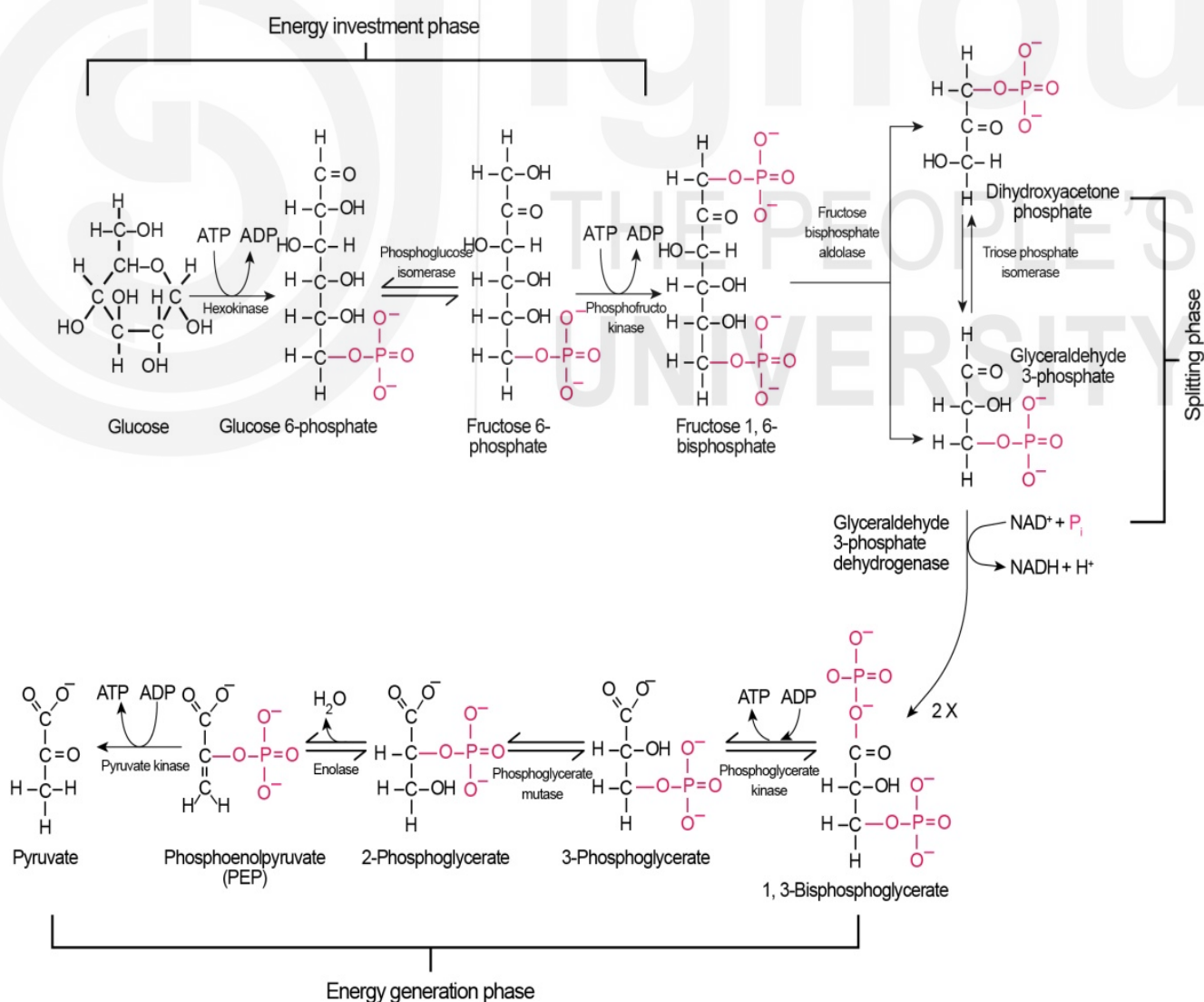
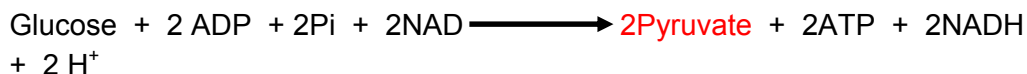


Fig. 14.1: Process of glycolysis.

Energy investment phase involves reactions for conversion of glucose to fructose-1,6- biphosphate with investment of two ATP molecules. Splitting phase results in splitting of 6-C molecule to two 3-C molecules which can interconvert. Energy generation phase proceeds with two 3-C molecules, therefore, all reactions will be doubled. It results in generation of energy rich molecules; 4 ATP by substrate level phosphorylation and 2 NADH.

Overall reaction



Sugars other than glucose such as fructose, galactose are converted into one of the intermediate of glycolysis. Now let us understand as to how it takes place.

14.2.1 Metabolism of Fructose

Fructose is a ketohexose. It is formed by the hydrolysis of sucrose (Table sugar). Sucrose as you will recall is a disaccharide formed of glucose and fructose. Fructose is also present in many food items (e.g., honey). It is metabolized in the liver. The major steps (Fig. 14.2) involved in its metabolism are as follows:-

1. Fructose is first converted into fructose-1-phosphate by **Fructokinase**.
2. Fructose-1-phosphate splits into glyceraldehyde and dihydroxyacetone phosphate (an intermediate of glycolysis) by **fructose-1-phosphate aldolase**.
3. Glyceraldehyde is converted into glyceraldehyde-3-phosphate by **triose kinase** and enters glycolysis.

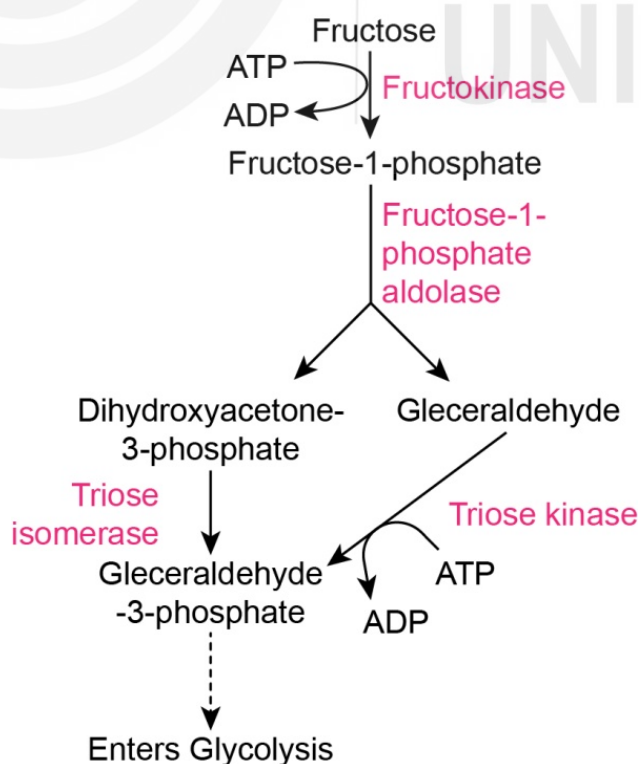


Fig. 14.2: Metabolism of fructose before it enters glycolysis.

14.2.2 Metabolism of Galactose

Galactose is also a hexose sugar. Its source is milk which contains lactose. Lactose is a disaccharide that gives glucose and galactose on hydrolysis. The main steps involved in its metabolism are as follows:-

1. Conversion of Galactose to Galactose-1-phosphate by **Galactokinase**.



2. Galactose-1-phosphate is converted into UDP-Galactose in the presence of galactose-1-phosphate uridylyltransferase. In this reaction UDP-glucose is the donor of uridinemonophosphate (UMP) and itself is converted to glucose-1-phosphate.
3. UDP-Galactose is converted into UDP-glucose by UDP galactose-4-epimerase. Sequence of reactions is shown in Fig. 14.3.

Glucose-1-phosphate formed from galactose is isomerized to glucose-6-phosphate, an intermediate of glycolysis by phosphoglucomutase.

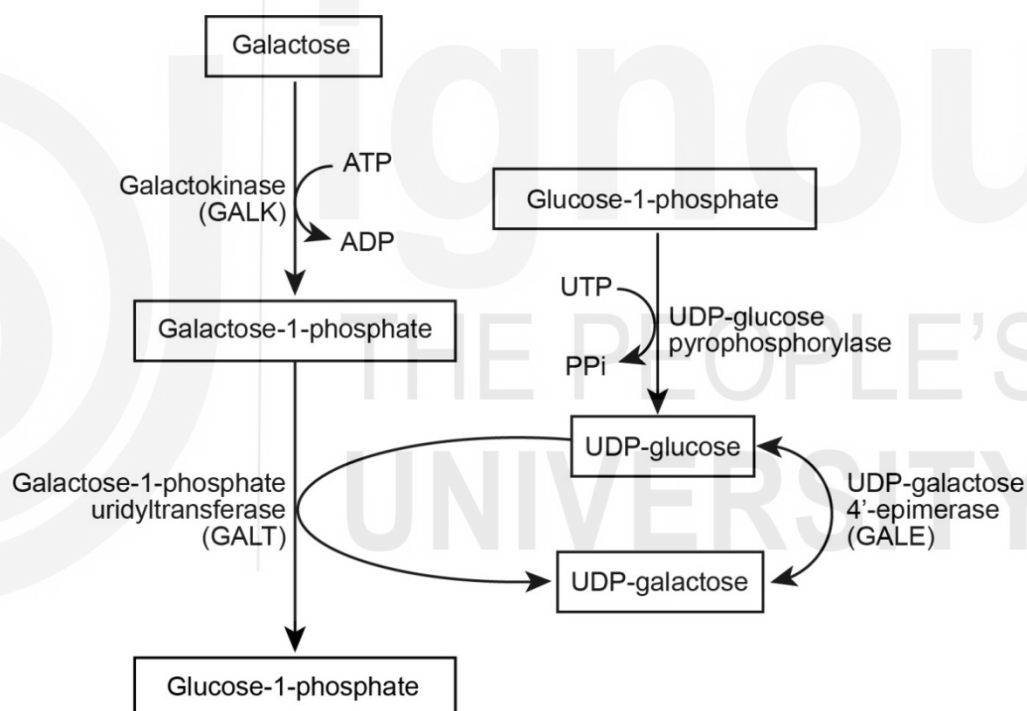


Fig. 14.3: Metabolism of galactose.

14.2.3 Regulation of Glycolysis

The enzymes which catalyse the irreversible steps in glycolysis regulate the pathway.

1. **Hexokinase:** This enzyme shows end product inhibition i.e. glucose-6-phosphate, the product of this reaction inhibits the enzyme to prevent its own accumulation.
2. **Phosphofructokinase (PFK):** This enzyme is the most important regulatory enzyme in glycolysis as it is the first committed enzyme of the pathway. Phosphofructokinase is an allosteric enzyme regulated by many allosteric effectors.

- Allosteric inhibitors include ATP, citrate and H^+ ion concentration (low pH).
- Allosteric activators comprise fructose 2, 6-bisphosphate, ADP, AMP and P_i .

Fructose 2, 6-bisphosphate is the most important regulatory factor controlling PFK. Fructose 2, 6-bisphosphate is formed from fructose-6-phosphate by the action of phosphofructokinase 2 (PFK-2). Fructose 2, 6-bisphosphatase breaks down fructose 2, 6 bisphosphate back into fructose-6-phosphate. In fact, PFK-2 and fructose 2, 6-bisphosphatase are the bi-functional enzyme activities of the same protein moiety. Both these activities are controlled by covalent modification regulated by cAMP in response to hormones insulin and glucagon.

3. **Pyruvate kinase:** This enzyme exists in two different forms:

- Dephosphorylated inactive form
- Phosphorylated active form

Inactivation of pyruvate kinase by phosphorylation is carried out by cAMP-dependent protein kinase. The hormone glucagon can prevent hepatic glycolysis by activation of cAMP dependent protein kinase. Enzyme phosphatase can convert inactive form of pyruvate kinase into active form by dephosphorylation. Figure 14.4 gives an overall view of regulation of glycolysis.

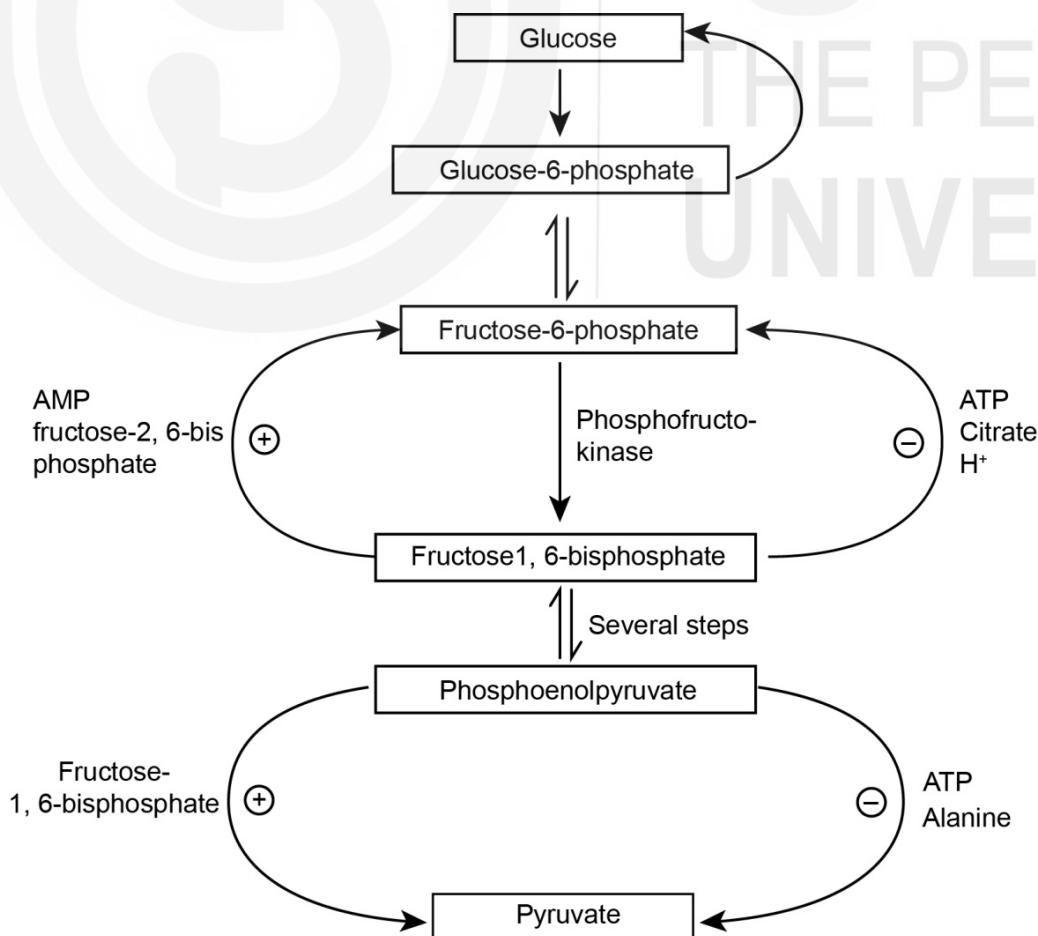


Fig.14.4: Allosteric modulators of glycolysis.

SAQ 1

Match the enzymes in **column A** with substrate/product pair in **column B**.

Column A	Column B
i) Hexokinase	(a) 3-Phosphoglycerate(3-PGA)/2-PGA
ii) Glyceraldehyde-3 phosphate dehydrogenase	(b) 2-phosphoglycerate / phosphoenol pyruvate
iii) Phosphoglyceratemutase	(c) fructose-1,6-bisphosphate/ glyceraldehyde 3-phosphate & DHAP
iv) Phosphofructokinase-I	(d) glyceraldehyde-3-phosphate/1,3-bisphosphoglycerate (BPG)
v) Enolase	(e) fructose-6-phosphate / fructose-1,6-bisphosphate
vi) Aldolase	(f) glucose-1-phosphate/ glucose-6-phosphate
vii) Phosphoglucomutase	(g) glucose/glucose-6-phosphate

14.3 FATE OF PYRUVATE

Breakdown of glucose into pyruvic acid is called glycolysis and further processing of pyruvic acid in anaerobes is called fermentation. Its main purpose is to regenerate NAD^+ so that ATP production by glycolysis can continue.

It may be of interest for you to know that earliest organisms lived in an atmosphere devoid of oxygen; therefore they had to develop strategies to derive energy from fuel molecules to survive under anaerobic conditions. Most of them depended on glycolysis for the breakdown of carbon. It is a near universal pathway as it is equally relevant in aerobic organisms. With the emergence of oxygen there has been shift towards a more efficient mode of energy generation with the complete breakdown of carbon to CO_2 and ATP generation by oxidative phosphorylation. Yet, most organisms / tissues have retained the ability to ferment under low oxygen such as skeletal muscles during strenuous muscular activity, and cornea of the eye. An extreme example is of erythrocytes that lack mitochondria and ferment glucose even in the presence of oxygen. In many niches anaerobic organisms still survive by utilising primitive catabolic pathways. **The process of generation of energy (ATP) by substrate level phosphorylation from incomplete oxidation of fuels like glucose under anaerobic conditions is known as fermentation.**

Let us learn about the reactions of pyruvate under anaerobic and aerobic conditions.

14.3.1 Anaerobic Respiration

Fermentation is an inefficient mode of energy generation as the end products are organic compounds rather than CO_2 that still conserve lot of energy. Let us study about lactate and alcohol fermentation reactions. In both examples you will note that there is no net change in the oxidation state of carbon which means that H: C ratio is same for glucose and the end product.

Lactic acid fermentation: Lactic acid is formed under low oxygen content during fermentation which is carried out by bacteria (*Lactobacillus* species), some fungi and rapidly contracting skeletal muscle. A single reaction catalysed by **lactate dehydrogenase (LDH)** reduces pyruvate to lactate (Fig. 14.5). NADH utilized in this step is obtained from the reaction catalyzed by glyceraldehyde-3-phosphate dehydrogenase in glycolysis. The formation of lactate allows the regeneration of NAD^+ which can be reused in glycolysis. Glycolysis followed by lactic acid fermentation results in net formation of 2 ATP for each molecule of glucose and no net production of NAD^+/NADH .

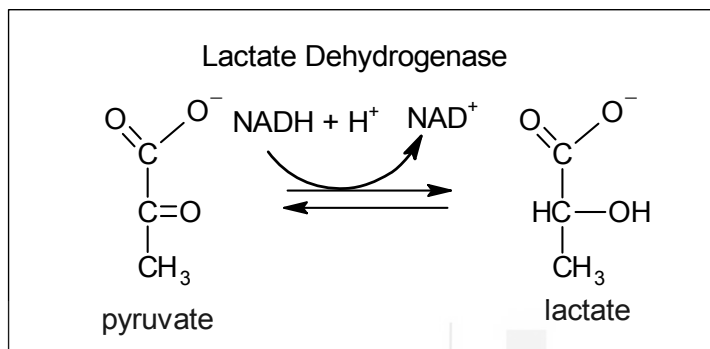


Fig. 14.5: Reduction of pyruvate to lactate. Pyruvate formed in glycolysis is reduced to lactate under anaerobic conditions to regenerate NAD^+ .

Alcohol fermentation: In some bacteria and fungi (e.g., yeast), pyruvate is fermented to ethyl alcohol in two steps. In the first step, pyruvate is decarboxylated irreversibly to acetaldehyde by a thiamine pyrophosphate (TPP) dependent **pyruvate decarboxylase**. It also requires Mg^{2+} . In general, the decarboxylation of α - keto acids like pyruvate is assisted by thiamine pyrophosphate (TPP). This enzyme is absent in animal tissues.

In the second step, acetaldehyde is reduced to ethyl alcohol by **alcohol dehydrogenase (Fig. 14.6)**. Ethyl alcohol is excreted by microorganisms as accumulation of alcohol beyond a certain limit can kill them. In many organisms including humans, the enzyme metabolises ethyl alcohol.

Alcohol fermentation is very important from industrial point of view and has been extensively exploited for beer and wine production and baking industry.

CO_2 released during alcohol fermentation gives the characteristic carbonation of champagne in brewing and allows dough to rise in baking.

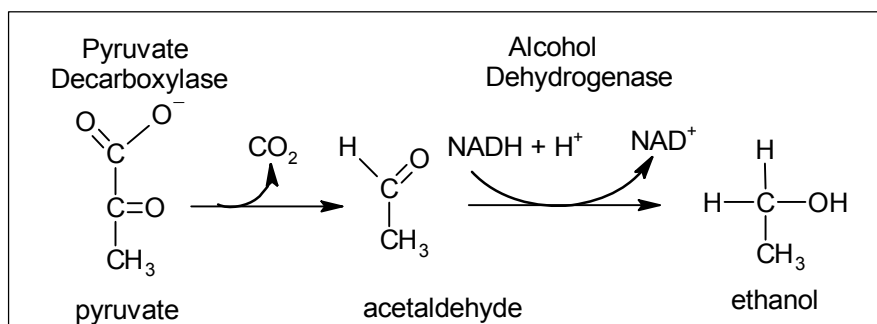


Fig. 14.6: The conversion of pyruvate to ethanol reoxidizes NADH.

14.3.2 Aerobic Respiration

Under aerobic conditions, pyruvate is converted into acetyl CoA (2-C) by oxidative decarboxylation, a reaction catalyzed by **pyruvate dehydrogenase multienzyme complex** present in mitochondria. This enzyme requires two

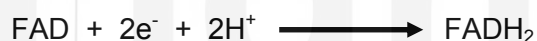
coenzymes (CoA and NAD⁺) and three prosthetic groups (TPP, FAD, lipoamide), of which all are regenerated except CoA (which is converted into Acetyl CoA). NADH produced enters ETC (Electron Transport Chain) for the production of ATP by oxidative phosphorylation. The overall reaction can be summarized as below:-



Pyruvate dehydrogenase complex (PDH) comprises 3 enzymes. It is present in mitochondrial matrix. It is a large enzyme complex containing many copies of each of the 3 enzymes E₁, E₂ and E₃.

- E₁ is **pyruvate dehydrogenase**: Its prosthetic group is TPP (thiamine pyrophosphate).
- E₂ is **dihydrolipoyl transacetylase**: Its prosthetic group is lipoamide.
- E₃ is **dihydrolipoyl dehydrogenase**: Its prosthetic group is FAD (flavin adenine dinucleotide).

FAD is permanent part of the complex whereas NAD is a coenzyme that reversibly binds to the enzymes. FAD accepts and donates 2 electrons with 2 protons (2H⁺).



TPP is a derivative of vitamin B₁. Its deficiency leads to *BeriBeri*, a disease which affects brain, because brain depends on glucose metabolism for energy (You will study in detail about vitamins in Unit 16 of this course). The reaction catalyzed by **pyruvate dehydrogenase complex** (Fig. 14.7) is summarized below:-

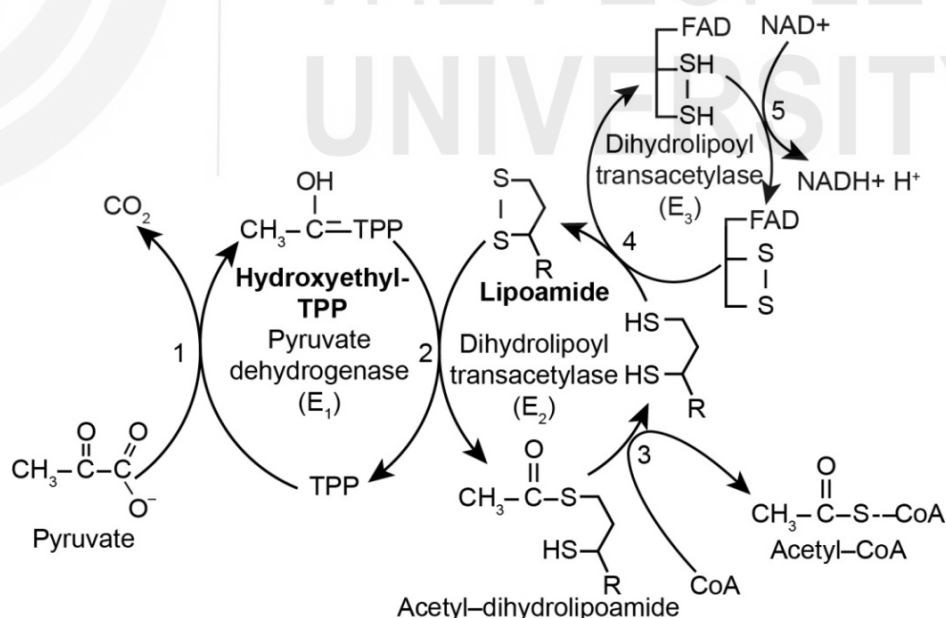


Fig. 14.7: Structural components of PDH and reactions catalyzed. (1) Pyruvate is decarboxylated by E₁ and TPP carries the remaining hydroxyl ethyl molecule to lipoamide arm of E₂ (2) E₂ converts it to acetyl group which is then transferred to CoA to form final product acetyl CoA (3) In the process, disulfide of lipoamide is reduced to two -SH, which is regenerated by oxidation by FAD at E₃ (4) FAD itself is reduced to FADH₂. (5) It is regenerated from FADH₂ by NAD⁺ which itself is reduced to NADH + H⁺.

Regulation of Pyruvate dehydrogenase complex (PDH)

PDH is the enzyme which catalyzes the conversion of pyruvic acid into acetyl CoA. This enzyme shows end product inhibition and is inhibited by acetyl CoA and NADH. PDH is also regulated by phosphorylation and dephosphorylation. The enzyme is active in its dephosphorylated form. PDH kinase converts PDH into its inactive form.

- ATP, NADH and acetyl CoA activate PDH kinase.
- NAD^+ , CoA and pyruvate inactivate PDH kinase.

Thus it can be seen that in the presence of high energy signals, PDH is turned off by activation of PDH kinase. PDH phosphatase converts PDH into its active form by removing the phosphate group.

➤ Ca^{2+} , Mg^{2+} and insulin promote the activity of PDH phosphatase.

Thus it can be seen that Ca^{2+} ions released during muscle contraction stimulate the activity of PDH for energy production.

SAQ 2

a) Choose the correct option given in the parantheses:

- The net yield of ATP during lactate fermentation is (2/4).
- is the example of human tissue that can ferment glucose to(skeletal muscle, lactate/skeletal muscle, ethyl alcohol).
- The end products of alcohol fermentation are and (ethanol; CO_2 /acetaldehyde; NADH).
- In both lactate and alcohol fermentation the purpose of going beyond glycolysis is to (regenerate more energy, regenerate NAD^+).

b) Match the enzyme component of PDH in Column A with its prosthetic group in Column B:

Column A	Column B
(i) Pyruvate dehydrogenase	(a) Lipoamide
(ii) Dihydrolipoyltransacetylase	(b) FAD
(iii) Dihydrolipoyl dehydrogenase	(c) TPP

14.4 KREB'S CYCLE/CITRIC ACID CYCLE

It is a common pathway for the complete oxidation of acetyl CoA into CO_2 and H_2O . This pathway is called a cycle because its end product oxaloacetate is reused to start another cycle. This is the central pathway connecting all the metabolic pathways; directly or indirectly.

HIGHLIGHTS OF CITRIC ACID CYCLE

Citric acid cycle is the final stage of oxidation of glucose. It is a common pathway for the catabolism of carbohydrates, lipids and proteins. Each acetyl CoA which enters citric acid cycle produces two molecules of CO₂, one molecule of GTP and reducing equivalents in the form of NADH and FADH₂ which enter ETC to form ATP.

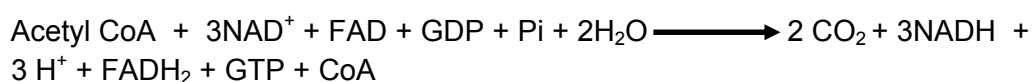
- This cycle is also called tricarboxylic acid cycle (TCA cycle) as tricarboxylic acids namely **citrate, cis aconitate and isocitrate** participate at the outset of the cycle
- This pathway should not be viewed as closed circle, since many compounds enter and leave this pathway.
- This pathway is both anabolic and catabolic in nature, hence it is also regarded as *amphibolic* pathway.
- Although there is no direct participation of oxygen in this cycle, but still this process takes place under aerobic conditions. This is due to the fact that NAD⁺ and FAD required to operate this cycle are regenerated only in the presence of oxygen.

The steps in the citric acid cycle are described below:

1. In the first reaction, acetyl CoA (2-C) condenses with oxaloacetate (4-C) in the presence of **citrate synthase** to form citric acid (hence the name of the cycle).
2. Citrate is isomerized to isocitrate in a two step reaction of dehydration followed by hydration. This reaction is catalysed by Fe²⁺ activated enzyme **aconitase** and involves an intermediate *cis*-aconitate (not shown).
3. **Isocitric dehydrogenase** converts isocitrate (6-C) into α-ketoglutarate (5-C) by oxidative decarboxylation. Formation of NADH and CO₂ occurs during this reaction.
4. α-ketoglutarate (5-C) gets converted to succinyl CoA (4-C) again by oxidative decarboxylation. In this reaction, NADH and CO₂ are liberated. **α-ketoglutarate dehydrogenase** catalysing this reaction requires five cofactors as in Pyruvate dehydrogenase complex. These include three prosthetic groups (TPP, lipoamide and FAD) and two coenzymes (CoA and NADH).
5. Succinyl CoA is converted to succinate by **succinate thiokinase**. This reaction is coupled with the phosphorylation of GDP to GTP (substrate level phosphorylation).
6. Succinate is oxidized to fumarate by **succinate dehydrogenase** in a reaction which results in the formation of FADH₂ from FAD.
7. **Fumarase** converts fumarate to malate with the addition of H₂O.
8. An oxidation reaction converts malate into oxaloacetate. The reaction catalyzed by **malate dehydrogenase** results in the synthesis of NADH.

Oxaloacetate regenerated in this cycle is again available to combine with another molecule of acetyl CoA to start another cycle (Fig. 14.8).

Overall reaction



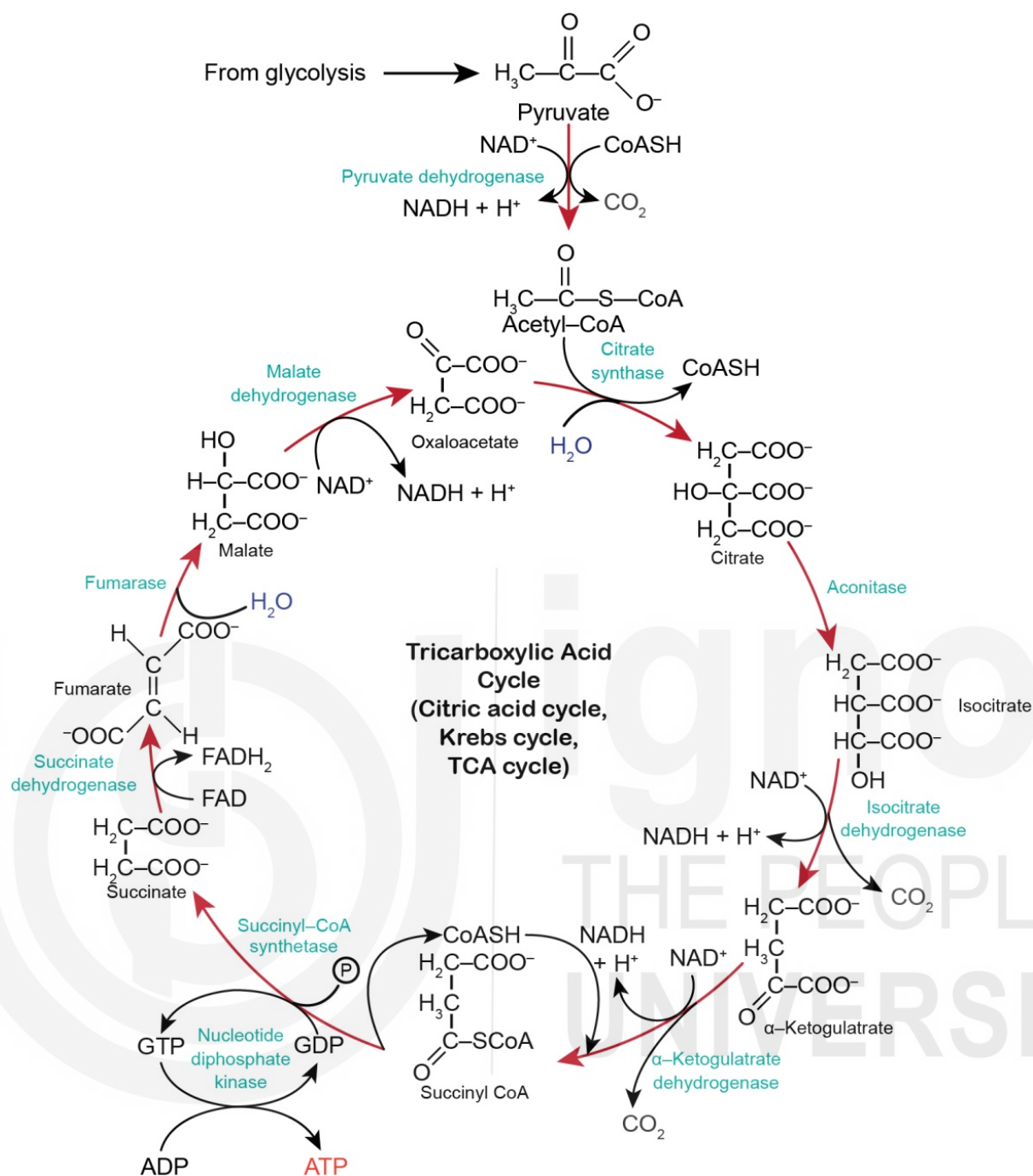


Fig. 14.8: Citric acid cycle.

14.4.1 Energetics of Citric Acid Cycle

Let us calculate how much energy (ATP) is extracted from glucose breakdown under aerobic conditions:

- The degradation of glucose to 2 pyruvate by glycolysis yields 2 ATP and 2 $\text{NADH} + 2\text{H}^+$.
- The oxidative decarboxylation of 2 pyruvate to 2 acetyl CoA generates 2 $\text{NADH} + 2\text{H}^+$.
- Oxidation of each acetyl CoA via citric acid cycle produces 3 NADH , 1 FADH_2 and 1 GTP . Therefore, two acetyl CoA will produce double the number of reducing equivalents and GTP .

- Oxidation of NADH and FADH₂ by ETC is coupled to oxidative phosphorylation. Each NADH produces 2.5 ATP whereas FADH₂ produces 1.5 ATP.
- The final count of ATP is: $10 \times 2.5 + 2 \times 1.5 + 4 = 32$.

The number of ATP produced varies from 30 – 32, depending on the shuttle operative in a given cell.

14.4.2 Regulation of Citric Acid Cycle

Three enzymes namely citrate synthase, isocitrate dehydrogenase and α -ketoglutarate dehydrogenase regulate citric acid cycle.

1. Citrate synthase activity is inhibited by ATP, NADH, acetyl CoA and succinyl CoA.
2. Isocitrate dehydrogenase activity is activated by ADP and inhibited by ATP and NADH.
3. α -Ketoglutarate dehydrogenase activity is inhibited by succinyl CoA and NADH.

It is important to understand that availability of ADP is very crucial for the continuous operation of citric acid cycle. If ADP levels are low, oxidation of NADH and FADH₂ through electron transport chain will stop. Accumulation of NADH and FADH₂ inhibits the above stated enzymes.

SAQ 3

How many molecules of CO₂, NADH and FADH₂ are produced in one citric acid cycle?

14.5 GLUCONEOGENESIS

Gluconeogenesis refers to synthesis of glucose from non-carbohydrate precursors such as lactate, pyruvate, glycerol, and products derived from glucogenic amino acids. These precursors are either converted to pyruvate or they may enter as intermediates at later points in the pathway such as DHAP and OAA.

HIGHLIGHTS OF GLUCONEOGENESIS

- It mainly occurs in the cytosol of the cell while some precursors may be formed in the mitochondria.
- Liver is the major organ which contributes to gluconeogenesis.
- Gluconeogenesis is very essential to meet basal requirements of the body for glucose during fasting conditions.
- Gluconeogenesis is effective to remove certain metabolites accumulated in the blood, e.g., lactate and glycerol.
- This pathway is significant to ensure continuous supply of glucose to the brain.

The partial degradation of most amino acids except lysine and leucine generates one or more gluconeogenic precursors. These amino acids are classified as glucogenic amino acids.

14.5.1 Gluconeogenesis from Pyruvate

The conversion of pyruvate or other non carbohydrate precursors to glucose is not a simple reversal of glycolysis. The two pathways differ in three essentially irreversible steps of glycolysis that account for most of the negative free energy change. We will study in detail how these three steps are bypassed in gluconeogenesis by separate enzymes. These three reactions are also irreversible under cellular conditions. As expected the two pathways are subject to reciprocal regulation at these three steps. Let us learn about the unique reactions of gluconeogenesis.

- a) **Conversion of pyruvate to phosphoenolpyruvate:** Pyruvate is initially converted into oxaloacetate by biotin dependent mitochondrial enzyme namely **pyruvate carboxylase**. This reaction requires ATP and CO_2 . Oxaloacetate being impermeable to mitochondrial membrane is converted to malate by **malate dehydrogenase** (an enzyme present both in cytosol and mitochondria). Malate is transported out of the mitochondrial membrane and is again converted into oxaloacetate by **malate dehydrogenase**. In the cytosol, oxaloacetate is converted into phosphoenolpyruvate by **phosphoenolpyruvate carboxykinase**. In this reaction, GTP is utilized. Thus in gluconeogenesis, 2 ATP molecules are used up in the conversion of pyruvate to phosphoenolpyruvate which is in contrast to only 1 ATP molecule produced in glycolysis for this reaction (Fig. 14.9).

Gluconeogenesis is the process by which glucose is produced from non carbohydrate precursors such as lactate, propionate and glycerol.

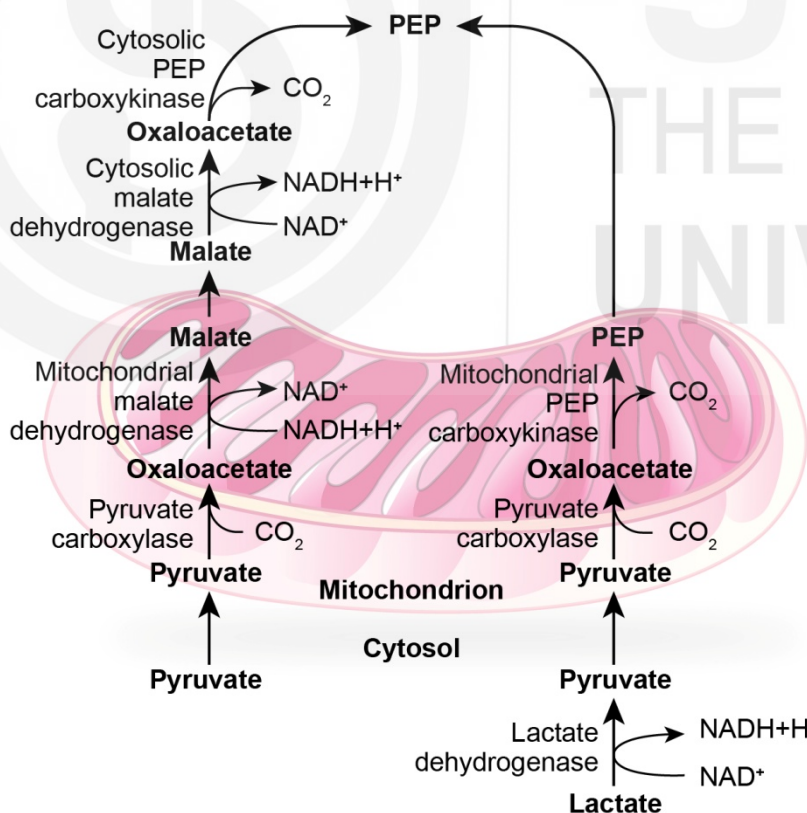


Fig. 14.9: The conversion of pyruvate to phosphoenol pyruvate.

- b) **Conversion of fructose-1,6-bisphosphate to fructose-6-phosphate:** The enzyme **fructose-1,6-bisphosphatase** converts fructose-1,6-bisphosphate into fructose-6-phosphate. This enzyme requires Mg^{2+} for its activity and is absent in smooth muscles.

- c) **Conversion of glucose-6-phosphate into glucose:** This conversion is carried by **glucose-6-phosphatase**, an enzyme mostly present in liver and kidney but absent in muscles and brain. The presence or absence of this enzyme in a tissue determines whether or not that tissue is capable of producing glucose by this process.

Figure 14.10 summarises the reactions of glycolysis and gluconeogenesis. The enzymes catalysing the unique steps are highlighted with different colours.

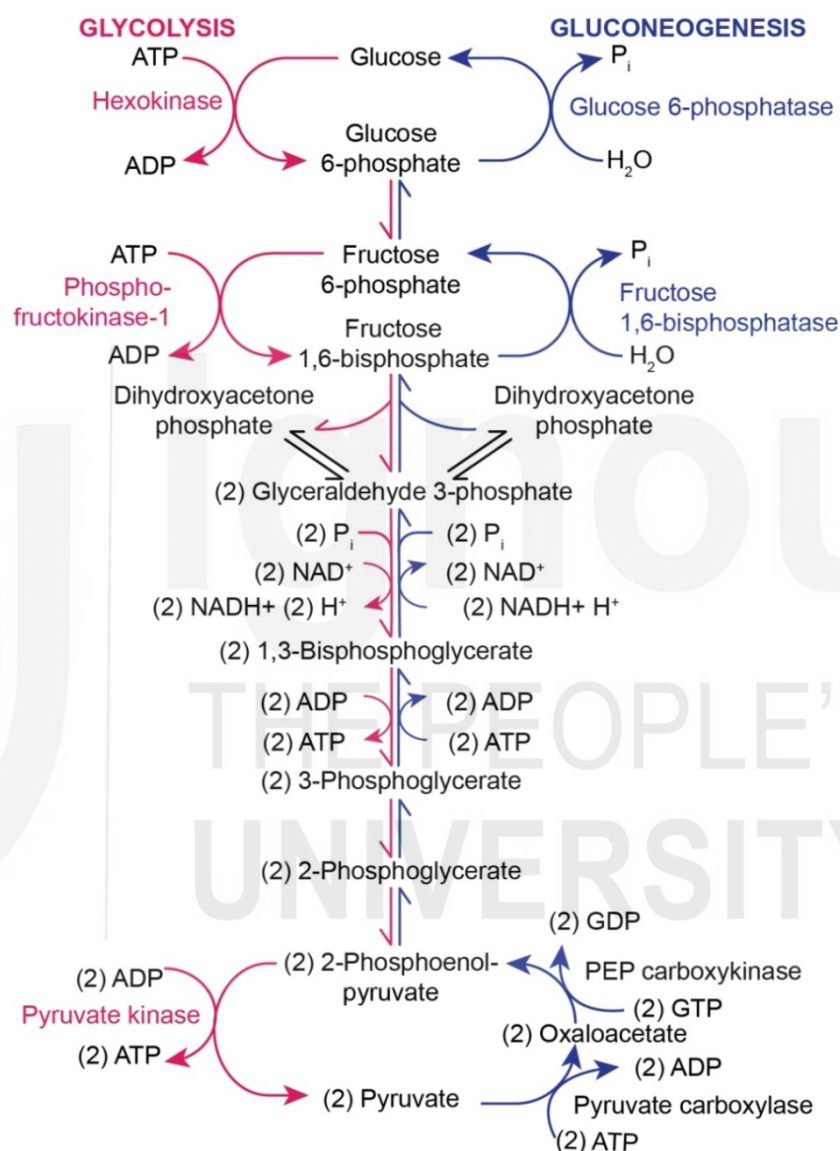


Fig. 14.10: Reactions of glycolysis and gluconeogenesis.

The synthesis of glucose starting from pyruvate is an energetically expensive process. The net reaction is:



It is clear from the net reaction that for each glucose formed from pyruvate, six high energy phosphate groups (4ATP and 2GTP) and 2 NADH are needed. It is much higher than the energy requirement if gluconeogenesis was a simple reversal of glycolysis. The increased energy cost is to make gluconeogenesis irreversible under cellular conditions.

14.5.2 Gluconeogenesis from Lactate

Lactate produced by anaerobic respiration in muscle cannot be utilized for the production of glucose due to lack of glucose-6-phosphatase and fructose-1,6-biphosphatase in muscle. Lactate is transported to liver through blood where it is oxidized to pyruvate. The latter is converted into glucose by gluconeogenesis in liver and glucose is transported back to the muscle by blood.

14.5.3 Gluconeogenesis from Propionate

Propionyl CoA is produced from the β -oxidation of odd chain fatty acids and break down of some amino acids such as methionine and isoleucine. It is converted into methyl malonyl CoA by the enzyme propionyl CoA carboxylase in the presence of ATP, biotin and CoA. Methylmalonyl CoA is converted into succinyl CoA which enters citric acid cycle. Oxaloacetate can produce glucose *via* gluconeogenesis.

14.5.4 Gluconeogenesis from Glycerol

Glycerol (produced in the hydrolysis of triglycerides) is initially converted into glycerol-3-phosphate by **glycerol kinase**. ATP is used in this reaction. Glycerol-3-phosphate is then converted into dihydroxyacetone phosphate by **glycerol-3-phosphate dehydrogenase**. Dihydroxyacetone phosphate being an intermediate of glycolysis can be used for the formation of glucose.

14.5.5 Gluconeogenesis from Amino Acids

The catabolism of glucogenic amino acids results in the formation of pyruvate or the intermediates of the citric acid cycle which ultimately are converted to glucose.

SAQ 4

Name the enzymes unique to the pathway of gluconeogenesis.

14.6 PENTOSE PHOSPHATE PATHWAY

The pentose phosphate pathway is an alternative to glycolysis for glucose metabolism. It is also known as hexose monophosphate shunt (HMP shunt) or phosphogluconate pathway.

HIGHLIGHTS OF PENTOSE PHOSPHATE PATHWAY

- It occurs in cell cytosol.
- This pathway is more anabolic in nature, since it results into biosynthesis of NADPH and pentoses.
- Dietary pentose sugars derived from the digestion of nucleic acids may be metabolized through this pathway.
- This pathway is involved in the interconversion of monomeric sugars.

- Upto 30% of glucose in liver can enter this pathway.
- This pathway is absent in skeletal muscles.

This pathway is divided into two distinct phases:

- A. **Oxidative phase**, an irreversible phase in which NADPH is generated.
- B. **Non Oxidative phase** is a reversible phase in which 5-carbon sugars are synthesized.

A. Oxidative phase

1. **Glucose-6-phosphate dehydrogenase** converts glucose-6-phosphate into 6-phosphogluconolactone. In this reaction NADPH + H⁺ is produced from NADP⁺.
2. 6-phosphogluconolactone is then hydrolyzed to 6-phosphogluconate by **gluconolactone hydrolase**.
3. In the next reaction catalysed by **phosphogluconate dehydrogenase**, NADPH + H⁺ is synthesized to form an unstable compound which undergoes decarboxylation to produce Ribulose-5 phosphate.

B. Non Oxidative phase

In this phase, interconversion of three, four, five and seven carbon monosaccharides takes place.

1. Enzymes **epimerase** and **ketoisomerase** convert Ribulose-5-phosphate (5-C) into Xylulose-5-phosphate (5-C) and Ribose-5-phosphate (5-C), respectively.
2. A two carbon moiety is transferred from xylulose-5-phosphate to ribose-5-phosphate in the presence of **transketolase** to give glyceraldehyde-3-phosphate (3-C) and sedoheptulose-7-phosphate (7-C). Transketolase is dependent on coenzyme TPP & Mg²⁺ for its activity.
3. **Transaldolase** catalyses the transfer of a three carbon moiety from sedoheptulose-7-phosphate to glyceraldehydes-3-phosphate to form fructose-6-phosphate (6-C) and erythrose-4-phosphate (4-C).
4. **Transketolase** later transfers a 2 carbon moiety from xylulose-5-phosphate to erythrose-4-phosphate to produce fructose-6-phosphate and glyceraldehydes-3-phosphate.
5. Fructose-6-phosphate and glyceraldehydes-3-phosphate then enter glycolysis and citric acid cycle for their catabolism. In this pathway, out of 6 molecules of glucose-6-phosphate, 5 molecules are regenerated with the production of 12 molecules of NADPH and 6 molecules of CO₂.

Pentose phosphate pathway is a significant pathway of glucose metabolism in which interconversion of sugars occurs. It results in the formation of tetroses and pentoses which are used for the synthesis of aromatic amino acids and nucleic acids, respectively. It also yields NADPH required for the synthesis of lipids and reduced glutathione.

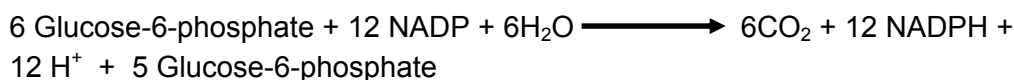
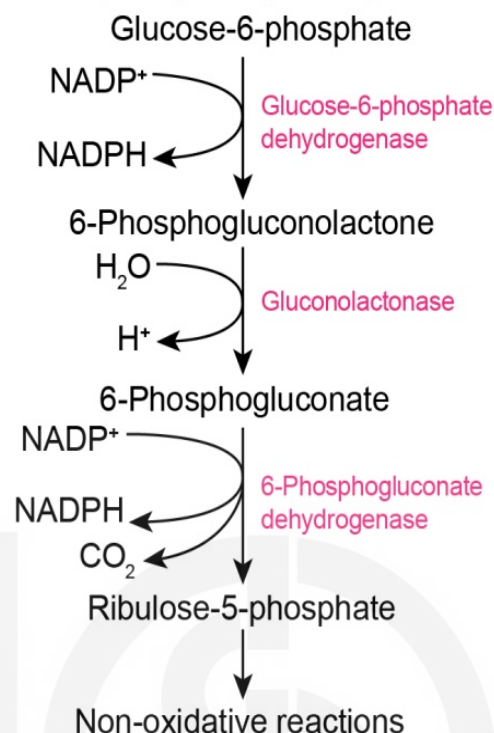
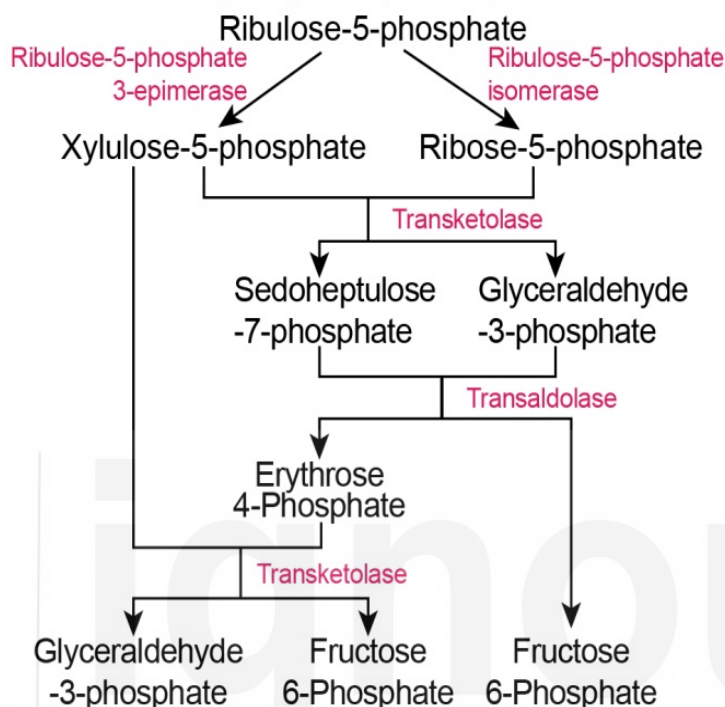
Overall reaction**Oxidative stage of Pentose phosphate pathway****Non-Oxidative stage of Pentose phosphate pathway**

Fig. 14.11: Reactions of pentose phosphate pathway: The pathway is depicted in two parts representing the oxidative and non-oxidative branches.

14.6.1 Significance of Pentose Phosphate Pathway

- Ribose-5-phosphate and erythrose-4-phosphate produced in this pathway are used in the synthesis of nucleic acids and aromatic amino acids respectively.
- Most of the body tissues (**liver, adipose tissue, adrenal gland, testes and lactating mammary gland**) involved in the biosynthesis of fatty acids and steroids use NADPH produced in Pentose phosphate pathway.
- This pathway also assumes significance in RBCs where a large amount of NADPH produced is used in the reduction of glutathione and thus prevention of oxidative stress. The inability to maintain reduced glutathione in RBCs leads to increased accumulation of peroxides mainly H_2O_2 that results into weakening of cell membrane and thus causes haemolysis. Glutathione removes peroxides and protects RBCs from oxidative damage.

SAQ 5

Write significance of pentose phosphate pathway.

14.6.2 COORDINATED REGULATION OF GLYCOGENEogenesis AND GLYCOLYSIS

You have learnt in the previous section that glycolysis and gluconeogenesis share seven out of the ten reactions. Therefore, a reciprocal and coordinated regulation is exercised at three irreversible steps hexokinase, phosphofructokinase and pyruvate kinase mainly governed by energy status and blood glucose levels.

Figure 14.12 shows coordinated regulation of the two pathways by allosteric modulators. You may note that the allosteric regulators act in opposite way on the two pathways.

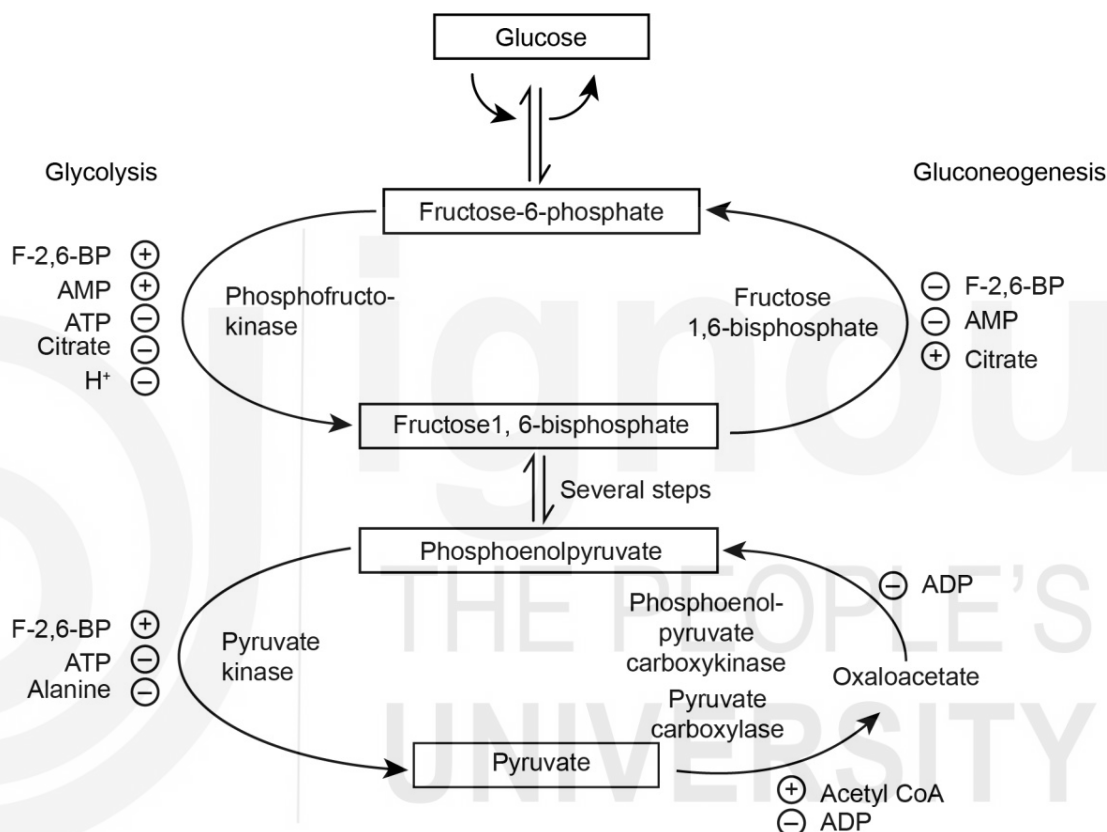


Fig. 14.12: Coordinated regulation of glycolysis and gluconeogenesis.

In nutshell, glycolysis is favoured when AMP or ADP is high and gluconeogenesis occurs when ATP, citrate or other cellular indicators are abundant.

Further, hormones insulin and glucagon, in response to blood glucose level alter the levels of fructose 2,6 bisphosphate and bring about **reversible covalent modification** of target enzymes.

Glucagon released in response to low blood glucose level binds to cell surface receptor and activates adenylyl cyclase to convert ATP to an intracellular second messenger, cyclic AMP (cAMP). cAMP in turn activates protein kinase A (PKA) which phosphorylates PFK-2/ FBPase-2, activating the phosphatase (Fig. 14.13) and simultaneously inhibiting PFK-2. The net effect is a reduction in fructose 2,6 bisphosphate level, creating conditions for gluconeogenesis and generating more glucose needed to replenish the blood glucose levels. Insulin has opposite effect.

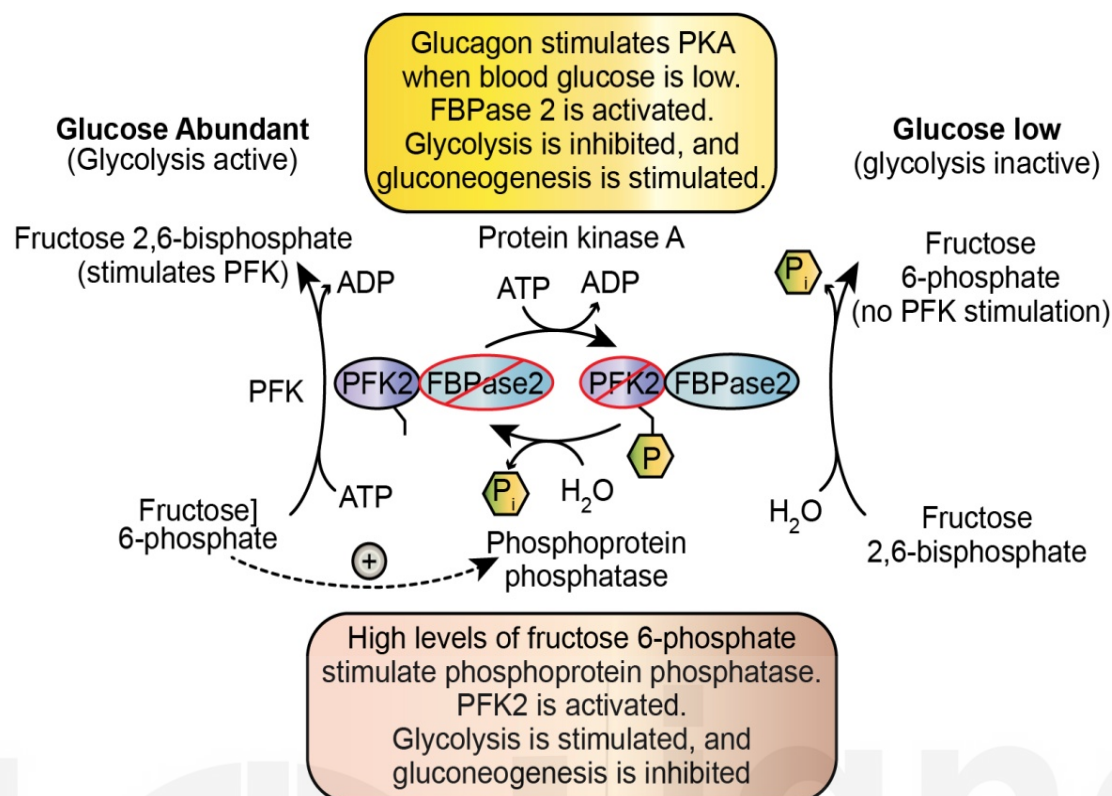


Fig. 14.13: Covalent modification of PFK2/FBPase and regulation of glycolysis and gluconeogenesis in response to hormones.

14.7 SUMMARY

Let us summarise what you have learnt so far:

- Carbohydrate metabolism is initiated as oxidation of glucose by glycolysis with release of some energy.
- Pyruvate is the end product of glycolysis which has different fates. Under anaerobic conditions, it undergoes fermentation and purpose is to regenerate NAD^+ for glycolysis to continue.
- Under aerobic conditions, pyruvate enters citric acid cycle where pyruvate is completely oxidized to CO_2 and H_2O with the release of energy and formation of reducing equivalents NADH and FADH_2 . These enter ETC to produce ATP.
- Pentose phosphate pathway involves interconversion of sugars and formation of tetroses required for the synthesis of aromatic amino acids and nucleic acids, respectively.
- When glucose level in the body is low, glucose is synthesized in the cell from non-carbohydrate precursors by gluconeogenesis. These precursors include pyruvate, lactate, propionate, glycerol and amino acids.
- Regulation of these pathways is at the level of irreversible reactions. It takes place through various mechanisms such as end product inhibition, reversible covalent modification of the enzymes or allosteric modulators.

14.8 TERMINAL QUESTIONS

1. What is substrate level phosphorylation?
2. Explain the importance of Krebs's cycle?
3. Define gluconeogenesis. Name five gluconeogenic substrates. Discuss the role of gluconeogenesis.
4. Describe the coordinated regulation of glycolysis and gluconeogenesis, taking mammalian liver as an example. What would happen if both pathways are simultaneously active at a high rate?
5. Explain as to how the three irreversible reactions of glycolysis are bypassed in gluconeogenesis.
6. Briefly explain the regulation of glycolysis. Why is step 3 and not step 1 of glycolysis the major control point?
7. Describe the two phases of glycolysis. What is the net outcome of each phase?

14.9 ANSWERS

Self Assessment Questions

1. (i) g; (ii) d; (iii) a; (iv) e; (v) b; (vi) c; (vii) f.
2. a) (i) 2
(ii) skeletal muscle and lactate
(iii) ethanol and CO_2
(iv) regenerate NAD^+
b) (i) c; (ii) a; (iii) b.
3. 2 molecules of CO_2 , 3 molecules of NADH and 1 molecules of FADH_2 are formed in each cycle of citric acid cycle.
4. (i) Fructose-1, 6-bisphosphatase- converts fructose-1, 6-phosphate to fructose-6-phosphate.
(ii) glucose-6-phosphatase converts glucose-6-phosphate into glucose.
(iii) phosphoenol pyruvate carboxykinase and pyruvate carboxylase convert pyruvate to phosphoenol pyruvate.
5.
 - Ribose-5-phosphate is used in the synthesis of nucleic acids and and erythrose-4-phosphate for aromatic amino acids,
 - NADPH produced is used in the biosynthesis of fatty acids and steroids
 - In RBCs, NADPH produced is used in the reduction of glutathione and thus helps in prevention of oxidative stress.

Terminal Questions

1. When ATP is synthesised by phosphoryl group transfer from activated phosphorylated substrate to ADP is known as substrate level phosphorylation, as for example the conversion of 1,3 BPG to 3- PGA.
2. Kreb's cycle is the central pathway of metabolism of carbohydrates, lipids and proteins. It produces reducing equivalents in the form of NADH and FADH₂ which enter ETC to produce ATP.
3. Gluconeogenesis refers to *de novo* synthesis of glucose from non-carbohydrate precursors. Five gluconeogenic substrates include lactate, pyruvate, propionate, glycerol, and glucogenic amino acids. The pathway plays an important role to ensure continuous supply of glucose to brain, meet basal requirements of the body for glucose during fasting and to remove certain metabolites which are harmful to cells if they are allowed to accumulate.
4. Coordinated and reciprocal regulation of glycolysis and gluconeogenesis are mainly governed by energy status and blood glucose levels. The reciprocal regulation of the two pathways is exercised at three irreversible steps. Refer to section 4.3.1 and 4.3.2 for more details.

If both pathways operate at a high rate simultaneously, no useful product will be formed and the energy released gets wasted as heat.
5. Three irreversible reactions of glycolysis catalysed by hexokinase, PFK-1 and pyruvate kinase are bypassed in gluconeogenesis by alternate reactions. Refer to section 4.2.1 for details.
6. The glycolytic pathway is regulated at the steps catalysed by hexokinase, phosphofructokinase-I and pyruvate kinase. Step 1 of glycolysis is not subject to stringent control because glucose-6-phosphate also fed to pathways such as glycogen synthesis and pentose phosphate pathway. Reaction 3 catalysed by PFK-I is the first unique step of glycolysis and the product is only directed to glycolysis. Therefore, it is more important for regulation of glycolysis. Refer to Fig.14.12 for an overview of regulation of glycolysis.
7. Investment (preparatory) and pay off phase. The investment phase consumes two molecules of ATP whereas the pay off phase results in formation of 4 ATP molecules by substrate level phosphorylation and 2 molecules of NADH. Refer to section 2.3 for more details.

CARBOHYDRATE METABOLISM-II

Structure

15.1	Introduction	Respiratory Complex-III: CoQH ₂ -Cytochrome-c Oxidoreductase
	Objectives	
15.2	Glycogen: A Storage Polymer	Respiratory Complex-IV: Cytochrome-c Oxidoreductase
15.3	Glycogenesis	
15.4	Glycogenolysis	15.7 Peter Mitchell's Chemiosmotic Hypothesis
15.5	Regulation of Glycogen Metabolism	15.8 F ₀ -F ₁ ATP Synthase Complex
15.6	Electron Transport Chain	15.9 Summary
	Respiratory Complex-I: NADH- CoQ Oxidoreductase	15.10 Terminal Questions
	Respiratory Complex-II: Succinate CoQ Oxidoreductase	15.11 Answers

15.1 INTRODUCTION

You have learnt about the metabolism of glucose as primary source of energy in the preceding unit. You may now appreciate how cells use different metabolic pathways such as glycolysis and TCA cycle or at times fermentation to extract energy from this biomolecule. The circulating level of blood glucose is stringently controlled by its metabolism. You also know that excess of glucose is stored in the form of glycogen in animals and starch in plants. These storage sugars are mobilised to provide energy and biosynthetic precursors.

In this unit, we shall discuss the synthesis (glycogenesis) and breakdown (glycogenolysis) of glycogen in animals as well as their regulation. We shall complete the unit with a discussion on electron transport chain and oxidative phosphorylation. In these processes, electrons produced from the reducing powers NADH/FADH₂ during catabolism of carbohydrates or lipids are transferred through chain of various electron carriers to oxygen finally and forming water. The process enables cell to maximise the yield of energy in the form of ATP synthesis.

Objectives

After studying this unit, you should be able to:

- ❖ describe the steps of glycogenesis,
- ❖ explain glycogenolysis and conversion of glucose 1-phosphate to glucose 6-phosphate,
- ❖ describe the coordinated regulation of glycogen metabolism;
- ❖ describe the structure and function of various components of mitochondrial respiratory (electron transport) chain,
- ❖ describe the organization of respiratory chain (electron transport) components into electron transport complexes, and
- ❖ explain the theory and complex involved in the process of ATP synthesis.

15.2 GLYCOGEN: A STORAGE POLYMER

Glycogen is an extensively branched homopolymer of glucose. It consists of approximately 6×10^5 glucose residues with a branch on average after every 8-10 residues. The sugars are linked by α (1 $\rightarrow$ 4) glycosidic bonds except at branch points where the linkage is α (1 $\rightarrow$ 6) (Fig. 15.1). The biological effect of branching is to increase the number of non-reducing ends and to increase its solubility. There are $(n+1)$ non-reducing ends; n is the number of branches. Both synthesis and degradation of glycogen begin with the non-reducing ends.

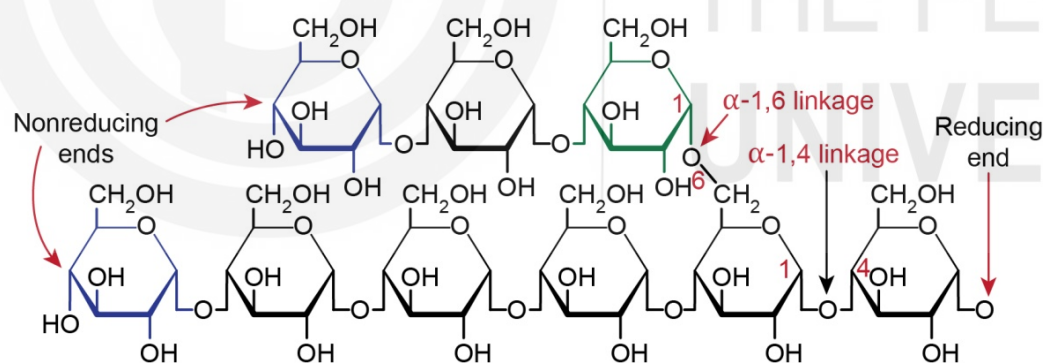


Fig. 15.1: Structure of glycogen.

Glycogen is stored as a heavily hydrated short term energy reserve in vertebrates and many microorganisms. In animals, it is especially abundant in the liver and muscles. Liver glycogen stores are used to provide glucose to other tissues, brain in particular. These stores can sustain glucose levels only up to 12-24 hours. In muscles, these stores are meant for in house use such as to support muscle contraction.

Glycogen is present in granules, distributed in cytosol. These granules also contain enzymes for synthesis and degradation of glycogen. A glycogen granule differs from a multienzyme complex such as PDH (pyruvate dehydrogenase) in that the enzymes are not present in defined ratios and it is structurally less organized.

One would wonder why in the first place both glycogen and fats were selected when they serve the same function. With reference to animals the possible reasons could be

- (a) they lack the ability to convert fatty acids to glucose and fat metabolism alone cannot maintain blood glucose levels,
- (b) fatty acids cannot be metabolised anaerobically, and
- (c) some tissues cannot use fats as efficiently as glycogen.

SAQ 1

What are the advantages of having a branched storage polymer?

In the next two sections, we will learn in details the reactions involved in the pathways of glycogen synthesis and degradation.

15.3 GLYCOGENESIS

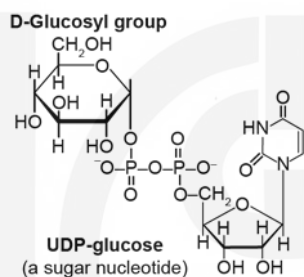


Fig. 15.2: Structure of UDP-Glucose.

The process of synthesis of glycogen from glucose is known as glycogenesis. In animals, glycogen is synthesised in almost all tissues but the most important sites are liver and muscles. As mentioned in Section 15.2 the purpose of synthesizing glycogen in both these locations is different.

The first step in glycogenesis is the synthesis of activated donor, UDP-glucose (Fig.15.2) from glucose 1-phosphate and UTP by **UDP-glucose pyrophosphorylase**. Generally, excess glucose in liver and muscles is first converted to glucose 6-phosphate by glucokinase (in liver) or hexokinase (in muscles) and then to glucose 1-phosphate by phosphoglucomutase (PGM). In liver, gluconeogenesis can directly provide glucose 6-phosphate (Fig.15.3).

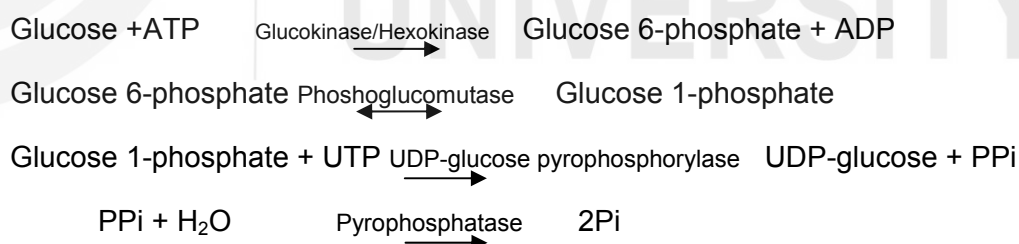


Fig. 15.3: Synthesis of UDP-glucose from glucose.

The reaction catalysed by UDP-glucose pyrophosphorylase proceeds in the direction of synthesis as the product pyrophosphate (PPi) is readily hydrolysed by a ubiquitous **inorganic pyrophosphatase**.

The polymerizing enzyme glycogen synthase is incapable of initiating glycogen synthesis. It is a chain elongating enzyme. The initial steps of polymerisation are primed by a protein **glycogenin**. It is both; an enzyme (glucosyl transferase) and a primer for assembling glycogen chains. The primer is synthesised by step by step addition of eight glucose residues from UDP-glucose by glucosyl transferase; the first one is attached to the tyrosine residue of glycogenin. Further elongation is taken over by glycogen synthase and glycogenin remains bound to the single reducing end.

Glycogen synthase catalyses the transfer of glucose residues from UDP-Glucose to the non-reducing end of the growing chain. It links glucose units by α -1, 4 glycosidic bonds (Fig.15.4). The synthase cannot introduce α -1, 6 linkages at branch points.

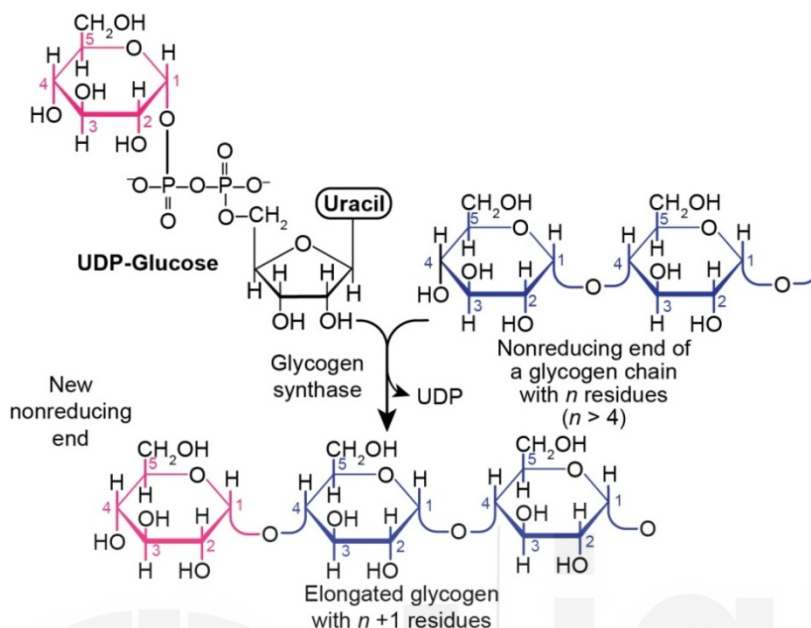


Fig. 15.4: Chain elongation by glycogen synthase.

The final stage is the conversion of a linear chain to a branched polymer by the **branching enzyme**; (amylase (1 $\rightarrow$ 4) to (1 $\rightarrow$ 6) transglycosylase or glycosyl (4 $\rightarrow$ 6) transferase). This enzyme transfers terminal fragment of five to eight glucose residues from the non reducing end of a glycogen branch that has at least eleven glucose residues to the C-6 hydroxyl of glucose in the same or another chain. The newly transferred oligosaccharide is linked by α (1 $\rightarrow$ 6) glycosidic bond. Figure 15.5 gives an overview of the complete glycogenesis pathway.

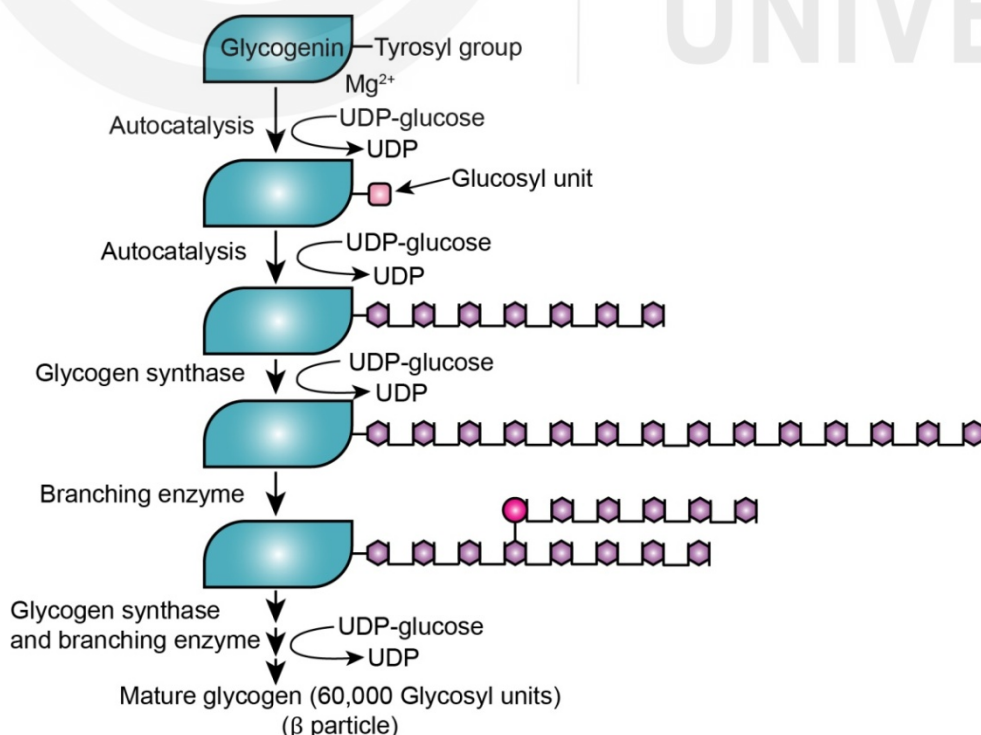


Fig. 15.5: Complete glycogenesis pathway.

Overall reaction involved in complete glycogenesis:

For each molecule of glucose incorporated into glycogen, two molecules of ATP are required. One is required for the phosphorylation of glucose and other for the conversion of UDP to UTP.

SAQ 2

- a) Differentiate between glycogenin and glycogen synthase.
 - b) Fill in the blanks:
 - (i) In humans glycogen is stored predominantly in and
 - (ii) Glucose is converted to glucose 6-phosphate by in liver.
 - (iii) Glycogen synthase adds glucose residues from the end of glycogen.
 - (iv) The primer for glycogen synthesis is
 - (v) The activated donor of glucose is synthesised from and by
 - (vi) The number of high energy phosphate bonds consumed to add a glucose residue to glycogen is
-

Glycogenolysis is the process of breakdown of glycogen to glucose-1 phosphate by glycogen phosphorylase. It is the key enzyme in glycogen breakdown and is subject to stringent regulation.

15.4 GLYCOGENOLYSIS

Glycogenolysis is the process of conversion of glycogen to glucose-1 phosphate (90%) and to a lesser extent to glucose (10%). The process of breakdown of glycogen is catalysed by a separate set of enzymes than those used for synthesis. It is initiated by **glycogen phosphorylase** but it stops cleaving four residues before a branch point. Further degradation is dependent on the activities of other enzymes that remodel the substrate for phosphorylase to continue its breaking down action.

Glycogen phosphorylase (homodimer) catalyses the sequential removal of glucose residues by phosphorolytic cleavage of α -1,4-glycosidic bonds from the non-reducing end of glycogen. The product released is glucose-1-phosphate (Fig.15.6).

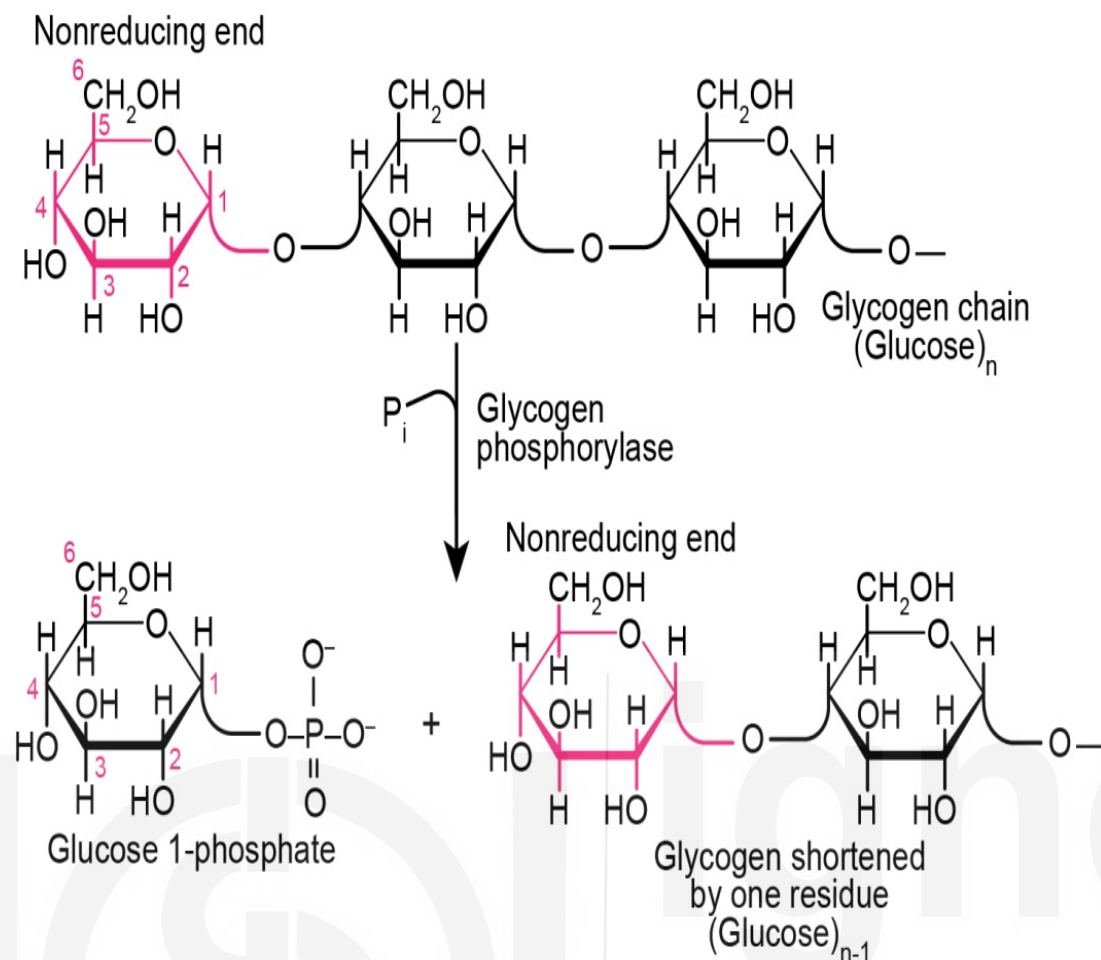


Fig. 15.6: Phosphorolytic cleavage of glycogen by glycogen phosphorylase.

The reaction proceeds in the direction of breakdown *in vivo* as the intracellular ratio of $[P_i] / [\text{glucose-1-phosphate}]$ is high (30-100). The enzyme requires pyridoxal phosphate (PLP). The release of phosphorylated sugar is energetically advantageous as compared to simple hydrolysis to glucose because it can enter into glycolysis or pentose phosphate pathway. In addition, the negatively charged sugar phosphate would not leave the cell.

The process continues until four glucose residues remain on either side of the branch point. The branched structure so formed is called **limit dextrin** and cannot be further metabolized by glycogen phosphorylase. The processing of the branched intermediate to an unbranched substrate suitable for phosphorylase is catalysed in eukaryotes by a bifunctional **debranching enzyme**. The two enzymes that catalyse two successive reactions are **glycosyltransferase** and **amylase α -1,6-glucosidase**.

The glycosyl transferase transfers a trisaccharide from one outer branch to the non reducing end of another branch so that only one glucose residue is left at the branch point. Now glucose at the branch point, linked via α -1,6 linkage is hydrolytically released by amylase α -1,6-glucosidase (Fig. 15.7). This reaction accounts for the minor product (10%) of glycogen breakdown. It may either be released (in case of liver) or phosphorylated before feeding to other pathways.

It is interesting to note that hydrolysis of dietary glycogen does not involve phosphorolysis but is degraded by the combined action of amylases, debranching enzymes and maltase to un-phosphorylated sugars, facilitating its absorption.

Glucose-1-phosphate and free glucose are produced in a ratio 8:1 in glycogenolysis.

At this stage glycogen phosphorylase can resume degradation of the unbranched product to glucose-1-phosphate.

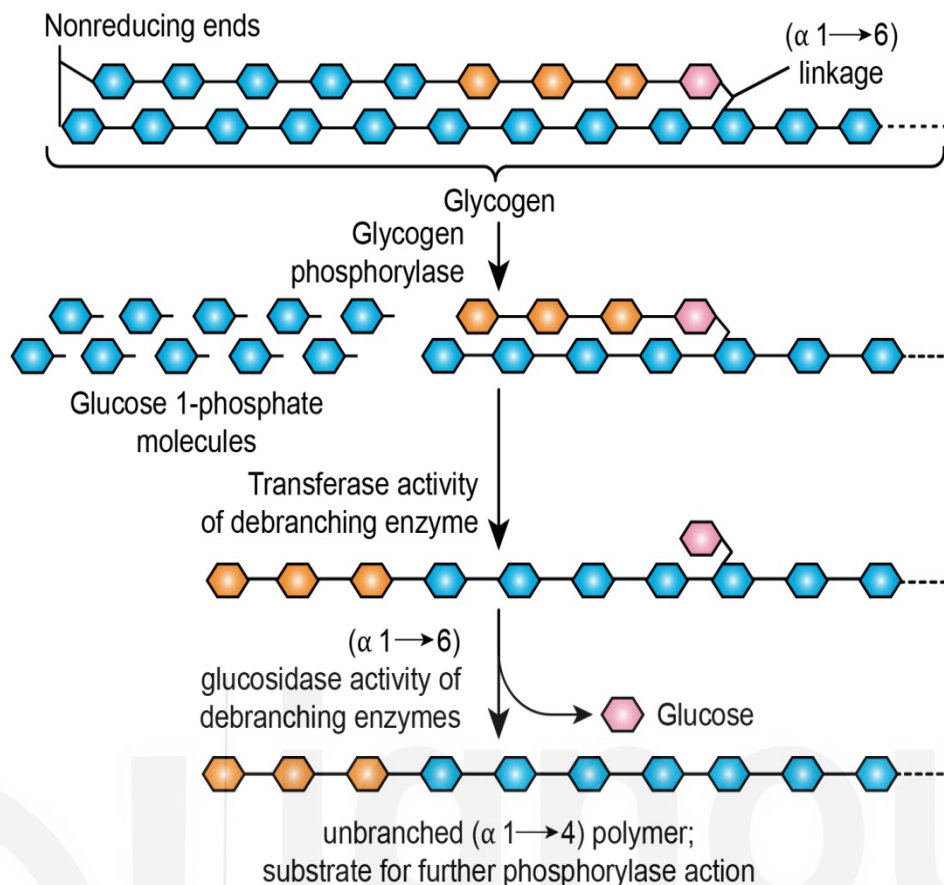


Fig. 15.7: Action of debranching enzyme on limit dextrin.

Glucose-1-phosphate is converted into glucose-6-phosphate by **phosphoglucomutase**, the fate of which is dependent on the tissue (Fig. 15.8). The reaction proceeds via an enzyme bound intermediate, glucose 1,6-bisphosphate.

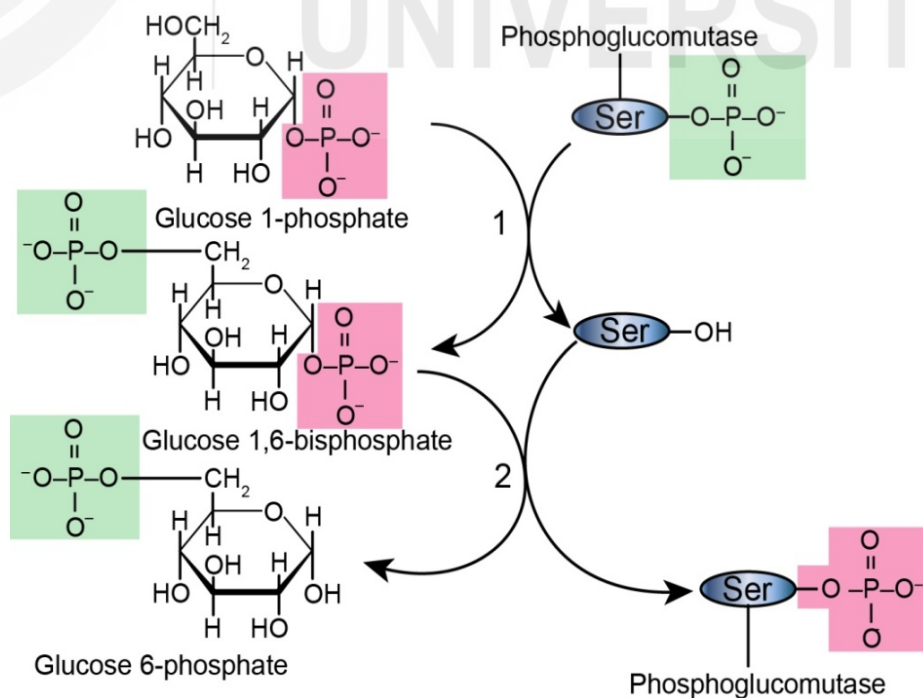


Fig. 15.8: Conversion of glucose-1-phosphate to glucose-6-phosphate by phosphoglucomutase (PGM).

The liver expresses a hydrolytic enzyme **glucose-6-phosphatase** which converts glucose-6-phosphate to glucose. Glucose is not a major fuel for the liver. Free glucose is released into circulation to be made available to other tissues especially between meals and muscular activity. You have already studied this enzyme in gluconeogenesis. Since glucose-6-phosphatase is absent in muscle and adipose tissue, therefore free glucose cannot be produced in these tissues. Instead, glucose-6-phosphate produced by glycogenolysis and PGM enters glycolysis to generate energy for muscle contraction. The fate of glucose-6-phosphate in different tissues is summarised in Figure 15.9.

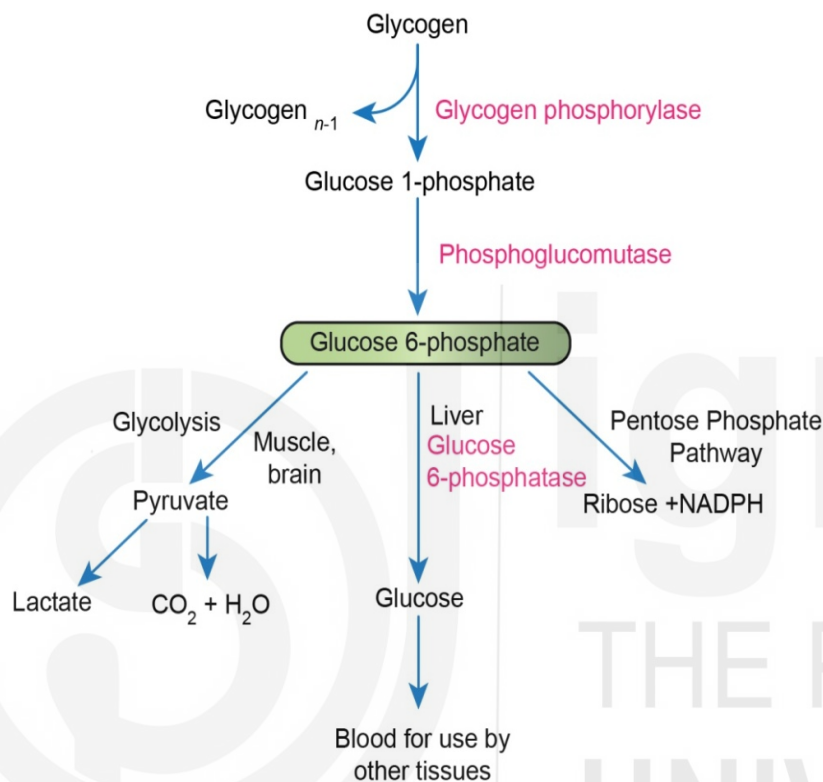


Fig. 15.9: Fate of glucose-6-phosphate in different tissues.

SAQ 3

Name the enzymatic activities of debranching enzyme.

15.5 REGULATION OF GLYCOGEN METABOLISM

Glycogenesis operates when blood glucose levels are high such as after a meal and glycogenolysis is favored when blood glucose levels are low, for instance during fasting or vigorous exercise.

The coordinated regulation of glycogenesis and glycogenolysis occurs both by allosteric modulators and hormones. The hormones act by inducing reciprocal covalent modifications. The major hormones are glucagon, epinephrine and insulin. While glucagon and epinephrine (adrenaline) trigger the breakdown of glycogen; insulin favors glycogen synthesis. Glucagon action is more pronounced on liver than muscle whereas epinephrine mainly acts on muscle.

The key enzymes of synthesis and degradation (glycogen synthase and phosphorylase) are the primary targets of regulation. The isozymes of glycogen phosphorylase present in liver and muscle respond differently to regulators which are in accordance with their role.

Carl and Gerty Cori showed the existence of two interconvertible forms of glycogen phosphorylase in skeletal muscles: *phosphorylase a* and *phosphorylase b*; the former was active while the latter was less active. The active form of phosphorylase is phosphorylated while less active/inactive form is dephosphorylated (Fig 15.10). Their inter conversion is triggered by hormones and allosteric modulators. The hormones induce an enzyme cascading effect leading to enormous amplification of the initial signal.

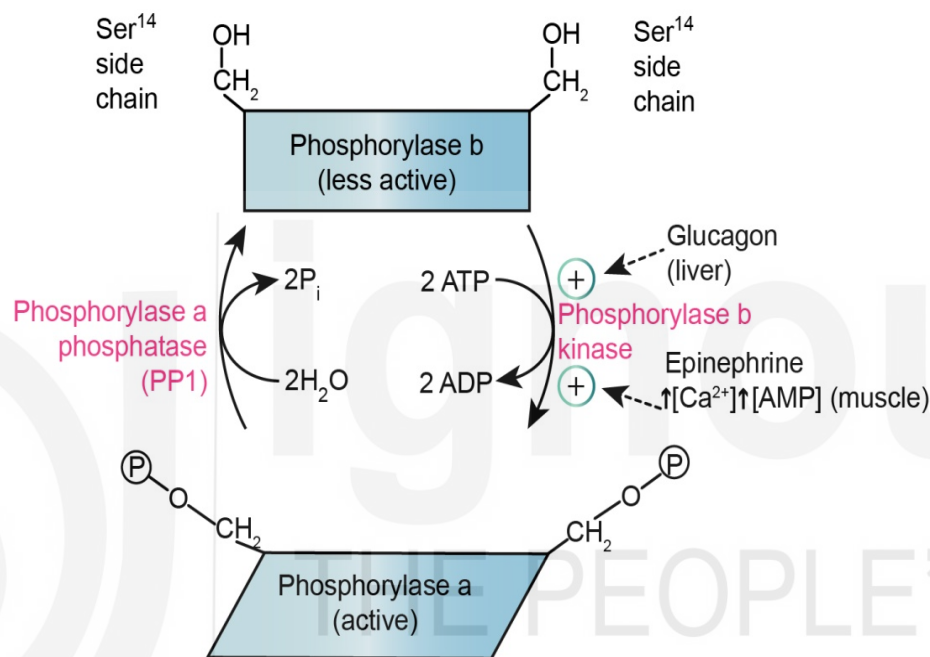


Fig. 15.10: The two interconvertible forms of glycogen phosphorylase.

Let us consider the regulation in both skeletal muscle and liver. During vigorous muscular activity or a fall in blood glucose, the hormone (epinephrine or glucagon) released acts through cell surface receptors on muscle or liver cells to induce the synthesis of an intracellular second messenger, cAMP. The second messenger activates protein kinase A (PKA) which sets into action by a cascade effect of phosphorylations.

The activated PKA phosphorylates phosphorylase- b kinase, activating it to phosphorylate glycogen *phosphorylase-b* converting it into active phosphorylase-a. Glycogen phosphorylase-a degrades glycogen to release glucose 1-phosphate to provide energy for muscle contraction or releases glucose from the liver by an action of glucose 6-phosphatase to maintain blood glucose levels (Fig 15.11).

Glycogen phosphorylase acts as sensor of glucose while glycogen synthase senses glucose -6-phosphate.

The allosteric control mechanisms differ in muscle and liver. In the muscle, calcium which is a signal for muscle contraction activates phosphorylase-b kinase and AMP (Adenosine mono phosphate) activates phosphorylase; both increase the rate of glycogen breakdown. ATP has an opposite effect to AMP. The liver isozyme of glycogen phosphorylase is allosterically inhibited by glucose when its concentration in blood comes to normal.

The conditions that favour glycogenolysis inhibit glycogenesis. The active form of glycogen synthase (a form) is converted into inactive form (b form) by phosphorylation. The enzyme remains phosphorylated unless glucose 6-phosphate (allosteric activator) is present. The reciprocal regulation in response to hormonal signals is depicted in Figure 15.11.

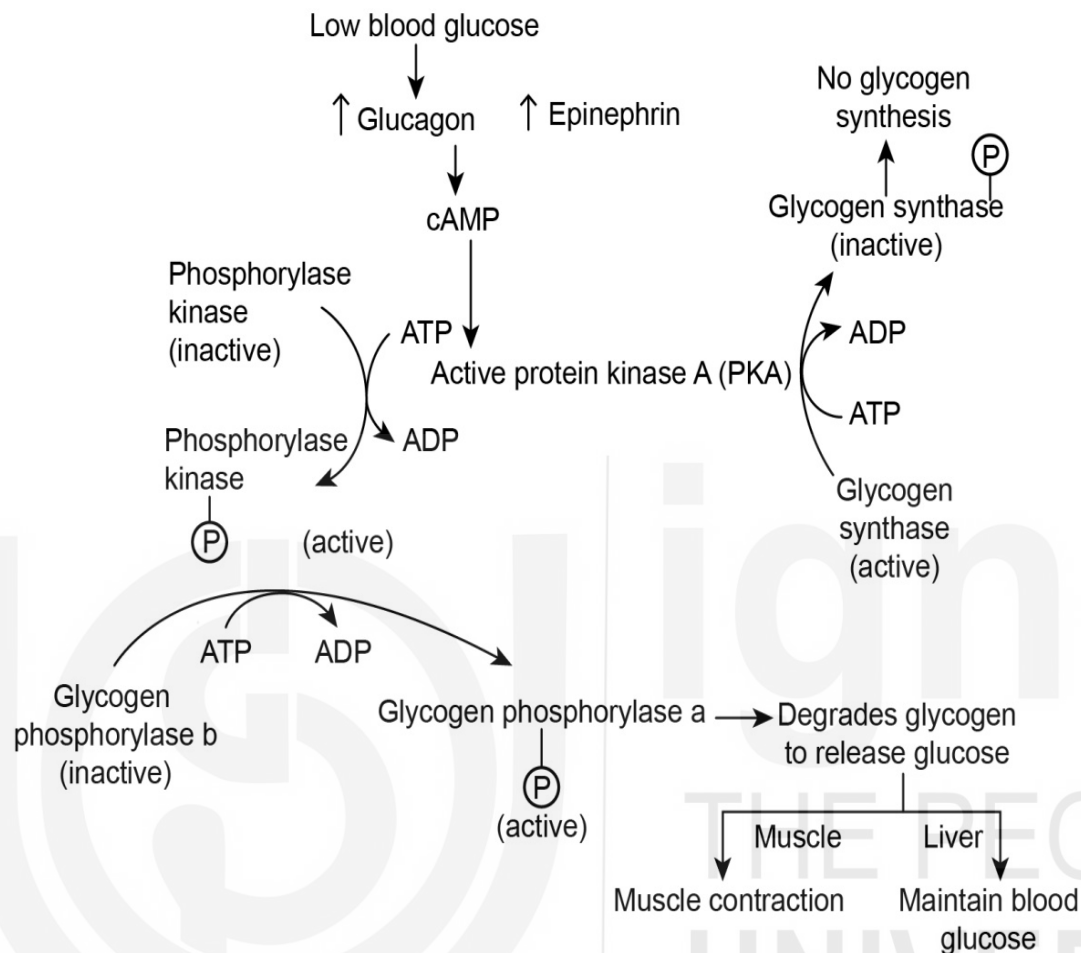


Fig. 15.11: Hormonal regulation of glycogen metabolism by reversible covalent modification.

Insulin is antagonistic to epinephrine. When blood glucose levels are high, insulin is secreted. It binds to target cell surface and inhibits the activity of adenylate cyclase on ATP to convert it to cAMP. Thus protein kinase A is not activated. Glycogen synthase remains active and the net result is glycogen synthesis.

SAQ 4

Indicate whether the phosphorylated form of the following enzymes is active or inactive?

- Glycogen phosphorylase
- Glycogen synthase
- Protein phosphatase

So far you have learnt about catabolic and anabolic reactions of carbohydrates. You may notice that energy released during catabolic reactions result in formation of energy rich molecules such as ATP, NADH and FADH_2 . While ATP serves as the energy currency of the cell; NADH and FADH_2 molecules act as reducing powers and have a pair of electrons each with high transfer potential. This potential is converted to ATP as their electrons are transferred to oxygen through a series of electron carriers. Sequential oxidations of these carriers generate a proton gradient. The proton motive force generated due to proton gradient is used to drive synthesis of ATP by the ATP synthesizing machinery, process called oxidative phosphorylation. ***Oxidative phosphorylation is the process in which ATP is formed as a result of the transfer of electrons from NADH or FADH_2 to O_2 by a series of electron carriers.***

Both electron transport chain and ATP synthesis take place in mitochondria justifying its role as “powerhouse” of the cell. It is within this organelle most of the energy derived from respiratory oxidation is captured. Oxidative phosphorylation is fundamental to all aspects of cellular life in aerobic organisms since it is their main source of useful energy. Let us discuss in details how cell gets at this task.

15.6 ELECTRON TRANSPORT CHAIN

Mitochondrial electron transport chain consists of a series of electron carriers, more often referred to as the respiratory chain. It consists of four protein complexes **NADH-Q oxidoreductase, Succinate-Q reductase, Q-cytochrome c oxidoreductase, and Cytochrome c oxidase** that are also known as **Complex I, II, III and IV**, respectively. Besides, several other iron-sulfur centers or proteins, ubiquinone and cytochromes b, c_1 , a and a_3 also participate. The sequence of these electron carriers in the respiratory chain is as given in figure 15.12. *Transfer of electrons from NADH or FADH_2 to O_2 by a series of electron carriers generates 26 of the 30 molecules of ATP that are formed when glucose is completely oxidized to CO_2 and H_2O .*

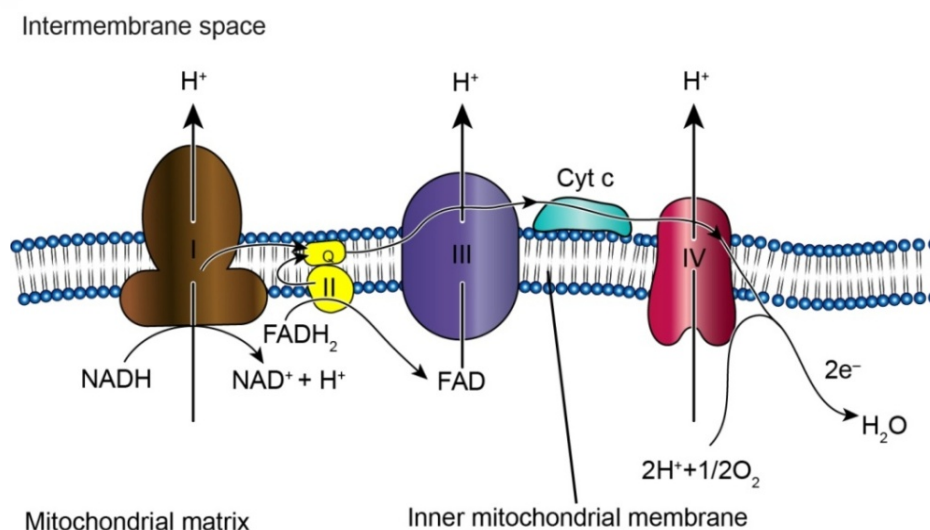


Fig. 15.12: Mitochondrial electron transport chain showing electron transport complexes.

15.6.1 Respiratory Complex-I: NADH-CoQ Oxidoreductase

The first complex of electron transport chain (ETC) catalyses the first step of electron transport, namely the transfer of electrons from NADH to coenzyme Q (CoQ). Complex-I, also known as NADH dehydrogenase, is an integral part of the inner mitochondrial membrane. It contains a flavoprotein (FMN) that oxidizes NADH and several iron-sulfur clusters, along with many other subunits.

The electron transport reaction occurs in several steps, with successive oxidation and reduction of the flavoprotein and the iron-sulfur moiety. It begins with the transfer of electrons from NADH to the flavoprotein. In the next step, the reduced flavoprotein is reoxidized and in turn, the oxidized form of the iron-sulfur protein is reduced. In the third step, the reduced iron-sulfur protein then donates its electrons to coenzyme Q, which becomes reduced to CoQH₂. Coenzyme Q is also called ubiquinone (Fig. 15.13). Transfer of two electrons from NADH to CoQ results in transport of four protons from mitochondrial matrix to intermembrane space.

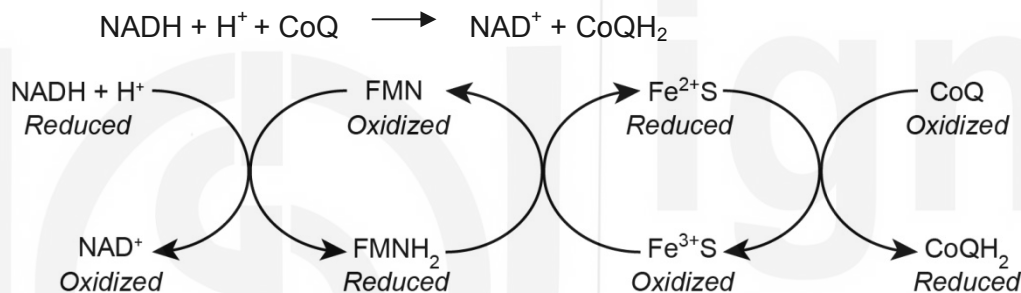


Fig. 15.13: Sequential electron transport from NADH to CoQ.

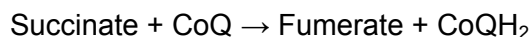
This overall reaction is one of those three reactions that are responsible for the proton transport that creates the proton gradient. The value of standard free-energy change ($G^{\circ} = -81 \text{ KJ mol}^{-1}$) points out to the reaction that is strongly exergonic, releasing adequate energy to drive the phosphorylation of ADP to ATP.

The final electron receptor of complex-I, coenzyme Q, is dynamic— that is to say, it is free to move in the membrane and to hand over the electrons it has gained to the third complex for further sending it to oxygen. You will again note that the second complex also transfers electrons from an oxidizable substrate to coenzyme Q.

15.6.2 Respiratory Complex-II: Succinate CoQ Oxidoreductase

It is the second of the four membrane-bound complexes, and also known as succinate dehydrogenase. Like Complex I, this also catalyses the transfer of electrons to coenzyme Q. However, in this case the substrate is succinate from the citric acid cycle, which is oxidized to fumarate by a flavin enzyme succinate dehydrogenase. FADH₂ generated remains as part of the enzyme and is later transferred to Fe-S clusters and then to CoQ. Unlike NADH, transfer of electrons from FADH₂ to CoQ does not lead to transport of protons from matrix to intermembrane space. As a result less ATP is generated from FADH₂ compared to NADH.

The overall reaction is



The standard free-energy change (ΔG°) is $-13.5 \text{ KJ mol}^{-1} = -3.2 \text{ Kcal mol}^{-1}$ makes the overall reaction exergonic. *However, there is not enough energy from this reaction to drive ATP production, and no hydrogen ions are pumped out of the matrix during this step. The energy dissipates as heat and helps to maintain body temperature in human beings.*

15.6.3 Respiratory Complex-III: CoQH₂-Cytochrome- c Oxidoreductase

Coenzyme Q, the uniquely mobile component of respiratory chain, is soluble in the lipid component of the mitochondrial membrane.

This third complex is also known as cytochrome reductase. This complex is an integral part of the inner mitochondrial membrane. Cytochrome reductase catalyses the oxidation of reduced coenzyme Q (CoQH₂). The electrons produced during this oxidation reaction are passed on to cytochrome c in a multistep process. The overall reaction is:



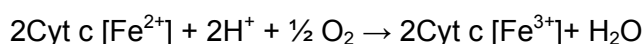
As we have seen that the oxidation of coenzyme Q involves two electrons, whereas the reduction of Fe^{3+} to Fe^{2+} (ferric to ferrous) requires only one electron. *Consequently, two molecules of cytochrome c are required for every molecule of coenzyme Q.* The components of this complex include cytochrome- b, cytochrome- c₁ and several iron-sulfur proteins. Cytochromes can carry electrons, but not hydrogen ions. This is another location where hydrogen ions leave the matrix. When reduced CoQH₂ is oxidized to CoQ, the hydrogen ion moves out on the other side of the membrane towards the inter membrane space.

Proton pumping, to which ATP production is coupled, takes place as a result of the reactions of this complex. The standard free-energy change (G°) is $-34.2 \text{ KJ} = -8.2 \text{ kcal}$ for each mole of NADH entering the electron transport chain. The phosphorylation of ADP requires $30.5 \text{ kJ mol}^{-1} = 7.3 \text{ kcal mol}^{-1}$, and the reaction catalysed by the third complex supplies enough energy to drive the production of ATP.

15.6.4 Respiratory Complex-IV: Cytochrome-c Oxidase

It catalyses the final steps of electron transport which is the transfer of electrons from cytochrome- c to oxygen. The reduced cytochrome oxidase is then oxidized by oxygen, which is itself reduced to water.

The overall reaction is



Proton pumping across the inner mitochondrial membrane also takes place as a result of this reaction. Like the other respiratory complexes, cytochrome oxidase is also an integral part of the inner mitochondrial membrane and contains cytochromes a and a₃, as well as two Cu^{2+} ions that are involved in the electron transport process.

Now, we are mentally ready to understand how the energy in this proton gradient is used for ATP synthesis, in short the mechanism of oxidative phosphorylation. Figure 15.14 summarizes the flow of electrons across the electron transport chain.

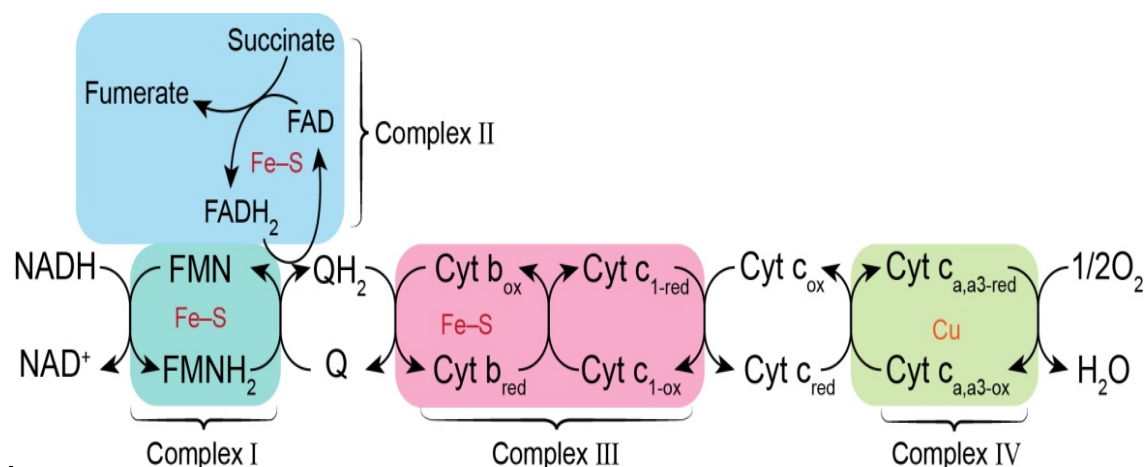


Fig. 15.14: Flow of reducing equivalents through respiratory complexes (electron transport chain) of mitochondria.

SAQ 5

- a) Fill in the blanks with the options given in the parentheses:
- (Succinate-CoQ reductase/Cytochrome bc₁ reductase) complex along the electron transport chain is not coupled to ATP synthesis.
 - (Cytochrome b/Coenzyme Q) component of the ETC accepts only one electron.
 - (FMN/ FAD) is a component of Succinate dehydrogenase in electron transport chain.
 - ATP synthesizing system is located on.....(outer/inner) mitochondrial membrane.
 - (O₂/FAD) has the highest redox potential.
- b) Match the following columns with each other.

S. No.	Column I	S. No.	Column II
i)	Respiratory Complex I	a)	Succinate-CoQ oxidoreductase/succinate dehydrogenase
ii)	Respiratory Complex II	b)	Cytochrome-c oxidase
iii)	Respiratory Complex III	c)	CoQH ₂ cytochrome-c oxidoreductase/cytochrome reductase
iv)	Respiratory Complex IV	d)	NADH-CoQ oxidoreductase/ NADH dehydrogenase

15.7 PETER MITCHELL'S CHEMIOSMOTIC HYPOTHESIS

Energy released by the oxidation reactions in the electron transport chain is used to drive the synthesis of ATP. The coupling between electron transport and oxidative phosphorylation has been explained successfully by the British scientist Peter Mitchell in 1961. Mitchell's theory is also known as **chemiosmotic theory**. Salient features of Mitchell's theory are as follows:

- Electron transport through the ETC generates a proton gradient which is stored in the form of electrochemical potential. To maintain the proton gradient intact inner mitochondrial membrane is required.
- Pumping of H^+ (protons) from the matrix to the intermembranous space results in increase of pH and the matrix becomes negatively charged with respect to the cytosol. This creates a pH gradient and electrical gradient across the inner membrane, both of which tends to attract protons back into the matrix from the cytosol (Fig. 15.15).
- The membrane-spanning enzyme, ATP synthase, catalyses the phosphorylation of ADP accompanied by the movement of H^+ ions across the intermembrane space into the matrix.

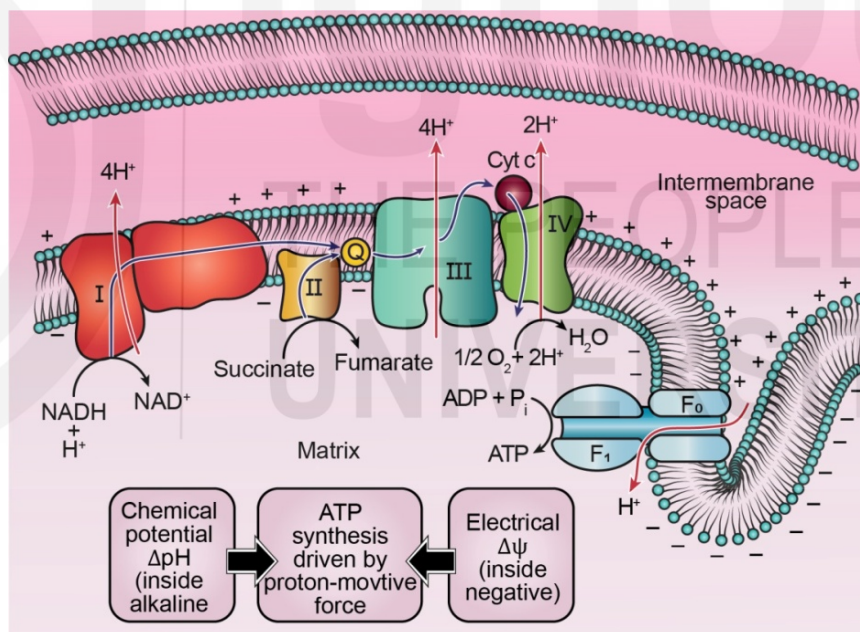


Fig. 15.15: Proton and electrochemical gradient across mitochondrial membrane.

The way in which the proton gradient leads to the production of ATP depends on ion channels through the inner mitochondrial membrane; these channels are the features of the structure of ATP synthase. Protons flow back into the matrix through ion channels in the ATP synthase; the F_0 part of the protein is the proton channel. Flow of protons is accompanied by formation of ATP, which takes place in the F_1 unit. The unique feature of chemiosmotic coupling is the direct linkage of the proton gradient to the phosphorylation reaction.

You will study in a bit detail about the F_0F_1 ATP synthase complex in next section. It is also known as mitochondrial F_0F_1 -ATPase.

John Walker and Paul Boyer have provided the structural verification for binding change mechanism for ATP synthesis and were awarded Nobel Prize for Chemistry in 1997.

Boyer proposed the rotational catalysis model for ATP synthesis.

<https://www.youtube.com/watch?v=At16hpHSE>

15.8 F₀-F₁ ATP SYNTHASE COMPLEX

The energy of the electrochemical potential (voltage drop) across the membrane is converted to the chemical energy of ATP by the coupling process. Figure 15.16 is a schematic diagram to show connection between mitochondrial electron transport and phosphorylation of ADP to give ATP.

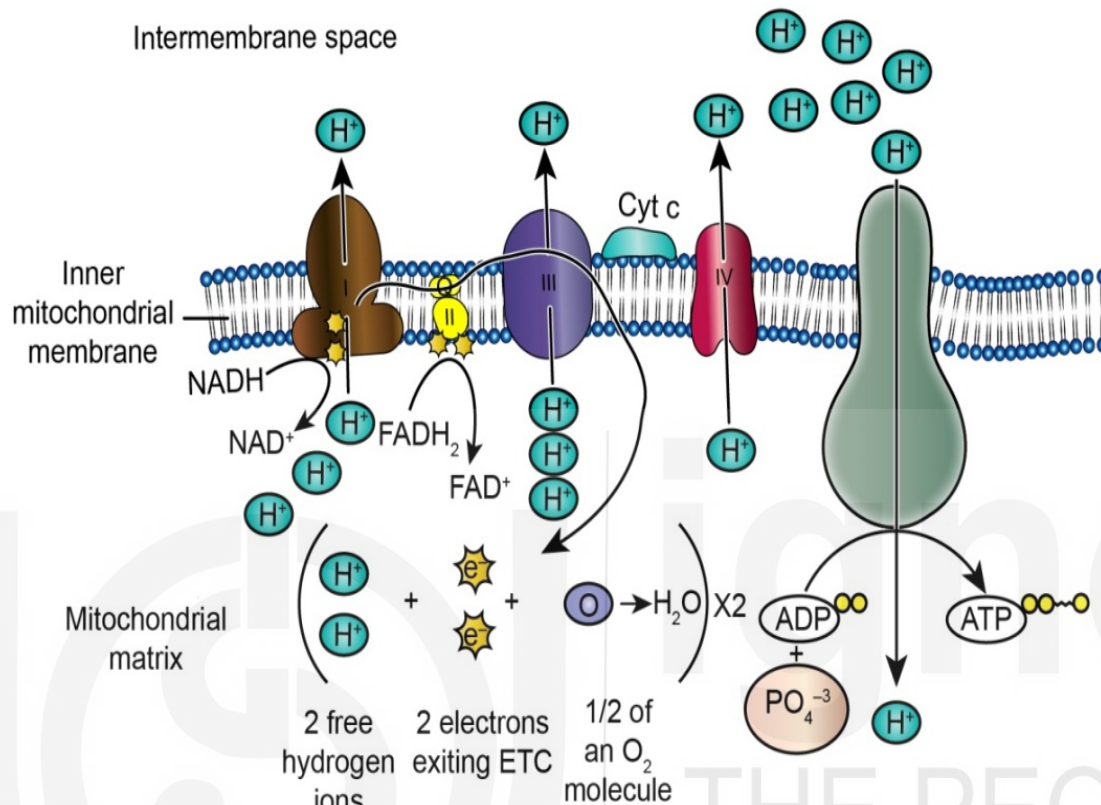


Fig. 15.16: A Schematic diagram to show connection between mitochondrial electron transport and phosphorylation of ADP to give ATP.

The coupling factor needed to link oxidation and phosphorylation is known as **ATP synthase**, a complex protein oligomer which is separate from the electron transport complexes. The complete protein spans the inner mitochondrial membrane and projects into the matrix as well. The portion of the protein that spans the membrane is called F₀ unit. It forms a transmembrane channel through which protons move to drive ATP synthesis. It consists of three hydrophobic subunits named a, b and c, with a subunit stoichiometry **a₁b₂c₁₀₋₁₅**. The spherical portion that projects into the matrix is called F₁ and consists of five different kinds of polypeptide chains named α, β, γ, δ, and ε with a subunit stoichiometry **α₃β₃γδ̵**. The whole protein complex F₀F₁ is called as ATP synthase (Fig 15.17). It is also known as mitochondrial ATPase because the reverse reaction of ATP hydrolysis, as well as phosphorylation is catalysed by the same enzyme. The purified F₁ particles catalyse ATP hydrolysis, the reverse reaction of the ATP synthase.

Oligomycin an antibiotic acts directly on ATP synthase and blocks the movement of protons through F₀.

It has been experimentally observed that flow of protons through F₀ drives the conformational change in ATP synthase which results in binding of the substrate on enzyme, synthesis and release of ATP. For every four protons transported into the matrix, one ATP is synthesised.

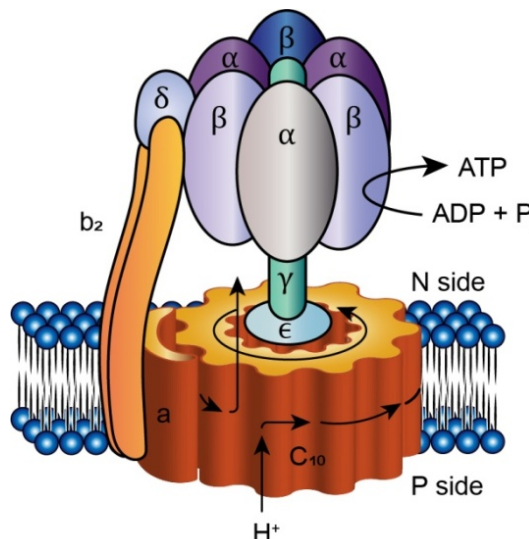


Fig. 15.17: The ATP synthase, a rotating molecular motor for ATP synthesis CC-SA.

It has been experimentally demonstrated that for each pair of electron entering the respiratory chain as NADH and FADH_2 and traversing the entire chain to O_2 , ~ 2.5 and ~ 1.5 moles of ATP are generated, respectively. You may note that similar calculations have been used in the previous unit to calculate the total number of ATP formed during various metabolic pathways.

SAQ 6

Fill in the blanks by appropriate word/name.

- enzyme makes ATP by chemiosmosis.
- Chemiosmosis hypothesis was proposed by
- Measure of potential energy stored as combination of proton and voltage gradients across membrane is termed as

15.9 SUMMARY

Let us summarise what we have learnt so far:

- In vertebrates and many microorganisms excess glucose is stored as glycogen. It is distributed in the cytosol as dense granules in liver and muscles. The granules also have enzymes of synthesis and degradation.
- Liver glycogen is primarily responsible for maintaining blood glucose levels whereas muscle glycogen stores are meant to meet the energy demands of the muscle itself.
- Glycogenesis (synthesis of glycogen) is catalysed by the enzyme glycogen synthase which adds glucose residues to a primer of at least 8 glucose residues linked by α -1, 4 linkages synthesised by an autocatalytic protein glycogenin. It is stimulated by insulin in response to high glucose levels especially after a carbohydrate rich meal.

- UDP-glucose is the activated glucose donor for glycogen synthesis. Both glycogenin and synthase add glucose residues from the non-reducing end of primer/glycogen.
- Branches in glycogen are introduced by the action of branching enzyme.
- Glycogenolysis is the process of breakdown of stored glycogen to glucose 1-phosphate. It is initiated by glycogen phosphorylase. It catalyses phosphorolytic cleavage from the non-reducing ends until four glucose residues remain on either side of the branch point. The structure so formed is called limit dextrin. The enzyme is pyridoxal phosphate dependent. .
- The highly branched limit dextrin is a substrate of bifunctional debranching enzyme. First, it transfers a fragment of 3 or 4 glucose residues from an outer branch to another branch or to an interior position of the same branch. Then amylo α -1,6-glucosidase hydrolyses the α -1,6 bond at the branch point releasing free glucose.
- Glucose-1-phosphate is converted into glucose-6-phosphate by phosphoglucomutase before it can enter different routes.
- Both glycogenesis and glycogenolysis are regulated by hormones primarily glucagon, epinephrine and insulin. The key enzymes regulated are glycogen phosphorylase and glycogen synthase. These are also under allosteric control.
- Mitochondrial electron transport chain consist of four protein complexes NADH-Q oxidoreductase, succinate-Q reductase, Q-cytochrome c oxidoreductase, and cytochrome c oxidase also known as complex I, II, III and IV respectively. It also involves several other iron-sulfur centers or proteins, ubiquinone and cytochromes b, c_1 , a and a_3 .
- These carriers transfer electrons from reduced energy carriers such as NADH and $FADH_2$ generated during catabolism of carbohydrates and lipids to oxygen. In this process, oxygen is reduced to water and a proton gradient is generated due to pumping of proton across the inner mitochondrial membrane.
- Chemiosmotic theory explains the coupling of ETC and oxidative phosphorylation.
- Proton gradient due to pumping of H^+ (protons) from the matrix to the intermembranous space results in increase of pH and the matrix becomes negatively charged with respect to the cytosol. This creates a pH gradient and electrical gradient across the inner membrane, both of which tends to attract protons back into the matrix from the cytosol.
- The membrane-spanning enzyme, ATP synthase, catalyses the phosphorylation of ADP accompanied by the movement of H^+ ions across the intermembrane space into the matrix.
- For each pair of electron entering the respiratory chain as NADH and $FADH_2$ and traversing the entire chain to O_2 , approximately 2.5 and 1.5 moles of ATP are generated respectively.

15.10 TERMINAL QUESTIONS

1. What is glycogenesis? Explain the steps involved in the process of glycogenesis.
2. Is glycogenolysis a reversal of glycogenesis? Explain.
3. What are the various components of mitochondrial electron transport chain?
4. Discuss the function of electron transport complex- I.
5. Give a brief description of chemiosmotic mechanism involved in oxidative phosphorylation.

15.11 ANSWERS

Self Assessment Questions

1. Branching increases the number of non reducing ends. Since synthesis and degradation of glycogen occur from the non reducing ends so a branched structure will allow reactions to take place simultaneously at multiple ends thereby increasing the overall rate. In addition, branching increases solubility.
2. a) Glycogenin is a glycosyltransferase that can initiate glycogen synthesis. It can add up to eight glucose residues; the first one is attached covalently to the phenolic hydroxyl of tyrosine.

Glycogen synthase is a chain elongating enzyme that adds glucose residues from the non reducing end of a primer. The polymer has approx. 6×10^5 glucose residues.
b) (i) Liver and muscle; (ii) glucokinase (hexokinase IV); (iii) Non reducing; (iv) Glycogenin; (v) Glc 1-phosphate, UTP, UDP pyrophosphorylase; (vi) 3.
3. (i) Glycosyl 4: 4 transferase and (ii) amylo α - 1,6-glucosidase.
4. (i) Active; (ii) Inactive; (iii) Inactive.
5. a) i) Succinate-Co Q reductase; ii) Coenzyme Q; iii) FMN; iv) inner; v) O_2 .
b) i) d; ii) a; iii) c; iv) b.
6. i) ATP synthase; ii) -Peter D. Mitchell; iii) proton motive force.

Terminal Questions

1. The synthesis of glycogen from glucose is known as glycogenesis. It is activated by insulin in response to high glucose levels especially after a carbohydrate rich meal. Although present in all tissues, it primarily takes place in cytosol of liver and skeletal muscles. Refer to Section 15.3 for details.

2. Glycogenolysis is not a reversal of glycogenesis. Both pathways use a different set of enzymes. Separate pathways allow greater flexibility and control. Refer to Section 15.3 and 15.4 for details.
3. Mitochondrial electron transport chain consists of four protein complexes **NADH-Q oxidoreductase, Succinate-Q reductase, Q-cytochrome c oxidoreductase, and Cytochrome c oxidase** that are also known as **Complex I, II, III and IV** respectively. It also involves several other iron-sulfur centres or proteins, ubiquinone and cytochromes b, c₁, a and a₃. Refer to Section 15.6 for more details.
4. **Respiratory Complex I: NADH-CoQ oxidoreductase**, the first complex of electron transport chain (ETC) catalyses the first step of electron transport, namely the transfer of electrons from NADH to coenzyme Q (CoQ). Refer to Section 15.6 for more details.
5. Energy released by the oxidation reactions in the electron transport chain is used to drive the synthesis of ATP. The coupling between electron transport and oxidative phosphorylation has been explained successfully by the British scientist Peter Mitchell in 1961. Mitchell's theory is also known as chemiosmotic theory. Refer to Section 15.7 for more details.

LIPID METABOLISM |

Structure

16.1	Introduction	16.6	<i>De novo</i> Lipogenesis
	Objectives		Transport of Acetyl Groups from Mitochondria to the Cytosol
16.2	Metabolism of Fatty Acids		Conversion of Acetyl CoA to Malonyl CoA
	Activation of Fatty Acids	16.7	Fatty Acid Synthase (FAS)
	Transport of Activated Fatty Acids		Repetitive Condensation of Two Carbon Units by FAS I
16.3	β Oxidation of Fatty Acids	16.8	Palmitate as Source of other Fatty Acids
	Saturated Fatty Acids	16.9	Regulation of Fatty Acid Synthesis
	Unsaturated Fatty Acids	16.10	Summary
	Energetics	16.11	Terminal Questions
	Regulation	16.12	Answers
16.4	Minor Pathways of Fatty Acid Oxidation		
16.5	Ketone Bodies		
	Synthesis of Ketone Bodies (Ketogenesis)		
	Utilisation		
	Ketosis		
	Regulation		

16.1 INTRODUCTION

We learnt in the previous units that carbohydrate metabolism plays a major role in generating energy for various body activities, but lipids become important once carbohydrates are used up. Lipids due to their hydrophobic nature can be stored in larger amount in the body and upon oxidation produce almost double the amount of energy when compared to carbohydrates or proteins. On weight basis, the caloric yield is 9 kcal/g of fat compared to 4 kcal/g of carbohydrate or protein.

In this unit we will discuss mainly the metabolic fate of triglycerides, phospholipids and steroids, together with products of their metabolism such as long-chain fatty acids, glycerol and ketone bodies. In addition, you will also be familiarized with the pathways involved in synthesis of fatty acids. As catabolism of fatty acids is associated with generation of energy, therefore, these pathways merge with carbohydrate metabolism at the level of oxidative phosphorylation and are regulated at both, hormonal level as well as the enzymes catalysing these reactions.

Fatty acids are the building blocks of many lipid classes including triacylglycerols and membrane lipids. In this unit, we shall primarily focus on metabolism of fatty acids. Details of reactions involved in their activation, transport to mitochondria; the site of their oxidation for generation of energy will be discussed.

We shall explain *de novo* biosynthesis of fatty acids; a process by which long chain fatty acids are made starting from acetyl CoA.

Role of citrate, citrate lyase, acetyl CoA carboxylase, ACC and fatty acid synthase (FAS) complex will be highlighted. We shall also discuss additional steps for elongation and desaturation of fatty acids.

Acetyl CoA formed by fatty acid oxidation has different fates. Primarily it is used for generating energy by participating in TCA cycle, ETC and oxidative phosphorylation as discussed in the last unit. A small amount of acetyl CoA is also converted to ketone bodies which are used as preferred source of energy by many extra hepatic tissues. We will describe the synthesis, utilisation and regulation of ketogenesis. Under conditions of starvation or low glucose level, formation of ketone bodies increases. We shall try to understand the clinical relevance of these metabolic conditions.

Objectives

After reading this unit you should be able to:

- ❖ explain mitochondrial β oxidation,
- ❖ describe reactions and importance of minor pathways of fatty acid oxidation,
- ❖ describe the synthesis and utilisation of ketone bodies,
- ❖ describe *de novo* biosynthesis of fatty acids,
- ❖ indicate steps involved in the movement of acetyl units as citrate from the mitochondria to cytosol and the role of citrate lyase, and
- ❖ enlist additional steps for elongation and unsaturation of fatty acids.

16.2 METABOLISM OF FATTY ACIDS

When we say that fats provide energy; it implies fatty acids which are oxidized to provide energy. Fatty acids for oxidation can be obtained from four sources: (i) food, (ii) stored fat deposits in the form of triglycerides (TGs) in adipose tissue, (iii) fats formed in other tissues for instance in liver from excess carbohydrates for export to other tissues and (iv) autophagy, i.e. by degradation of cell's own organelles. Some organisms use all four sources while others may get it from one or more of these sources. Vertebrates such as humans get it from all four sources during starvation. In animals, triglycerides fulfill a significant but variable extent of our energy requirements. In some organs like liver, heart and skeletal muscle more than 50% of energy is generated from fat breakdown. The dependence of plants on fat reserves for energy is limited.

Whatever the source of fatty acids, these need to be activated before oxidation. The activation and oxidation of fatty acids are compartmentalized in different parts of the cell. This places the need for a mechanism to transport an activated fatty acid to the site of β -oxidation.

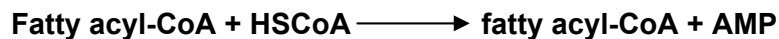
16.2.1 Activation of Fatty Acids

The oxidation of fatty acids is preceded by its activation to fatty acyl CoA. The activation step is catalysed by acyl-CoA synthetase (fatty acid thiokinase), localised in the outer mitochondrial membrane (and even ER). Different isozymes of acyl-CoA synthetase exist which differ in their chain length specificities.

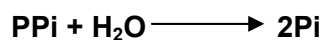
The activation of fatty acids was demonstrated by **Paul Berg** to occur in two steps. First an AMP (Adenosine mono phosphate) is transferred from ATP to the carboxyl group of fatty acid to form an enzyme bound **acyl adenylate intermediate**.



In the next step the $-\text{SH}$ group of HSCoA attacks the acyl adenylate to release AMP and form fatty acyl CoA.



The reaction is driven forward by the hydrolysis of pyrophosphate (PPi) by the ubiquitous pyrophosphatase.



The synthesis of a single thioester bond in essence has consumed an equivalent of two high energy bonds (pyrophosphate hydrolysis) to achieve irreversibility.

16.2.2 Transport of Activated Fatty Acids

In 1948 Eugene P. Kennedy and Albert Lehninger showed that in animal cells fatty acids are oxidised in the mitochondrial matrix. We have learnt in the previous section that activation occurs in the outer mitochondrial membrane,

therefore, these need to be transported across the inner mitochondrial membrane to reach matrix for oxidation. Fatty acids with shorter chain lengths (12 or fewer carbons) do not need any transporters to enter mitochondria. However, longer fatty acids are not able to cross the mitochondrial membrane and depend on **carnitine shuttle (Fig.16.1)**. Basically, it involves following steps:

1. The activated fatty acid is transiently transferred to **carnitine** to form fatty acyl carnitine complex catalysed by carnitine acyl transferase I present in the outer mitochondrial membrane. The entry of fatty acids is, therefore, regulated by controlling the activity of carnitine acyl transferase.
2. Tagged fatty acyl carnitine complex then enters mitochondrial matrix by facilitated diffusion through acyl carnitine/carnitine transporter present in the inner mitochondrial membrane.
3. In the final step, the fatty acyl moiety is transferred to intra-mitochondrial CoA to reform fatty acyl CoA by the enzyme carnitine acyl transferase II. Regenerated carnitine is returned back through acyl carnitine/carnitine transporter to participate in another cycle.

Cells maintain separate pools of coenzyme A and fatty acyl-CoA. The mitochondrial pool of coenzyme is generally used for oxidation of biomolecules whereas cytosolic pool is for synthesis of fatty acids.

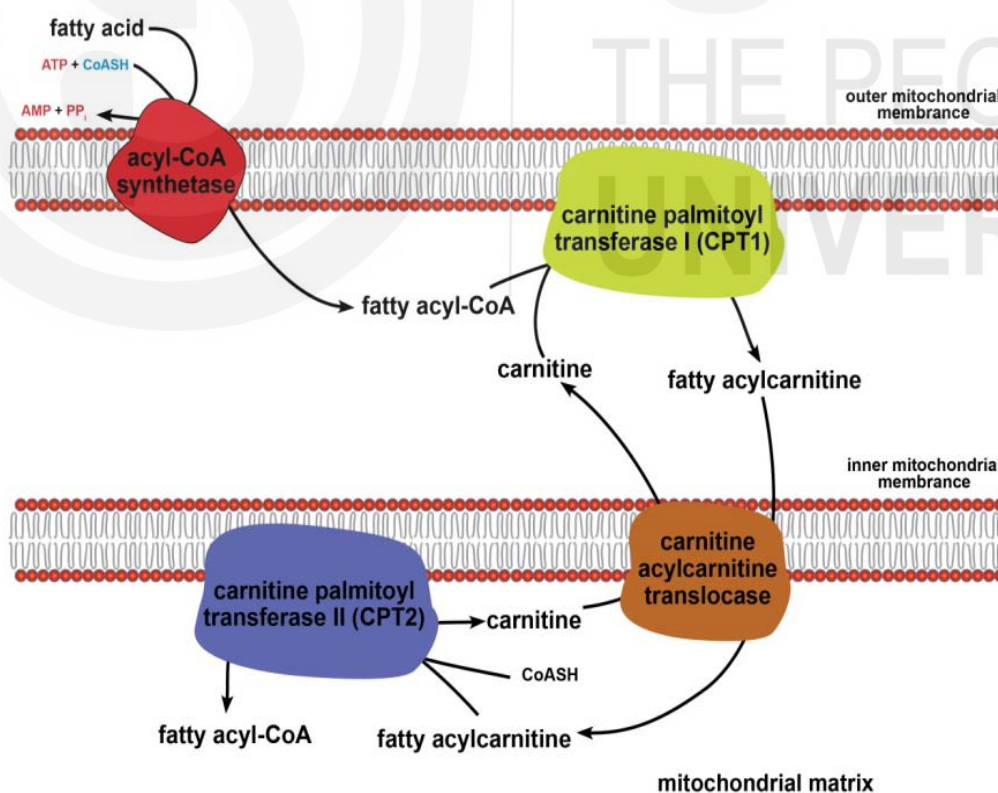


Fig. 16.1: The transport of long chain fatty acids to mitochondria. The names transferase I and II in this figure indicate that in this example palmitic acid is being activated and transported to mitochondrial matrix for oxidation.

SAQ 1

Write the function and location of following enzymes involved in activation and transport of fatty acids:

- (a) Carnitine acyl transferase I
- (b) Acyl CoA synthetase
- (c) Carnitine acyl carnitine translocase

16.3 β OXIDATION OF FATTY ACIDS

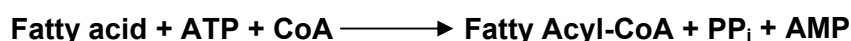
The oxidation of fatty acids is the central energy yielding pathway in animals, many protists and some bacteria. Major pathway is by oxidation at β -carbon. There are other minor pathways also operating in the cells. Let us look at these pathways one by one.

An initial clue to the mechanism of fatty acid oxidation in animals came from the work of **Georg Franz Knoop** in 1904. He used synthetic label to trace the fate of labelled fatty acids fed to dogs. Knoop deduced from these results that fatty acids are degraded by oxidation at the β -carbon.

16.3.1 Saturated Fatty Acids

The β -oxidation of saturated fatty acids occurs in four steps: oxidation, hydration, oxidation and thiolysis (Fig. 16.2). It is a spiral pathway in which each round of oxidation generates acetyl CoA, NADH and FADH₂. The process is so called β -oxidation pathway because of sequential removal of 2-carbon units by oxidation at the β -carbon position (C-3) of the fatty acyl-CoA molecule starting from the carboxyl end. The number of cycles of β -oxidation for a fatty acid of n carbon atoms is $(n / 2) - 1$. Therefore, a 16 carbon fatty acid goes through seven rounds of β -oxidation pathway and produces eight molecules of acetyl CoA.

As mentioned earlier short chain fatty acids enter directly into mitochondrial matrix where they are activated by mitochondrial fatty acyl CoA synthetase (also called acyl-CoA ligase or thiokinase) to fatty acyl CoA.



The activated fatty acids are now ready for oxidation.

The first step is catalysed by isozymes of acyl CoA dehydrogenase. Each isozyme is specific for a range of fatty acid chain lengths. It is a flavoenzyme with a tightly but noncovalently bound FAD prosthetic group. The enzyme is bound to the inner mitochondrial enzyme. It introduces a trans- double bond between C-2 and C-3 carbon atoms. The reduced prosthetic group is reoxidised by the electron transport chain which results in the production of 1.5 ATP per electron pair.

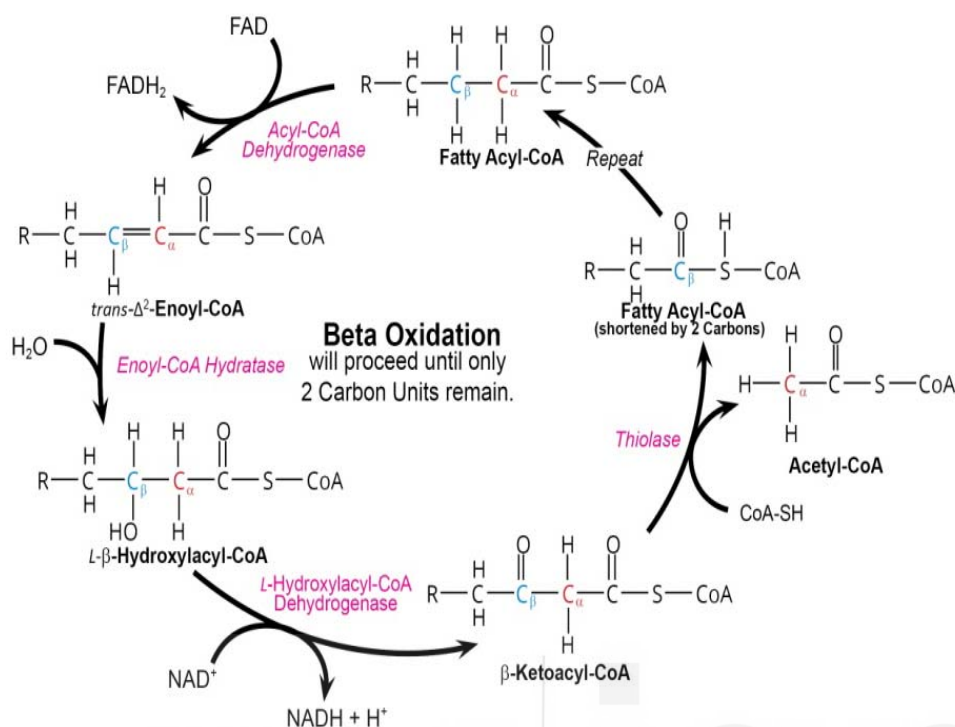


Fig. 16.2: The β -oxidation pathway.

In the next step, water is added across the trans-double bond. The stereospecific hydration catalysed by enoyl CoA hydratase forms L-3-hydroxyacyl CoA. The third step is the oxidation of secondary hydroxyl to a keto group. It is catalysed by NAD^+ dependent dehydrogenase which is specific for the L-isomer of hydroxy acyl CoA. The reoxidation of NADH by the ETC results in 2.5 ATP per electron pair.

The last step is the thiolysis of β -ketoacyl CoA releasing acetylCoA and shortening the chain by 2 carbons and generating a new activated carboxyl group. **The reaction is called thiolysis** as the enzyme **thiolase cleaves** with the thiol group of coenzyme A.

Fatty acids with odd number of carbon atoms also occur in nature. In general they represent a minor species but are common in many plants and some microbes. The oxidation of these fatty acids also occurs by β -oxidation. The difference comes in the last step of thiolysis that yields propionyl CoA and acetyl CoA instead of two molecules of acetyl CoA. In animals, propionyl CoA is converted to succinyl CoA, a TCA cycle intermediate while plants convert propionyl CoA to acetylCoA.

16.3.2 Unsaturated Fatty Acids

The oxidation of unsaturated fatty acids proceeds the same way as for the saturated fatty acids except that it requires few additional enzymes. These enzymes essentially help to resume β -oxidation cycle. We will consider now the oxidation of a monounsaturated and a poly unsaturated fatty acid (PUFA). You may recall from the study of lipids that most naturally occurring unsaturated fatty acids have unconjugated (in case there are more than one) cis double bonds. The cis double bond containing fatty acid is not a substrate for the enoyl CoA hydratase.

You would have noticed that the first three steps of β -oxidation are analogous to TCA cycle reactions that convert succinate to malate.

A common monounsaturated fatty acid in eukaryotes is oleic acid (18 carbon fatty acid) that has a *cis* double bond between C-9 and C10. The initial activation, transport and three cycles of β -oxidation go on as explained earlier. The Δ^3 -*cis* dodecenoylCoA is not a substrate for the enoyl CoA hydratase. The additional enzyme Δ^3, Δ^2 **enoyl CoA isomerase** comes into play. It shifts the position (from C-3 to C-2) and configuration (*cis*- to *trans*) of the double bond. The resultant **trans- Δ^2 -dodecenoyl CoA** bypasses the first step of β -oxidation and is hydrated to L- β -hydroxydodecanoylCoA. The first step is an energy yielding step and therefore, ATP yield will be less than an 18 carbon saturated fatty acid. After completing this round, four more cycles of β -oxidation will finally produce nine molecules of acetylCoA (Fig. 16.3).

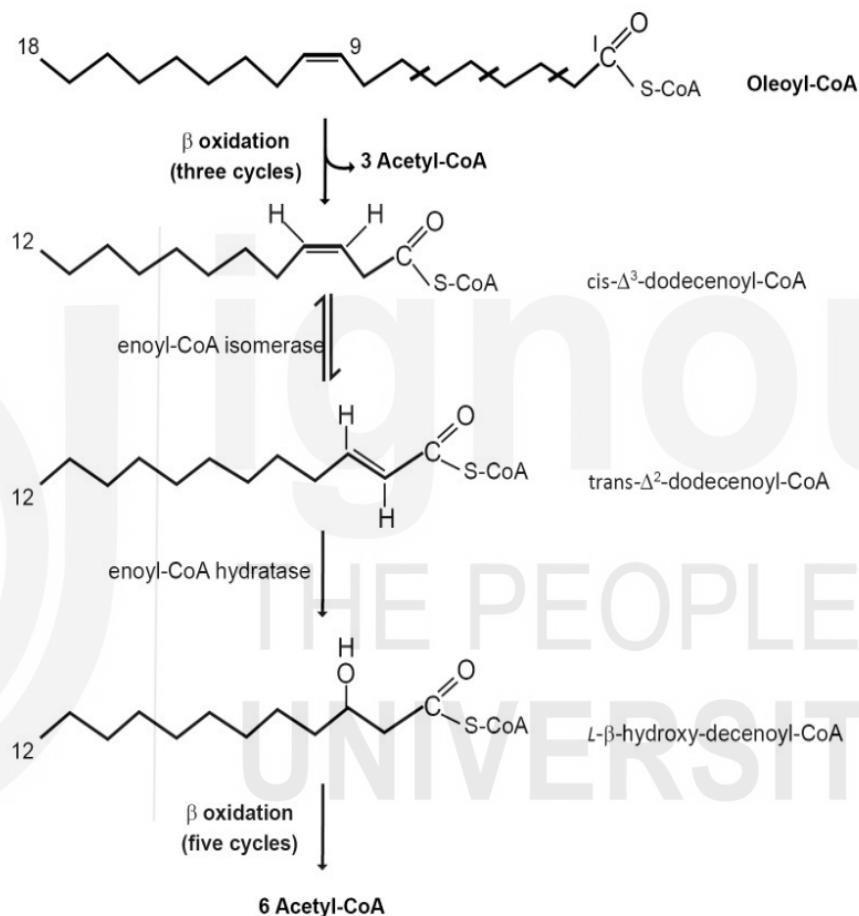


Fig. 16.3: β -oxidation of oleic acid (a monoenoic fatty acid).

In case of polyunsaturated fatty acids, the action of **2, 4-dienoyl CoA reductase and enoyl CoA epimerase** is required to pass the barriers to β -oxidation. The pathway shown in Figure 16.4 depicts the breakdown of linoleic acid. The oxidation of linoleic acid goes through three rounds of β -oxidation as oleic acid. The 12 carbon intermediate, *cis*- $\Delta^3, \text{cis-}\Delta^6$ dodedienoyl CoA is not a substrate of β -oxidation as both the position and configuration of double bonds have to be changed. The enzyme enoyl CoA isomerase isomerizes it to trans- $\Delta^2, \text{cis-}\Delta^3$ allowing one round of β -oxidation from step 2 onwards. By this time, four molecules of acetyl CoA have been released and the 10 carbon dienoyl CoA has trans- $\Delta^2, \text{cis-}\Delta^4$ double bonds. The final hurdle is overcome by the combined action of NADPH dependent 2,4-dienoylCoA reductase and enoyl CoA isomerase to trans- Δ^2 -decenoyl CoA. The resumption of β -oxidation (4cycles) generates five more acetyl CoA.

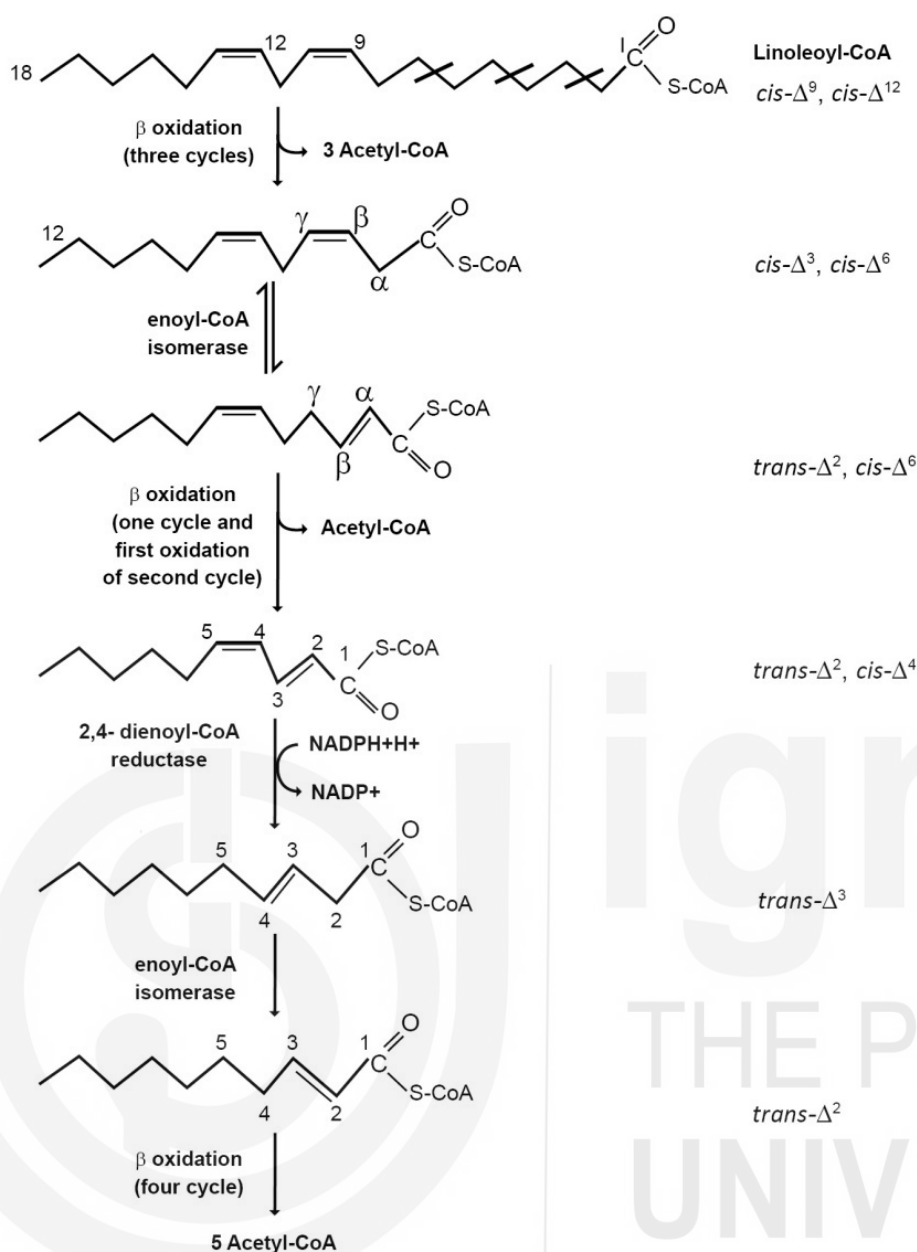


Fig. 16.4: β - oxidation of a poly unsaturated fatty acid.

16.3.3 Energetics

Let us calculate the ATP yield from the complete oxidation of a saturated even number fatty acid, for example palmitoyl CoA (a thioester of a 16 carbon fatty acid). It begins with β - oxidation (7cycles) of an activated fatty acid to produce 8 moles of acetyl CoA and reducing equivalents:



Now recall from unit 10 the fate of acetyl CoA via the citric acid cycle. Each molecule of acetyl CoA generates 3 moles of NADH, 1 mole of FADH_2 and one GTP (or ATP) and 2 CO_2 .



Further 1 mole of FADH_2 gives 1.5 moles of ATP and 1 mole of NADH generates 2.5 moles of ATP when reoxidised by the ETC. The final ATP tally is:

1. FADH_2 : $8 \times 1 = 8$ (TCA cycle) + 7 (β - oxidation) = 15 ATP: $15 \times 1.5 = 22.5$ (oxidative phosphorylation)
2. NADH: $8 \times 3 = 24$ (TCA cycle) + 7 (β - oxidation) = 31 ATP : $31 \times 2.5 = 77.5$ ATP (ETC)
3. GTP (or ATP): $8 \times 1 = 8$ (substrate level phosphorylation in TCA cycle). In organisms producing GTP (Guanosine tri phosphate) it is readily converted to ATP by a kinase.

ATP generated by complete oxidation of one mole of Palmitoyl CoA is the sum of 1 to 3: **$22.5 + 77.5 + 8 = 108$**

The overall equation for the complete oxidation of palmitoyl CoA is the outcome of the three stages:

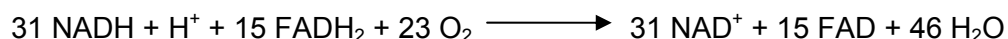
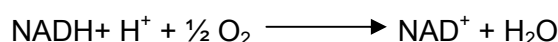


The activation of a fatty acid prior to oxidation consumes an equivalent of 2 moles of ATP. Recall the reaction catalysed by fatty acyl CoA synthetase:



As a result, a net of $(108 - 2) = 106$ ATP moles are generated from complete oxidation of palmitic acid to CO_2 and H_2O .

The oxidation of fatty acids is an important source of **metabolic water** especially in **hibernating animals, migratory birds, killer whale and camels**. The reoxidation of NADH and FADH_2 produces water by reducing the terminal acceptor oxygen.



The net outcome shows 23 H_2O as stage I and II together consume 23 H_2O molecules (46-23).

Each mole of ATP under standard conditions generates 30.5 KJ/ mol of energy; total energy being $106 \times 30.5 = 3230$ KJ/ mol. The total free energy released by oxidation of 1 mole of palmitic acid is 9800 KJ/mol in laboratory conditions. Therefore, the energy conserved as ATP (3230 KJ/mol) under standard conditions (25°C , 1 molar concentrations of substrate and product) from one mole of palmitic acid is approx. 33%. However, the actual efficiency (energy recovery) under cellular conditions is greater than 60% as the concentrations of substrates and products vary and are far from standard concentrations.

Do you know that during hibernation grizzly bear depends entirely on fat stores to replenish water, maintenance of body temperature and energy generation for basal metabolic activities?

16.3.4 Regulation of β -oxidation

The β -oxidation of fatty acids is a tightly regulated pathway as it results in consumption of valuable fuel. How slow or fast a fatty acyl CoA enters mitochondria is dependent on the activity of **acyl carnitine transferase I**, a component of the carnitine shuttle. Once the fatty acyl CoA gains entry it is destined to undergo degradation by β -oxidation. The transferase is allosterically inhibited by **malonyl CoA**, a key intermediate of fatty acid biosynthesis for incorporation into TG (Triglycerides) and phospholipids.

The concentration of malonyl CoA is high in a well fed state when there is much more glucose than required for oxidation or stored as glycogen. In such a situation the animal switches to fatty acid synthesis for fat storage by diverting acetylCoA to malonyl CoA synthesis. Thus β -oxidation is blocked when fatty acid synthesis is favored. The reverse scenario is observed during starvation; the rate of lipolysis is increased and free fatty acids released inhibit the synthesis of malonyl-CoA.

The high ratio of $[NADH] / [NAD^+]$ and acetylCoA inhibit respectively, β -hydroxyacyl-CoA dehydrogenase and thiolase. These metabolites signal energy sufficiency and thereby regulate the pace of β -oxidation.

The above short term regulatory mechanisms allow fast adjustments by altering the activity of existing enzymes. Apart from minute to minute regulation, the enzymes of lipid oxidation and components of the carnitine shuttle are subject to transcriptional regulation that results in changes in the number of enzyme molecules.

SAQ 2

- What are the end product(s) of β -oxidation of odd and even chain fatty acid?
- As compared to the oxidation of a saturated even chain fatty acid name the additional enzymes required for oxidation of the following fatty acids:
 - Monoenoic fatty acid
 - Polyunsaturated fatty acid

16.4 MINOR PATHWAYS OF FATTY ACID OXIDATION

There are two minor pathways of fatty acid oxidation, namely, α - and ω -oxidation. As the name suggests, oxidation occurs at either α - or ω -carbon instead of β -carbon.

ω -oxidation

The **oxidation of fatty acids from the ω -end** (farthest from the carboxyl group) **constitutes ω -oxidation**. It essentially involves the stepwise oxidation of the terminal methyl group to a carboxylic group. The resultant dicarboxylic acids are β -oxidised usually from both ends of the fatty acid to shorter dicarboxylic acids (bilateral β -oxidation).

Verkade and his coworkers (1933) first proposed ω -oxidation of fatty acids to occur in vivo. They showed large amounts of dicarboxylic acids to be excreted in the urine when medium or long chain fatty acids were administered to animals.

The enzymes involved in ω -oxidation are located in the endoplasmic reticulum of liver and kidney of vertebrates. Although it is a minor pathway, however, in case β -oxidation is blocked from the carboxyl end due to inefficient substrate utilisation, oxidation from the ω -end allows β -oxidation to take place. It is considered as a subsidiary oxidation pathway of β -oxidation. In some organisms it is a way to generate ω - hydroxy fatty acids. These acids are important components of royal jelly fed to queen bees; in skin glycolipids, etc. Generally, medium chain fatty acids (10-12 carbon) are oxidised.

α - oxidation

The oxidation of 3- methyl branched fatty acids is initiated by α –oxidation in which fatty acids are shortened by one carbon at a time. It is used to overcome a block in β oxidation. Once a substrate for β oxidation is formed it takes over the further oxidation. The process occurs in peroxisomes. It is reported in yeast, plants and mammals. The oxidation of phytanic acid (3, 7, 11, 15 tetra methyl hexadecanoic acid) is the best known example. Phytanic acid is an oxidation product of phytol, a side chain of chlorophyll and vitamin K2. The origin of phytanic acid is dietary sources such as fish, lamb, beef, cow's milk and foods derived from milk.

Normally, phytanic acid is present in very low amounts in the serum; however, defect in genes for some of the enzymes of this pathway results in a rare autosomal recessive disorder called **Refsum's disease**. Excessive amounts of this fatty acid accumulate in the tissues and serum leading to the neural degeneration and abnormalities in skin and bones. Diets low in phytanic acid has shown significant improvement in these patients especially if dietary control is started early in childhood.

SAQ 3

Fill in the blanks:

- i) The enzymes involved in ω oxidation of fatty acids are located in and of vertebrates.
 - ii) Refsum's disease results in accumulation of in tissues and is due to defect of oxidation pathway of fatty acids.
 - iii) Branched fatty acids having group on are initially oxidised by α - oxidation before β -oxidation resumes.
-

16.5 KETONE BODIES

Acetoacetate, β - hydroxyl butyrate and acetone are collectively called ketone bodies. The liver hepatocyte is the major site of their synthesis. All normal individuals partition a portion of acetyl CoA towards ketone body formation. They function as water soluble transportable, form of acetyl units and are relatively less toxic than fatty acids. Acetoacetate and 3-hydroxy butyrate are normal fuels of respiration in preference for glucose for skeletal and heart muscle and renal cortex. Let us look at the reactions of synthesis of ketone bodies.

16.5.1 Synthesis of Ketone Bodies (ketogenesis)

The synthesis of ketone bodies (Fig.16.5) in the liver mitochondria begins with the condensation of two molecules of acetylCoA by **thiolase** (acetyl CoA acetyl transferase) forming acetoacetyl CoA. The next step is the condensation of acetoacetyl CoA with another molecule of acetylCoA by **HMG CoA synthase**. The product of condensation, β -hydroxy- β -methylglutaryl-CoA (HMG-CoA) is then split by HMG-CoA lyase to acetylCoA and acetoacetate. The lyase is present only in the mitochondria.

The other two ketone bodies can be synthesised from acetoacetate. It may be reduced by **β -hydroxy butyrate dehydrogenase** to D- β -hydroxy butyrate. Recall β -oxidation of fatty acids produces the L-stereoisomer of D-hydroxy acyl CoA. Finally, acetoacetate may be decarboxylated non-enzymatically or by acetoacetate decarboxylase to acetone, a volatile compound which is exhaled. The liver releases acetoacetate and β -hydroxy butyrate into circulation to be picked up by extra-hepatic tissues.

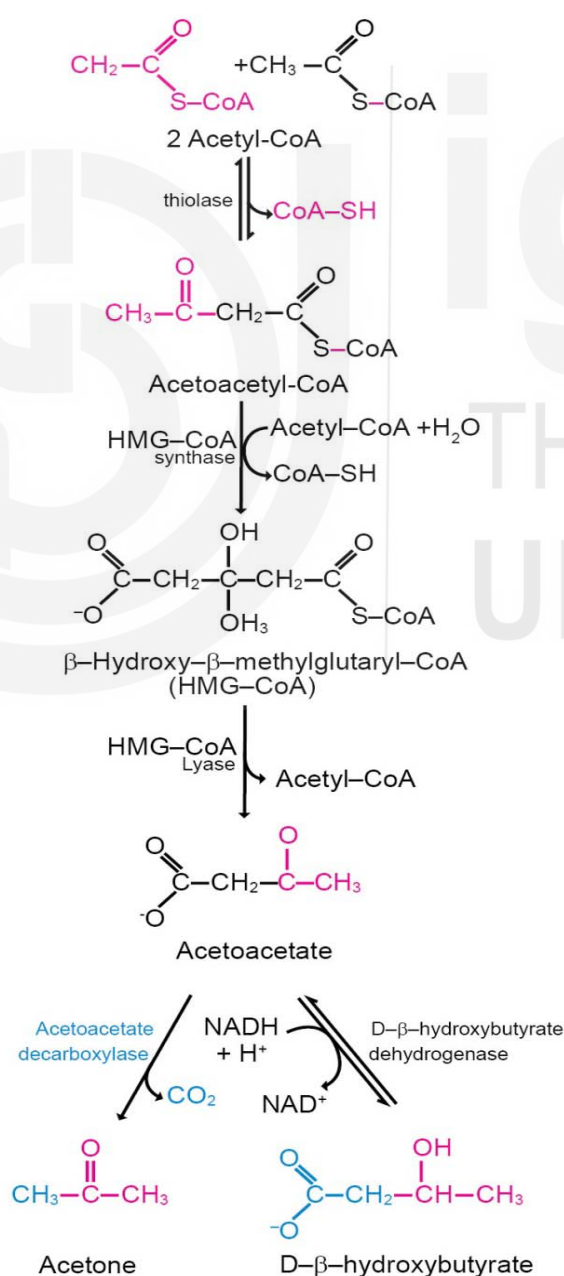


Fig. 16.5: The pathway of ketogenesis.

16.5.2 Utilisation

The extrahepatic tissues convert ketone bodies back to acetyl CoA in the mitochondria (Fig.16.6). An enzyme missing in the liver and present in all tissues holds the key to utilisation of ketone bodies, no doubt to varying extent. The enzyme is **β -ketoacyl CoA transferase** (or thiophorase) that activates acetoacetate by transferring CoA group from succinyl CoA. The next step is the thiolytic cleavage by acetoacetyl CoA thiolase. In case of β -hydroxybutyrate; it is first oxidised to acetoacetate by β -hydroxy butyrate dehydrogenase. The resultant acetylCoA units can now enter the TCA cycle to complete oxidation. The dehydrogenase and thiolase function in both synthesis and utilisation of ketone bodies.

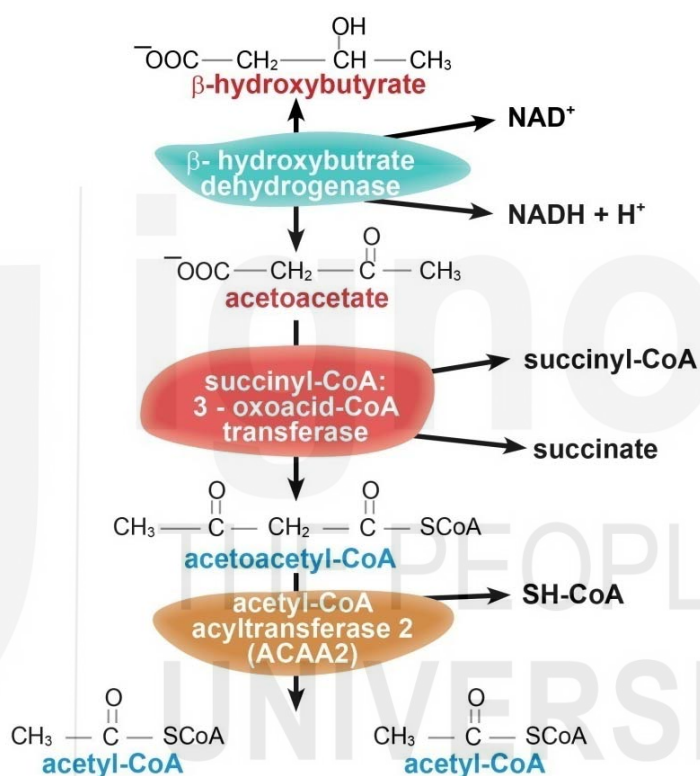


Fig. 16.6: The conversion of ketone bodies to acetyl CoA in extrahepatic tissues.

16.5.3 Ketosis

Ketogenesis occurs at moderate levels under normal physiological conditions as well as when carbohydrates are not available in adequate amounts. However, when ketone bodies are synthesized at higher than normal amounts, their levels also increase markedly in blood and urine. These conditions are known as **ketonemia** and **ketonuria**, respectively.

The ketone bodies are moderately strong acids and are buffered in blood and tissues. Their excretion leads to progressive decrease in buffer cation leading to a drop in blood pH. These results in a condition called **ketoacidosis**. It is commonly observed in prolonged starvation, untreated type I diabetes and even under conditions of high fat feeding. Moreover, because of volatile nature of acetone, its odour is detected in the breath of such patients. The triad of ketonemia, ketonuria and acetone odor is collectively termed **ketosis**.

SAQ 4

- Name the ketone bodies.
- Define ketogenesis.
- Name two conditions that result in ketosis.

16.5.4 Regulation

The importance of the pathway can be judged from the fact that liver has the potential to make as much acetoacetate as equivalent to half of its weight every day. The substrate for ketone bodies formation is fatty acid. Therefore, factors regulating mobilization of fatty acids from the adipose tissue are important in controlling ketogenesis. Figure 16.7 explains as to how interplay of hormones, enzymes and substrates involved in ketogenesis regulates this process in a coordinated manner.

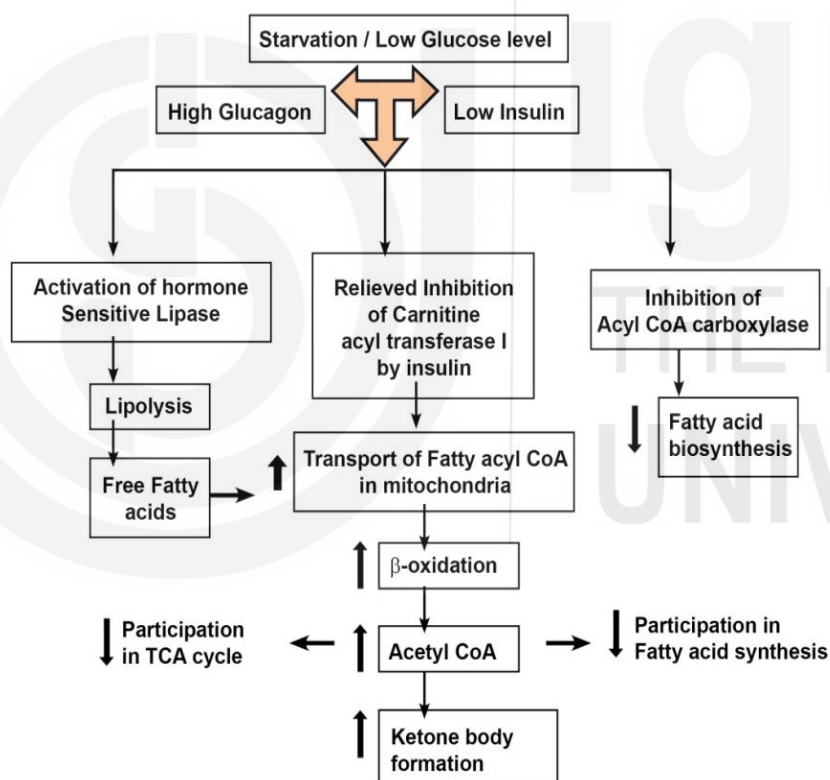


Fig. 16.7: Regulation of ketone bodies formation.

During starvation the amount of carbohydrates is limited. This results in secretion of less insulin and more of glucagon by pancreas. This in turn activates hormone sensitive lipase resulting in enhanced lipolysis and release of free fatty acids from adipose tissues. These fatty acids are transported via blood to liver where they are activated and rapidly transported across the mitochondrial membrane which is linked to carnitine as low levels of insulin relieve the inhibition of carnitine acyl transferase. Increased fatty acyl CoA level results in their increased β oxidation and formation of acetyl CoA. In the liver acetyl CoA can have one of the following three fates:

1. Acetyl CoA can condense with oxaloacetate and enter TCA cycle. In case of low carbohydrates levels, oxaloacetate is used for glucose synthesis, therefore acetyl CoA can minimally be utilized by this pathway. This is the molecular basis of the statement, 'fats burn in the flame of carbohydrates'.
2. Conversion of acetyl CoA to malonyl CoA for biosynthesis of fatty acids. This pathway is also inhibited as you can see in the figure 16.7 that acetyl CoA carboxylase which catalyses this step is inhibited by increased glucagon levels. At the same time β -oxidation is favored due to high activity of carnitine palmitoyl transferase-I.
3. The only route available for excess acetyl units is ketogenesis. This can lead to ketosis seen in diabetics and starvation. In these conditions even the brain adapts to utilisation of acetoacetate. During prolonged starvation, 75% of the fuel needs of the brain are met by acetoacetate.

So far we have learnt about catabolic aspect of lipids which can serve as fuel for cells. However, the discussion is not complete until we learn about how fatty acids are synthesized.

16.6 DE NOVO LIPOGENESIS

De novo is a Latin word which means "taken from new". *De novo* synthesis indicates that complex biomolecules are made beginning from the simple precursors and not by recycling of partially degraded intermediates.

The synthesis of fatty acids starting from simple precursor (acetyl CoA) is known as *de novo* lipogenesis. In animals (and yeast), it occurs primarily in the cytosol of various tissues such as liver, adipose (fat) tissue, central nervous system and lactating mammary glands. In plants, enzymes of this process are present in the chloroplast. Lipogenesis is an endergonic reductive process. The source of reductant is NADPH. The intermediates of fatty acid synthesis are covalently linked to an acyl carrier protein (ACP).

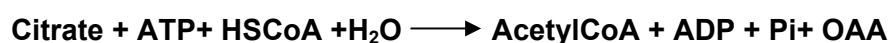
The fatty acid chain is elongated in steps of two and the 2 carbon units are derived from acetylCoA. The actual 2C donor is malonyl CoA. Different steps of fatty acid synthesis are catalysed by fatty acid synthase complex (FAS). The organization of FAS varies among different groups; it is multienzyme in some and multifunctional in others.

Let us elaborate how fatty acid synthesis occurs in animals.

16.6.1 Transport of Acetyl Groups from Mitochondria to the Cytosol

Acetyl CoA is the starting material for fatty acid synthesis. It is formed in the mitochondria from pyruvate oxidation and metabolism of carbon skeleton of some amino acids. Acetyl CoA produced by β -oxidation of fatty acids does not contribute towards biosynthesis of fatty acids. Since biosynthesis of fatty acids occurs in cytosol and mitochondrial inner membrane is not readily permeable to acetyl CoA, citrate functions as a carrier of acetyl groups from the mitochondria to the cytosol (Fig.16.8).

Mitochondrial acetyl-CoA first condenses with oxaloacetate (OAA) to form citrate in reaction catalyzed by citrate synthase. Citrate is then transported across the inner membrane by **citrate transporter**. Once in the cytosol, citrate is cleaved by **citrate lyase** to acetyl CoA and oxaloacetate in an ATP dependent reaction.



Finally, oxaloacetate is converted to either pyruvate or malate and shuttled back to mitochondria via pyruvate transporter and malate α -ketoglutarate transporter, respectively. Both malate and pyruvate can be converted to oxaloacetate in the mitochondria by malate dehydrogenase and pyruvate carboxylase, respectively. An added advantage of the malic enzyme catalysed step is the cytosolic generation of NADPH to support reductive synthesis of fatty acids. The synthesis of palmitate requires eight molecules of acetyl CoA and if all malate produced in the cytosol from citrate is oxidatively decarboxylated then 8 out of the 14 NADPH required may come from this reaction.

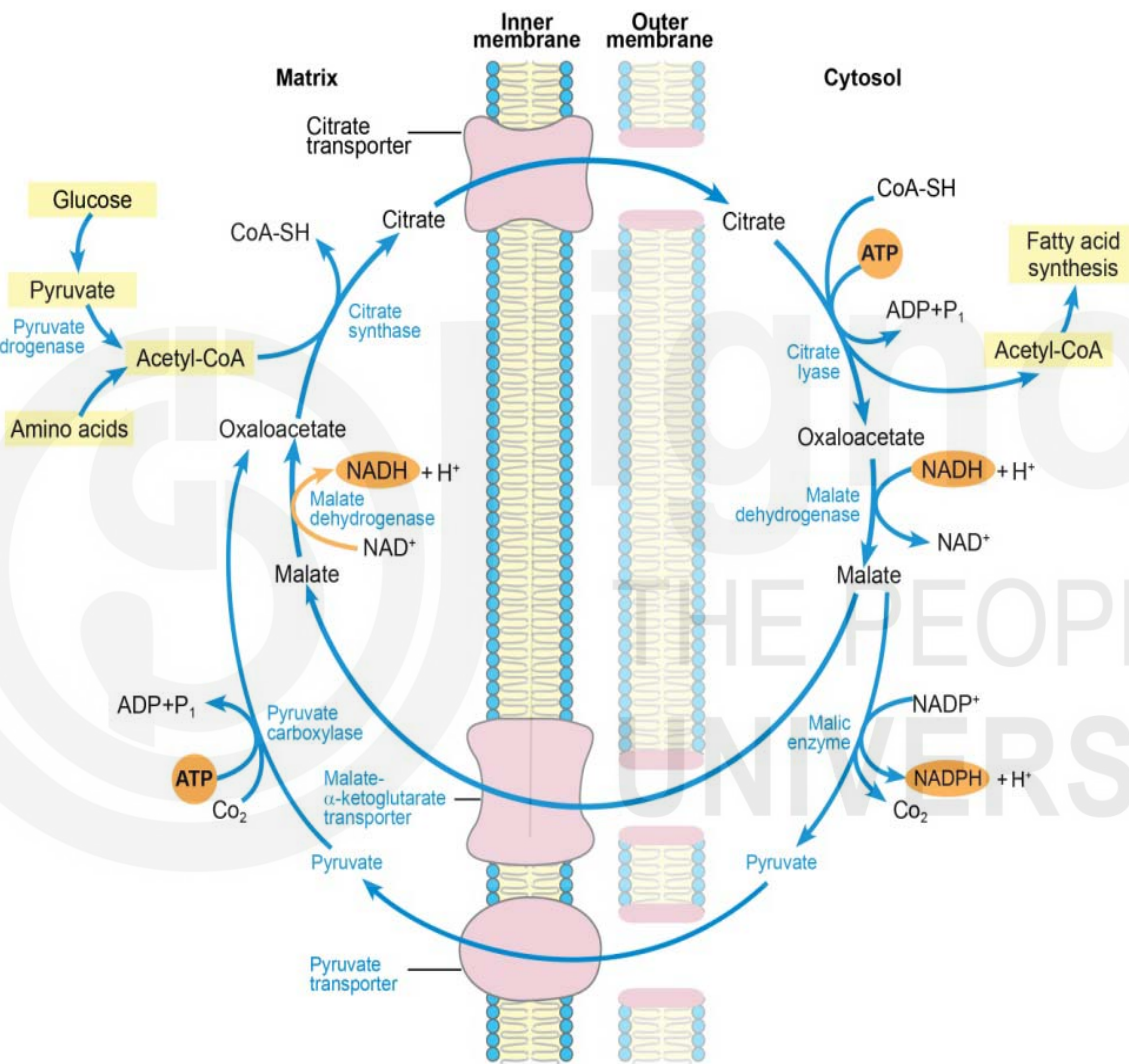


Fig. 16.8: Transport of citrate across mitochondrial membrane.

The *de novo* synthesis of fatty acids is carried out by two enzymes; acetyl CoA carboxylase (ACC) and fatty acid synthase (FAS).

16.6.2 Conversion of Acetyl CoA to Malonyl CoA

The synthesis of malonyl CoA from acetyl CoA is catalysed by acetyl CoA carboxylase (ACC). It is an irreversible and committed step of fatty acid synthesis. In mammals and yeast, ACC is a multifunctional enzyme (230kD) having both enzymatic activities and biotin carboxyl carrier. The reaction takes place in two steps (Fig.16.9):

- (i) Biotin carboxylase catalyses carboxylation of biotin at the expense of ATP hydrolysis, forming carboxy-biotin; a temporary activated carrier of CO_2 . With the formation of carboxy-biotin, ADP and P_i are released before the binding of the second substrate, acetyl CoA.
- (ii) The transcarboxylase transfers the activated CO_2 from carboxy-biotin to acetyl CoA, releasing malonyl CoA.

Acetyl-CoA carboxylase

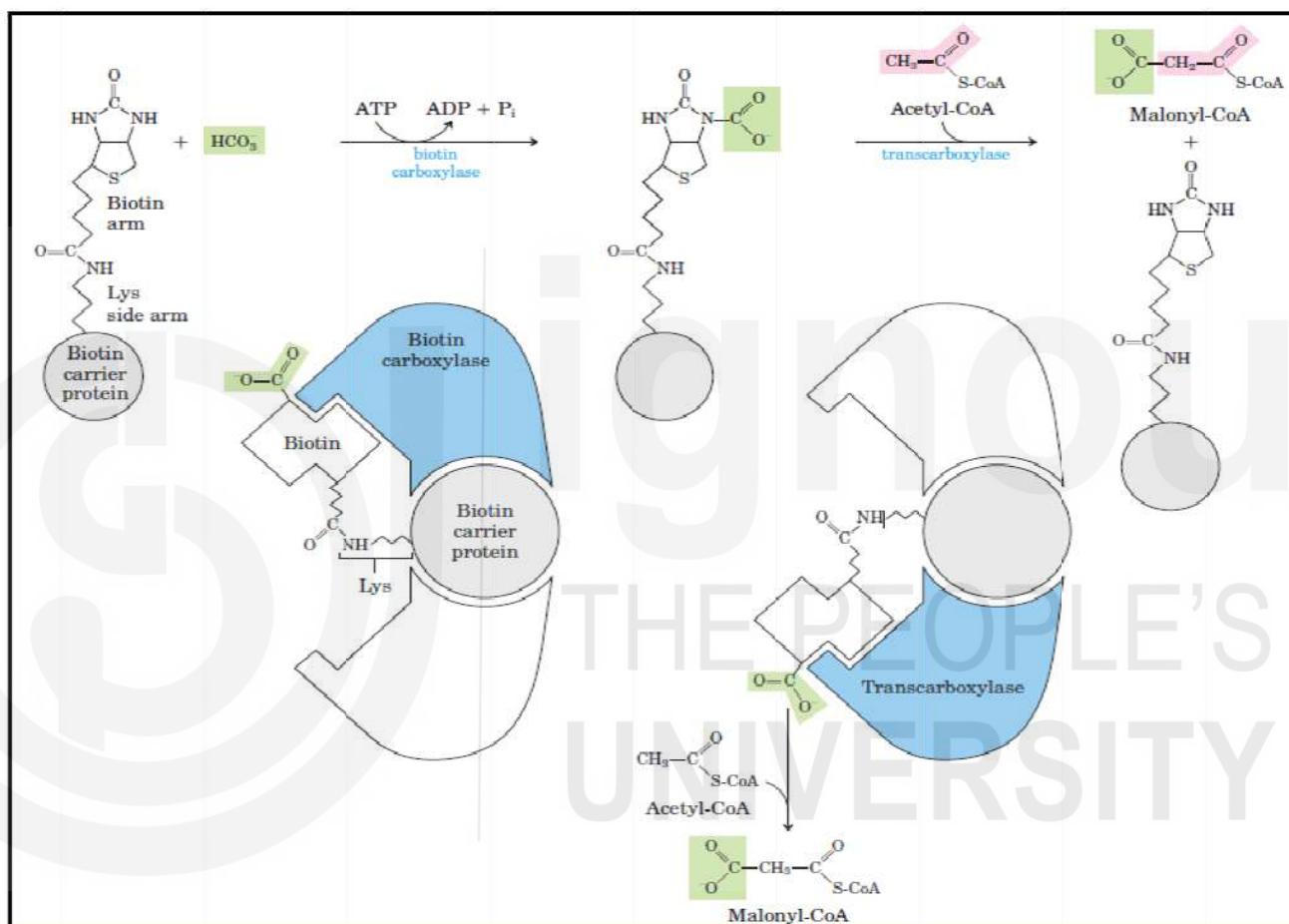
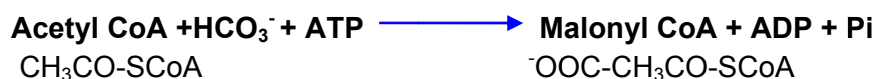


Fig. 16.9: Synthesis of malonyl CoA by acetyl CoA carboxylase.

Before we discuss about the final step of chain elongation of fatty acid, let us discuss in detail the organisation of FAS.

16.7 FATTY ACID SYNTHASE (FAS)

The synthesis of saturated fatty acid is catalysed by fatty acid synthase (FAS) complex which brings about the reductive synthesis of fatty acids. The seven catalytic units are organised either as domains of a multifunctional enzyme (FAS I) or as separate polypeptides (FAS II). The basic chemistry of fatty acid synthesis is conserved through evolution.

FAS-I is present in vertebrates and fungi. The animal FAS is a homodimer with each chain folded into three domains; domain 1 is substrate entry and condensation unit, domain 2 is reduction unit and domain 3 is the release unit

The fungal FAS system has two multifunctional polypeptides in which three active sites reside on the α -subunit and four on the β -subunit. These subunits assemble into a large $\alpha_6\beta_6$ hetero dodecameric form.

(Fig. 16.10). The two identical subunits are arranged anti-parallel such that domain I of each chain of the dimer interacts with domain 2 and 3 of the other chain. Although multiple enzymes are present on same chain in FAS I, their reactions are spatially isolated and the flexible arm of ACP carries intermediates from one active site to another. Thus two functional units consist of domains from different chains. In this system only the final product (palmitate) synthesised after multiple cycles is released.

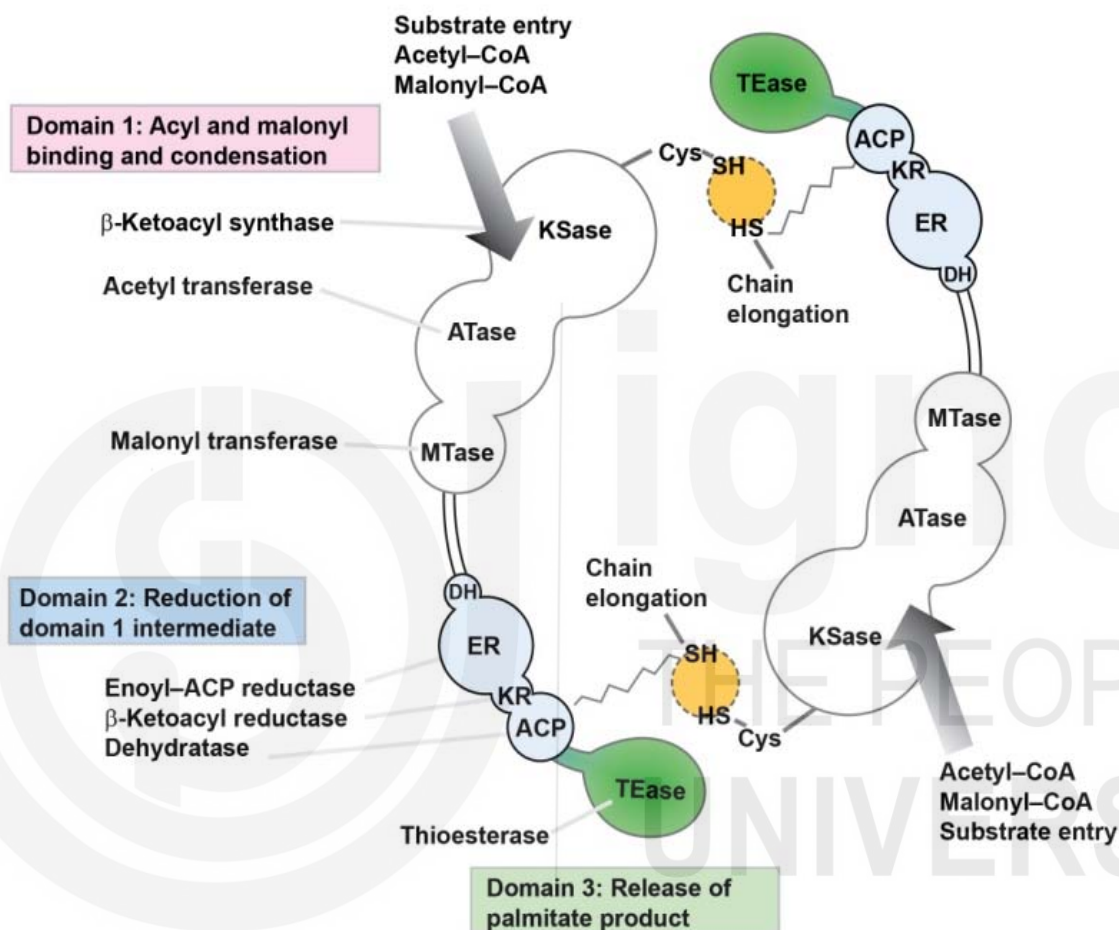


Fig. 16.10: Animal fatty acid synthase.

In FAS II each step is catalysed by a separate enzyme. The intermediates are released and then diffuse to the next enzyme; they may also be diverted to other routes. The FAS II generally ends up producing a variety of fatty acids. It is present in plants, bacteria and vertebrate mitochondria.

16.7.1 Repetitive condensation of two carbon units by FAS-I

The mammalian FAS I essentially performs four reactions that result in chain elongation by two carbon. These steps are repeated in each cycle until the final product is formed. The 2C unit is derived from malonyl CoA except in the first round in which acetyl CoA 'primes' synthesis. In each round condensation is accompanied by decarboxylation. The complex has seven catalytic activities and ACP (Table 16.1).

Table 16.1: The activities of mammalian Fatty Acid Synthase I complex.

S.No	Enzyme/ Protein	Function
1.	Acyl Carrier Protein (ACP)	Carries acyl groups.
2.	Acetyl CoA ACP Transacetylase (AT)	Transfers acyl group from CoA to cysteine of KS/ CE
3.	Malonyl CoA ACP Transferase (MT)	Transfers malonyl group from CoA to ACP
4.	β - ketoacyl ACP Synthase (KS) // Condensing enzyme (CE)	Condenses acyl and malonyl groups
5.	β - ketoacyl ACP Reductase (KR)	Reduces β - keto group to β - hydroxyl group
6.	β - hydroxyacyl ACP Dehydratase (HD)	Removes water from β - hydroxyacyl ACP, creating double bond
7.	Enoyl ACP Reductase (ER)	Reduces double bond, forming saturated acyl ACP
8	Thioesterase (TE)	Hydrolyses the thioester linkage and releases palmitate.

Out of these 7 components, ACP is a non enzymatic protein and specifically carries the acyl intermediates during the process. The complex facilitates four reactions. To begin with, the acetyl group of acetyl-CoA is transferred to the cysteine -SH group of the β -ketoacyl-ACP synthase. This step is catalyzed by **acetyl-CoA-ACP transacetylase**.

In the *second step*, the malonyl group from malonyl-CoA is transferred to the -SH group of ACP by the enzyme **malonyl-CoA-ACP transferase**, also part of the complex. At this stage acetyl CoA and malonyl CoA positioned very close to each other are charged for the preparation of the long chain of the fatty acid in the four reactions:

1. **Condensation:** Acetyl group is transferred to the malonyl CoA attached to ACP. The two molecules condense to form Acetoacetyl ACP with the release of CO_2 . This is the same CO_2 molecule which was added in the beginning to form malonyl CoA from acetyl CoA.
2. **Reduction:** Carbonyl group present in Acetoacetyl ACP undergoes reduction to form D- β - hydroxybutyryl-ACP. NADPH is the electron donor in the reaction.
3. **Dehydration:** A molecule of water (H_2O) is removed to form a double bond containing product, *trans*- Δ^2 - butenoyl-ACP.
4. **Reduction of the double bond:** Finally the double bond of *trans*- Δ^2 - butenoyl-ACP is reduced to form butyryl-ACP. Another NADPH donates the electrons for this reduction.

The reactions as catalysed by different enzymes are shown in the Figure 16.11.

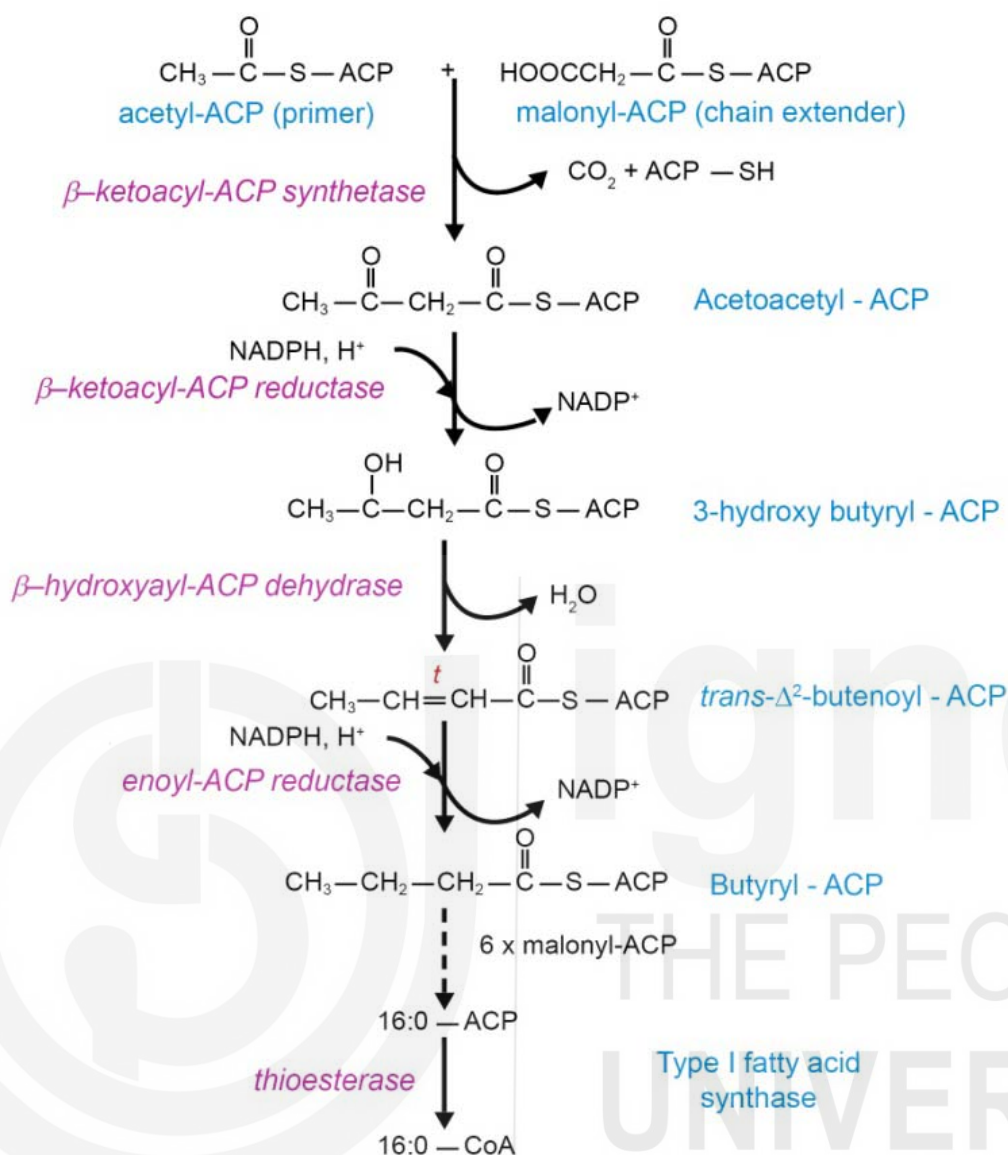
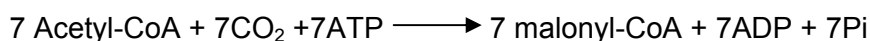


Fig. 16.11: Reactions catalysed by fatty acid synthase complex.

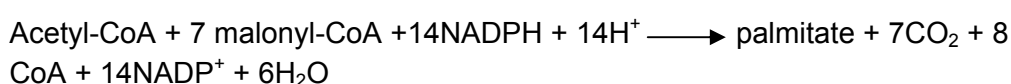
In the next round butyryl-ACP gets condensed with malonyl ACP to continue elongation. The process is repeated 7 times to synthesize 16 carbon palmitate. Once a C-16 acyl ACP is formed, the thioesterase (TE) hydrolyses it to release palmitate. The enzyme from different sources varies in the chain length of fatty acid released.

Before we proceed to study the elongation and unsaturation of fatty acids let us calculate the cost of palmitate synthesis starting from acetyl-CoA:

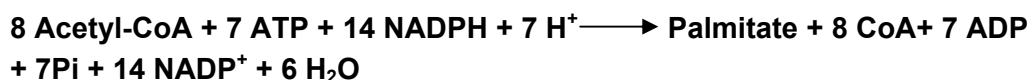
(i) Synthesis of seven malonyl-CoA molecules:



(ii) Seven cycles of condensation and reduction:



The **net reaction** for the synthesis of palmitate (C-16) is:



You have noticed that all carbons except the two at the ω -end (C-15 and C-16 in case of palmitate) are derived from malonyl CoA. Those at ω -end come directly from acetyl CoA.

SAQ 5

- a) Arrange the following steps of palmitate biosynthesis in order of occurrence:
 - (i) Conversion of acetyl CoA to malonyl CoA
 - (ii) Elongation of fatty acyl chain
 - (iii) Transport of acetyl CoA from mitochondria
 - b) Fill in the blanks:
 - (i) moles of acetyl CoA, ... ATP and NADPH are used to synthesise one mole of palmitate.
 - (ii) The subcellular site of *de novo* lipogenesis is in animals and in plants.
 - (iii) FAS I complex has activities on the same chain.
 - (iv) The source of NADPH for lipogenesis in animals is and
-

16.8 PALMITATE AS SOURCE OF OTHER FATTY ACIDS

Palmitate (a C-16 fatty acid) is the major product of the fatty acid synthase complex. In plants and animals, the most important fatty acids are the C-18 fatty acids, namely stearic, oleic, linoleic and linolenic. Palmitate may be lengthened to form stearate (18 : 0) or even larger saturated fatty acids by further addition of acetyl groups through the action of fatty acid elongation systems present in the smooth endoplasmic reticulum and mitochondria in the case of animals and in the soluble cytosol in the case of plants. The elongation pathways use coenzyme-A as acyl carrier rather than acyl carrier protein (ACP) of fatty acid synthase complex and use NADPH instead of FADH₂ for saturation of double bond.

Monounsaturated fatty acid oleate is synthesized by desaturase enzyme system also present in the SER of animal cells. Desaturases belong to mixed function oxidases. The pathways are depicted in the Figure 16.12.

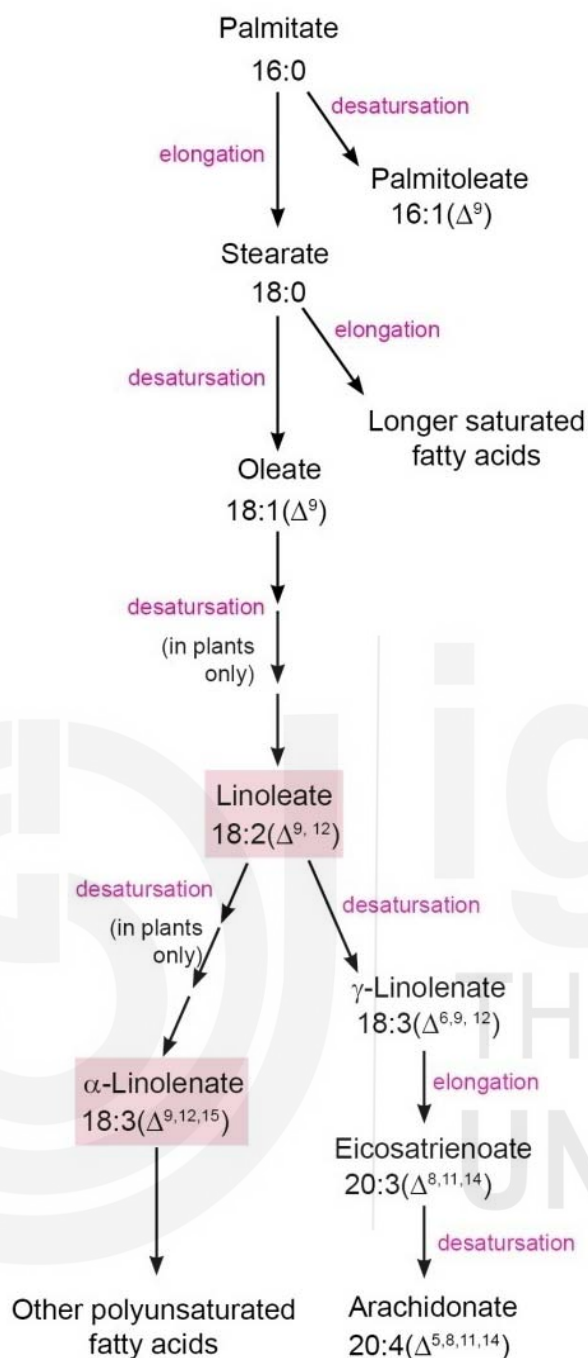


Fig. 16.12: Formation of different fatty acids from palmitate.

You can make out from the Figure 16.12 that animals have the enzyme system only to introduce single unsaturated bond in the fatty acid chain but plants have the ability to add more than one double bond. Therefore, animals cannot synthesize polyunsaturated fatty acids such as linoleic and linolenic acids hence considered essential fatty acids which they need to take from the plant diet.

Now that you have gone through the detailed process for the oxidation and biosynthesis of fatty acids, you will appreciate that the two pathways are not a simple reversal but catalysed by different set of enzymes that are independently regulated. In Table 16.2 a comparison of synthesis and degradation is presented.

Table 16.2: Comparison of fatty acid synthesis and degradation.

Characteristic	β - oxidation	Fatty acid synthesis
Subcellular location	Mitochondria / peroxisomes	Cytosol / chloroplasts (plants)
Acyl carrier	HS CoA	ACP / HSCoA
e ⁻ acceptor / donor	FAD and NAD ⁺	NADPH
2-C product / donor	Acetyl CoA / acetyl CoA & propionyl CoA	Malonyl CoA and acetyl CoA
Direction of synthesis / degradation	From carboxyl to methyl (ω) end.	From methyl to carboxyl end.
Isomer of hydroxy acyl CoA intermediate	L-isomer	D-isomer
Organisation of enzymes	Separate / some isoforms are multifunctional	Multifunctional / separate FAS enzymes
Regulation	Carnitine acyl transferase I is inhibited by malonyl CoA.	ACC is the prime target; citrate (+) and palmitoyl CoA (-) are modulators.

SAQ 6

- Linoleic and linolenic acids are essential fatty acids. Give reasons.
- How do β - oxidation and synthesis of fatty acids differ in terms of subcellular location, acyl carriers and regulation?

16.9 REGULATION OF FATTY ACID SYNTHESIS

The reaction catalysed by ACC plays a key role in controlling fatty acid synthesis and degradation. In animals this is mediated by two isoforms of ACC. The short term regulation is by both allosteric modulators and reversible covalent modifications.

The positive modulator of ACC is **citrate**. It signals abundance of energy and building blocks. One of the effects of citrate is to promote the conversion of inactive octamer into catalytically active polymers. It increases rate of the reaction (V_{max}) without affecting the affinity for its substrates (K_m). The negative modulator is **palmitoyl CoA**, the end product of fatty acid synthesis in animals. It causes the active polymer to disassemble into inactive octamer. Palmitoyl CoA also inhibits the translocase for transport of citrate, FAS and glucose 6-phosphate dehydrogenase (G6PD), the first enzyme of hexose monophosphate (HMP) shunt responsible for providing NADPH (Fig. 16.13).

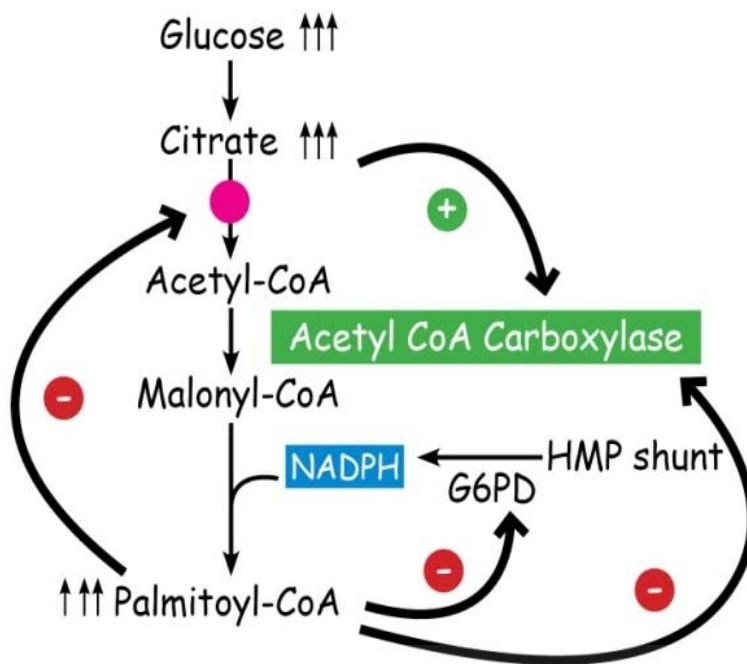


Fig.16.13: Allosteric regulation of fatty acid synthesis.

The reversible phosphorylation/dephosphorylation regulates the activity of ACC in response to global signals (glucagon, epinephrine and insulin). Phosphorylation by AMP dependent protein kinase (AMPK) or cAMP dependent protein kinase (PKA) converts the enzyme into inactive form. Glucagon and epinephrine activate PKA which inhibits the phosphatase by phosphorylating it. This keeps ACC in an inactive phosphorylated state. The phosphorylated enzyme is less sensitive to activation by citrate. Insulin on the other hand activates ACC by stimulating its dephosphorylation (Fig. 16.14).

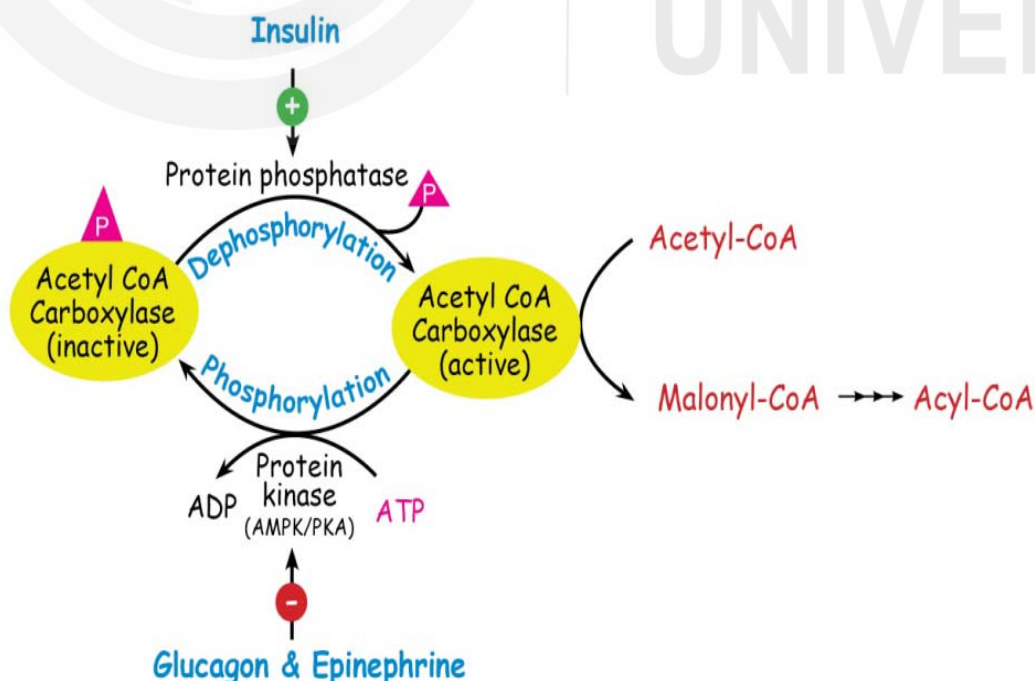


Fig.16.14: Regulation of fatty acid biosynthesis in animals by covalent modifications.

In fact, citrate plays an important role in deciding whether acetyl CoA enters TCA cycle or is diverted for fatty acid synthesis. When there are enough fuel, concentrations of mitochondrial acetyl CoA and ATP increase. As a result citrate is transported out to cytosol where it is both a precursor for acetyl CoA as well as allosteric activator of ACC, resulting in *de novo* fatty acid synthesis.

The long term control brings about a net change in the amount of enzymes by altering the rates of synthesis and degradation. Insulin stimulates the synthesis of both ACC and FAS.

SAQ 7

- (i) The chain elongation of preformed fatty acids occurs in and
 - (ii) enzymes introduce double bonds in fatty acids.
 - (iii) cannot synthesize linoleic and α -linolenic acid.
 - (iv) plays a key role in the overall control of *de novo* lipogenesis in animals.
-

16.10 SUMMARY

Let us summarise what we have learnt so far:

- Fats provide energy by primarily by oxidation of fatty acids in mitochondria.
- Vertebrates such as humans get fatty acids from four sources during starvation: food, stored fat deposits in the form of triglycerides (TGs), fats formed in other tissues for instance in liver from excess carbohydrates for export to other tissues and autophagy, i.e. by degradation of cell's own organelles.
- Mitochondria are the primary site of fatty acid oxidation. However, fatty acids need to be activated before they are oxidized and both these processes take place in different cell compartments.
- Fatty acids are activated to fatty acyl CoA in the outer mitochondrial membrane and transported via carnitine shuttle to mitochondrial matrix for oxidation.
- The oxidation of fatty acids is the central energy yielding pathway in animals, many protists and some bacteria. Major pathway is by oxidation at β -carbon, hence known as β oxidation of fatty acids.
- The β -oxidation of saturated fatty acids occurs in four steps: oxidation, hydration, oxidation and thiolysis. It is a spiral pathway in which each round of oxidation generates acetyl CoA, NADH and FADH₂.

- The number of cycles of β -oxidation for a fatty acid of n carbon atoms is $(n / 2) - 1$.
- Oxidation of fatty acids with even number of carbons produces acetyl CoA, while oxidation of those with odd number produces propionyl CoA and acetyl CoA.
- Fatty acids are also oxidized by two minor but important pathways namely, α - and ω - oxidation.
- A part of acetyl CoA is converted to acetoacetate, β - hydroxyl butyrate and acetone collectively called ketone bodies in all normal individuals. The liver hepatocyte is the major site of their synthesis.
- Ketone bodies function as water soluble transportable form of acetyl units and are relatively less toxic than fatty acids. Acetoacetate and 3-hydroxy butyrate are normal fuels of respiration in preference for glucose for skeletal and heart muscle and renal cortex.
- Formation of small amounts of ketone bodies is normal physiological process, however, during prolonged starvation, untreated type I diabetes and even under conditions of high fat feeding these are synthesized in large amounts resulting in decrease of blood pH due to their acidic nature and their increased secretion in urine.
- The synthesis of fatty acids starting from simple precursor (acetyl CoA) is known as *de novo* lipogenesis. In animals, it occurs primarily in the cytosol of various tissues such as liver, adipose (fat) tissue, central nervous system and lactating mammary glands.
- Synthesis of the fatty acid chain takes place in steps of two and the 2 carbon units are derived from acetyl CoA. Different steps of fatty acid synthesis are catalysed by fatty acid synthase complex (FAS).
- FAS is a complex of seven enzyme activities and one non enzymic carrier protein. Palmitate (a C-16 fatty acid) is the major product of the fatty acid synthase complex which is converted to other fatty acids except essential fatty acids mediated by elongase enzymes.

16.11 TERMINAL QUESTIONS

1. Explain the processes of activation and transport of fatty acids to mitochondrial matrix for their oxidation.
2. Explain, how many ATPs are produced by complete oxidation of stearic acid?
3. Describe ketogenesis. Is it a normal physiological process?
4. Write the name and function of enzyme and non enzyme components of fatty acid synthase (FAS) complex.
5. Explain, how is fatty acid synthesis regulated?

16.13 ANSWERS

Self Assessment Questions

1.
 - (i) Carnitine acyl transferase I is present in the outer mitochondrial membrane and helps in transfer of activated fatty acid to carnitine shuttle.
 - (ii) Acyl CoA synthetase is present in the outer mitochondrial membrane and activate fatty acid to fatty acyl CoA.
 - (iii) Carnitine acyl carnitine translocase is present on the inner mitochondrial membrane and transfers fatty acyl CoA complex from outer mitochondrial membrane to mitochondrial matrix.
2.
 - a)
 - (i) Acetyl CoA and propionyl CoA,
 - (ii) acetyl CoA
 - b)
 - (i) Enoyl CoA isomerase
 - (ii) 2, 4-dienoyl CoA reductase and enoyl CoA isomerase
3.
 - (i) Endoplasmic reticulum of liver and kidney
 - (ii) phytanic acid and α -oxidation
 - (iii) methyl group; β -carbon
4.
 - (i) Acetoacetate, acetone and β -hydroxyl butyrate,
 - (ii) The process of formation of ketone bodies;
 - (iii) Diabetes and starvation
5.
 - a) The order is (iii), (i) and (ii)
 - b) (i) 8; 7; 14, (ii) Cytosol; chloroplast, (iii) Seven, (iv) oxidative pentose pathway and oxidative decarboxylation of malate.
6.
 - a) Our body does not contain the enzymes necessary for synthesis of these fatty acids, therefore, these need to be supplemented in our food.
 - b) Please refer to table 16.2.
7.
 - i) Mitochondria and ER; ii) desaturases, iii) animals, (iv) ACC

Terminal Questions

1. Fatty acids are activated to fatty acyl CoA in the outer mitochondrial membrane and transported via carnitine shuttle to mitochondrial matrix before they undergo β oxidation. For more details refer to the section 16.2.
2. The total ATP yield of oxidation of stearic acid is 120 ATP.

3. Ketogenesis is the process of formation of ketone bodies from acetyl CoA. Refer to the section 16.5 for detailed reactions.

Ketogenesis occurs at moderate levels under normal physiological conditions as well as when carbohydrates are not available in required amounts. They are preferred fuels for some tissues like the skeletal and heart muscles.

4. FAS is a complex of seven enzymes and one non enzyme protein carrier. For more details refer to the section 16.7.
5. Fatty synthesis is regulated by both allosteric modulators and reversible covalent modifications. Refer to the section 16.9 for more details.



PROTEIN METABOLISM |

Structure

17.1	Introduction Objectives	17.5	Overview of Amino Acid Biosynthesis
17.2	Metabolic Fates of Amino Acids	17.6	Regulation of Urea cycle
17.3	Nitrogen Economy and Protein Turnover	17.7	Summary
17.4	Amino Acid Metabolism Transamination Deamination Transport of ammonia Urea (Krebs and Hensleit) Cycle	17.8	Terminal Questions
		17.9	Answers

17.1 INTRODUCTION

So far you have studied about carbohydrate and lipid metabolism in the previous units. In this unit you will study about the metabolism of proteins. As you know, protein has diverse roles to play; it makes the structural tissue for muscles and tendons, transports oxygen as hemoglobin, catalyses biochemical reactions as enzymes and plays regulatory role as hormones. Interestingly, our body is capable of synthesising wide variety of these proteins that are needed for growth, repair, maintenance and regulatory functions. However, proteins in excess or during starvation are catabolized to supply energy. We will study in detail about the metabolic fates of amino acids, the building blocks of proteins as well as various steps of amino acid degradation in this unit. We will also focus on biosynthesis of amino acids at the end.

Objectives

After studying this unit, you should be able to:

- ❖ state the metabolic fates of amino group in an amino acid,
- ❖ differentiate between transamination and deamination,

- ❖ explain the pathway for safe conversion and excretion of highly toxic ammonia to harmless urea,
- ❖ give an overview of amino acid biosynthesis, and
- ❖ differentiate between short term and long term regulation of urea cycle.

17.2 METABOLIC FATES OF AMINO ACIDS

During digestion, proteins are converted into their constituent amino acids by the action of various proteolytic enzymes. Amino acids are then absorbed into blood and transported to the liver. They are used in the body to synthesise new proteins for growth and repair. They are also required in small proportion for the synthesis of specialized nitrogenous molecules such as epinephrine and nor-epinephrine, neurotransmitters and precursors of purines and pyrimidines, the nitrogenous bases of nucleic acid (Fig. 17.1).

Unlike carbohydrates and triglycerides, which are stored, proteins cannot be stored for later use. In fact, any amino acid which is not needed for immediate biosynthetic needs is deaminated and the carbon skeleton is used as a metabolic fuel. As a rule, the overall process of amino acid degradation in higher animals is a complete combustion of the carbon skeleton, and excretion of most of the ammonia arising in the mammalian body from the degradation of amino acids and other nitrogenous materials in the form of urea.

The carbon skeletons of amino acids undergo oxidative degradation to moieties that can enter the tricarboxylic acid cycle (TCA) for oxidation. The amino groups of most of the L-amino acids are removed by transamination.

Purines and pyrimidines are the building blocks of nucleic acids. These are heterocyclic nitrogenous bases. Purines include adenine and guanine, and are double ringed while pyrimidines include cytosine, thymine and uracil, and are single ring structures.

The primary source of amino acid is the protein which we take in our food. However, amino acids are also synthesised in the body, as well as derived from breakdown of worn out tissues. These amino acids are added to the body fluid to form the '**amino acid pool**' which contains all the twenty different kinds of L- α - amino acids found in the proteins. It is small and dynamic, i.e., turns over rapidly, and hence is also called as 'labile amino acid pool'.

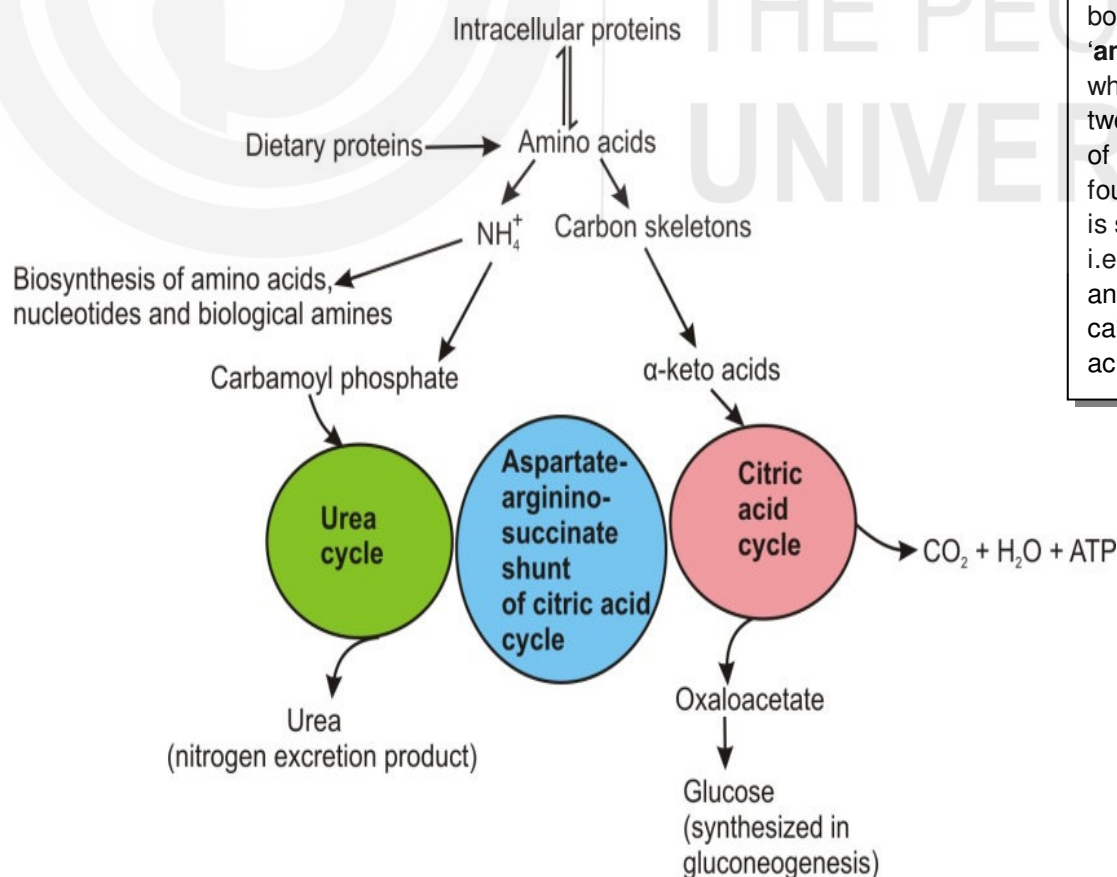


Fig. 17.1: Schematic representation of the metabolic fate of α - amino acids.

17.3 NITROGEN ECONOMY AND PROTEIN TURNOVER

Nitrogen economy is the supply and utilisation of utilisable nitrogen, i.e., nitrogen available in the form of ammonia. Almost all organisms possess the ability to convert ammonia into organic nitrogen compounds, i.e., substances containing C-N bonds. However, only few organisms like nitrogen fixing bacteria possess the ability to fix freely available atmospheric nitrogen (N_2) into ammonia (NH_3).

Proteins destined for degradation are labelled with Ubiquitin. Polyubiquitinated proteins are degraded by proteasomes, a protein complex which degrades unneeded or damaged proteins by proteolysis.

Protein turnover is a very tightly regulated process. Dietary proteins are hydrolysed to amino acids and are absorbed into the bloodstream. The amino acids obtained after protein degradation are used for synthesising new proteins and other nitrogen containing biomolecules. So, nitrogen balance tells us the relationship between the amount of nitrogen ingested in the form of nitrogenous substances, i.e., proteins in the diet and the amount of nitrogen excreted from the body in the form of nitrogenous end products (largely as urea in humans). It is an indicator of the overall protein metabolism of the body. A state of nitrogen balance is achieved by a healthy person when the dietary intake is balanced by the excretion of urea wastes. If the nitrogen excretion is less than the content of the diet, a positive nitrogen balance indicates the formation of protein as it happens in growth and recovery from disease. If nitrogen excretion is greater than the nitrogen content of the diet, the person is said to be in negative nitrogen balance. This is usually interpreted as an indication of tissue destruction.

SAQ 1

State whether the following statements are True or False.

- Like carbohydrates and triglycerides, proteins can also be stored for later use.
- The carbon skeletons of amino acids undergo oxidative degradation to moieties that can enter the tricarboxylic acid cycle for oxidation.
- The amino groups of most of the L-amino acids are removed by deamination.
- Nitrogen balance is the ratio of intake of nitrogenous compounds/output of nitrogenous metabolites from the body.
- Positive nitrogen balance means that there is a net gain of protein by the body.
- In negative nitrogen balance the excretion of nitrogenous metabolites is less than the dietary intake of nitrogenous compounds.

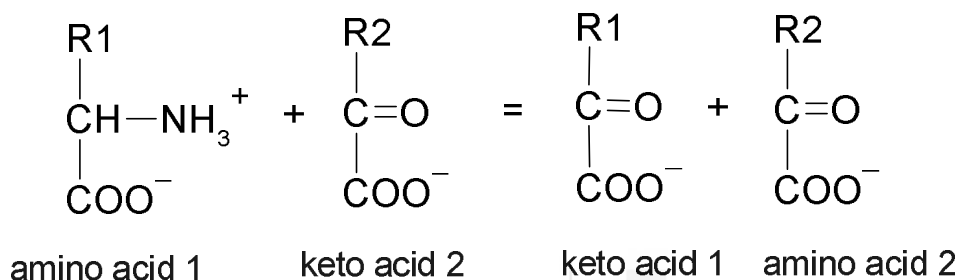
17.4 AMINO ACID METABOLISM

Amino acids are the building blocks of proteins, and these need to be metabolized. Now we will consider various stages involved in the metabolism of an amino acid with emphasis on ammonia as it is highly toxic to human beings and mammals and needs to be excreted out.

Metabolism of amino group can be divided into four steps: transamination, deamination, transport of ammonia to liver and the urea cycle. Let us go through each step one by one.

17.4.1 Transamination

Transamination is a process which brings about the transfer of amino group from donor amino acid to a recipient *keto* acid under the influence of enzyme *transaminase*. Once the transfer of amino group takes place the donor amino acid becomes *keto* acid and the recipient *keto* acid becomes the amino acid (Equation 17.1).



Equation 17.1

After looking at the above equation now what do you observe? You can say that transamination reaction results into inter conversion of a pair of amino acids and a pair of *keto* acids. This reaction is catalysed by a group of enzymes known as *transaminases* or *aminotransferases* and requires pyridoxal phosphate (coenzyme form of vitamin B₆) as the prosthetic group for enzymatic activity. Generally the keto acid 2 which receives the amino group is α-ketoglutarate and gets converted into glutamate (amino acid 2). α-ketoglutarate then undergoes deamination, the second step of amino acid metabolism. Here you must note that *glutamate and glutamine play a very critical role in nitrogen metabolism, they act as a collection point of amino group*.

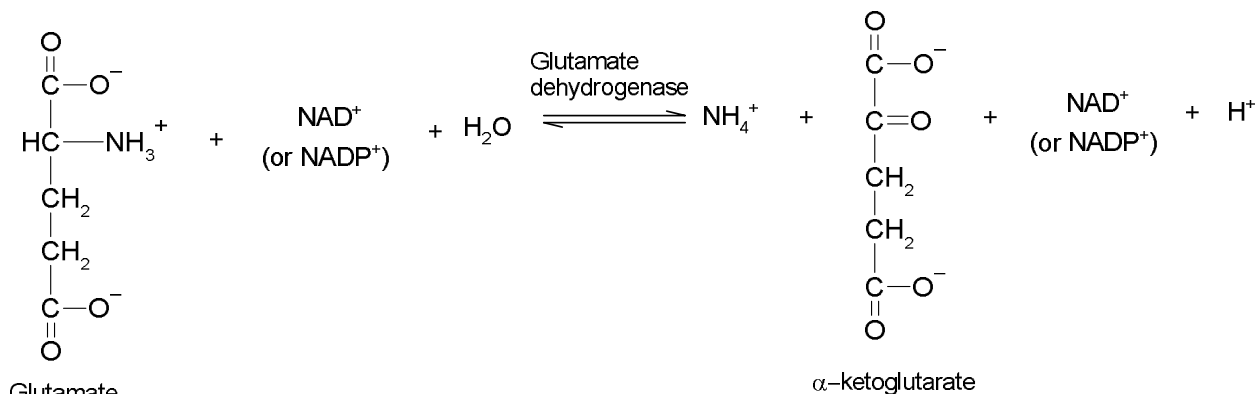
Measurement of alanine aminotransferase (ALT also called glutamate-pyruvate transaminase, GPT) and aspartate aminotransferase (AST, also called glutamate-oxaloacetate transaminase, GOT) levels in blood serum is important in some medical diagnoses. SGPT and SGOT (S for serum) provide diagnostic information about the heart and liver damage caused due to heart attack, drug toxicity or infection.

17.4.2 Deamination

Deamination is the removal of an amine group from the α-amino acids to form α-keto acid catalysed by *deaminases*. Generally, it takes place in liver and kidney cells. During this process the amino group is removed and it is further converted to urea.

The process of deamination occurs in the mitochondria of liver cells. In the hepatocytes, glutamate is transported from the cytosol into the mitochondria, where it undergoes oxidative deamination. The reaction is catalysed by the enzyme *glutamate dehydrogenase*. Let us go through this reaction in detail.

L-Glutamate Dehydrogenase (GDH Reaction) - This is a reaction of great biological significance. It is a reversible oxidative deamination reaction of glutamic acid by L- glutamate dehydrogenase (Equation 17.2), a zinc containing enzyme. This enzyme is widely distributed in mammalian tissues. Glutamate dehydrogenase can use either NAD⁺ or NADP⁺ as co-substrate. Liver glutamate dehydrogenase has many allosteric modifiers. ATP and GTP inhibit the enzyme while ADP activates it.



Equation 17.2

Some organisms like birds, amphibians and reptiles excrete nitrogen waste products as uric acid and are known as uricotelic. Those organisms (e.g. mammals) that dispose of nitrogenous waste products in the form of urea are referred to as "ureotelic" organisms. Many aquatic animals that excrete ammonia into the surrounding water by diffusion through the skin are called ammonotelic organisms.

Glutamine is a non-toxic, neutral compound which can readily cross the cell membrane, while glutamate being negatively charged fails to pass the membrane.

The reaction catalysed by glutamate dehydrogenase is reversible, and functions both in amino acid catabolism as well as their biosynthesis. It represents a mechanism by which ammonia is taken up by α -ketoglutaric acid and converted to glutamic acid. The amino group of glutamic acid may then be reversibly transferred to many different amino acids by transamination reaction. Thus, this reaction serves as a mechanism for incorporating ammonia into amino acids, and links protein and carbohydrate metabolism through the TCA cycle to synthesise amino acids. Conversely, by transamination, amino groups of amino acids are transferred to α -ketoglutaric acid to form glutamic acid, and then get disposed off as ammonia *via* the reaction depicted above.

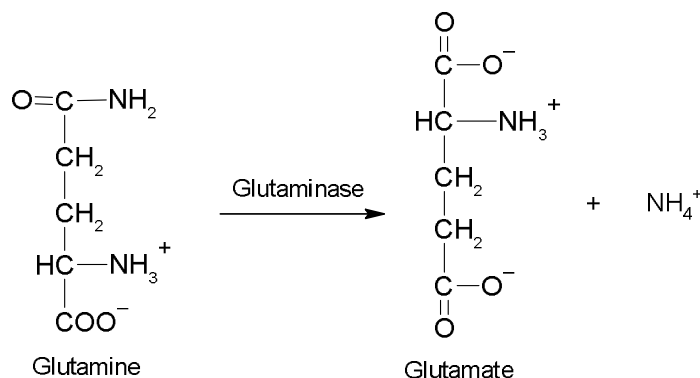
17.4.3 Transport of Ammonia

The deamination of amino acids in the body tissues forms large amounts of ammonia from glutamate. By the action of glutamate dehydrogenase on glutamate, ammonia is released. Another source of ammonia is glutamine, which is the chief form in which ammonia is transported in extracellular fluids. Liberation of amide nitrogen of glutamine as ammonia occurs by hydrolytic removal of ammonia, in a reaction catalysed by glutaminase. However, some of the amino acids (e.g. serine, threonine, cysteine, homocysteine and histidine) directly liberate ammonia through special deamination processes. Ammonia is also formed in other metabolic processes like deamination of adenylic acid, catabolism of pyrimidines and oxidation of amines by amine oxidases.

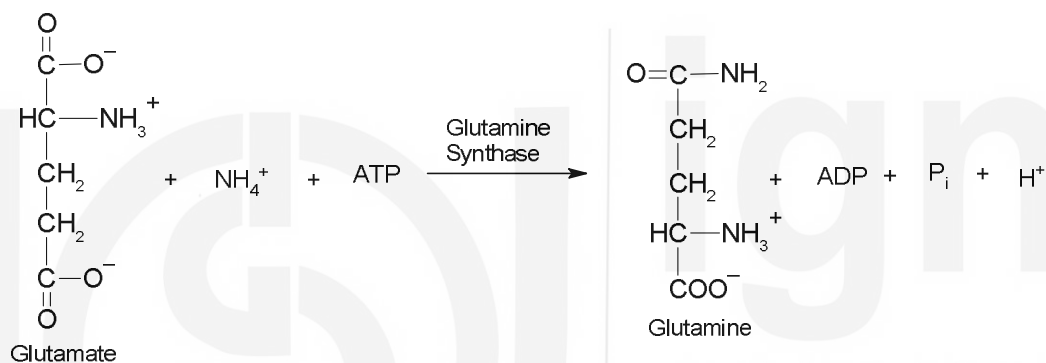
In addition to ammonia formed in the tissues, a considerable quantity is produced by the gastrointestinal tract which harbours bacterial flora. Kidneys also produce ammonia and add it to the blood. Considerable amount of ammonia may be excreted in the urine. This ammonia is produced by renal tubular cells from intracellular amino acids, especially glutamine. Consequently the concentration of ammonia in portal blood is higher than that in systemic circulation.

Since ammonia is toxic to animals, excess ammonia generated in extra-hepatic tissues (other than liver) is converted into a nontoxic compound before export from extra-hepatic tissues into the blood and then to the liver or kidneys. This transport function is accomplished by **L-glutamine** and not by glutamate which is so critical to amino group metabolism. Within liver

mitochondria glutaminase converts glutamine into glutamate and releases amino group (Equation 17.3). In many tissues, ammonia enzymatically combines with glutamate to yield glutamine by the action of glutamine synthetase (Equation 17.4). Glutamine is, thus, the major transport form of ammonia.



Equation 17.3



Equation 17.4

Alanine is another important molecule in the transport of amino groups for to transporting them from muscle to the liver (Glucose-alanine cycle, refer to Fig. 17.2). In muscle, excess amino groups are generally transferred to pyruvate to form alanine by the action of alanine aminotransferase. Alanine then passes into the blood and is carried to the liver.

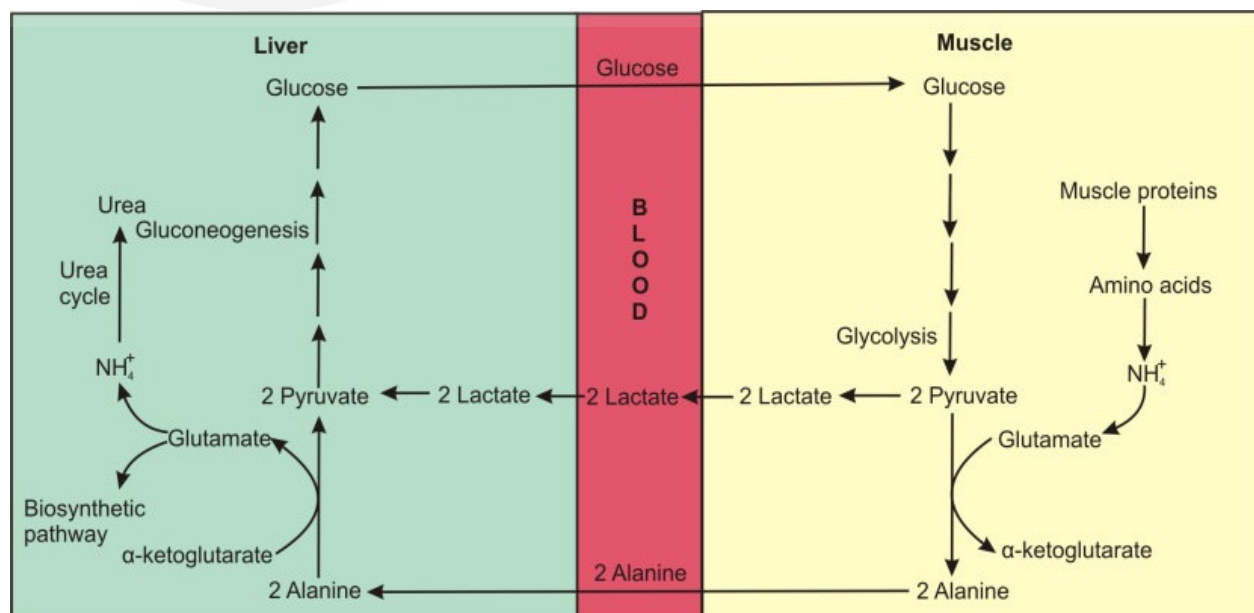


Fig. 17.2: Glucose-alanine cycle.

17.4.4 Urea (Krebs-Hensleit) Cycle

We discuss here the disposal of toxic ammonia as non-toxic urea in organisms in a set of reactions occurring in cyclic form, collectively known as *urea cycle*. The urea cycle (also known as ornithine cycle) takes place in the liver, initial two reactions occur in the mitochondria, and the rest, in cytosol.

In the human system, urea constitutes about 80-90% of the nitrogen excreted. Liver is the principal site of urea formation. Once formed, urea is excreted in the urine. It is essentially non-utilisable. Amount of urea excreted is a direct reflection of the extent of amino acid catabolism and varies considerably with dietary protein intake.

The overall process of urea formation was established by Hans Krebs and Kurt Hensleit in 1932. The urea cycle consists of five reactions:

two mitochondrial and three cytosolic. The cycle converts two amino groups, one from NH_4^+ and one from Aspartate, and a carbon atom from HCO_3^- , to the relatively nontoxic excretion product urea ($\text{NH}_2\text{-CO-NH}_2$) at the cost of four "high-energy" phosphate bonds (3 ATP hydrolysed to 2 ADP and one AMP). Let us look at each step (Fig. 17.3):

Formation of carbamoyl phosphate: Initial reaction in the process of urea biosynthesis results in the formation of carbamoyl phosphate from CO_2 and NH_3 . ATP, Mg^{++} and N-acetylglutamic acid are required. This reaction is catalysed by liver mitochondrial enzyme carbamoyl phosphate synthetase 1 (CPS1). N-acetyl glutamic acid has a positive allosteric effect on CPS1. Arginine acts as a specific stimulator for N-acetyl glutamate synthesis.

Formation of Citrulline: Citrulline is formed from ornithine and carbomyl phosphate. This reaction is catalysed by mitochondrial enzyme, L-ornithine transcarboxylase.

Formation of arginino-succinate: Synthesis of arginino-succinate from aspartate and citrulline requires ATP. The reaction is catalysed by argininosuccinatesynthetase, a liver cystolic enzyme.

Formation of arginine: Cleavage of arginino-succinate produces arginine and fumarate. The reaction is catalysed by a cold labile enzyme, argininosuccinate lyase also known as argininosuccinase.

Formation of urea from arginine: Arginine is hydrolysed to ornithine and urea by the enzyme arginase. Ornithine re-enters the urea cycle. Arginase is activated by Co^{++} or Mn^{++} ions, but ornithine and lysine are competitive inhibitors of arginase.

Defect in any of the five enzymes of urea cycle leads to ammonia intoxication, e.g., Hyperammonemia type I occurs due to a defect in carbamoyl phosphate synthetase I and Hyperammonemia type II due to the defect in ornithine transcarbamoylase enzyme. Ammonia is highly toxic to central nervous system and can cause coma and death.

In nut shell, metabolic fates of amino group can be summed up as under:

1. **Biosynthesis of glutamic acid from ammonia and α -ketoglutaric acid by the action of glutamate dehydrogenase enzyme.**
2. **Biosynthesis of non-essential amino acids through deamination reaction (transamination by transaminases with the help of ketoglutaric acid followed by deamination of the produced glutamic acid by glutamate dehydrogenase enzyme).**
3. **Biosynthesis of glutamine by glutamine synthetase enzyme.**
4. **Biosynthesis of urea is the main fate of ammonia.**
5. **Small amounts of ammonia are excreted in urine.**

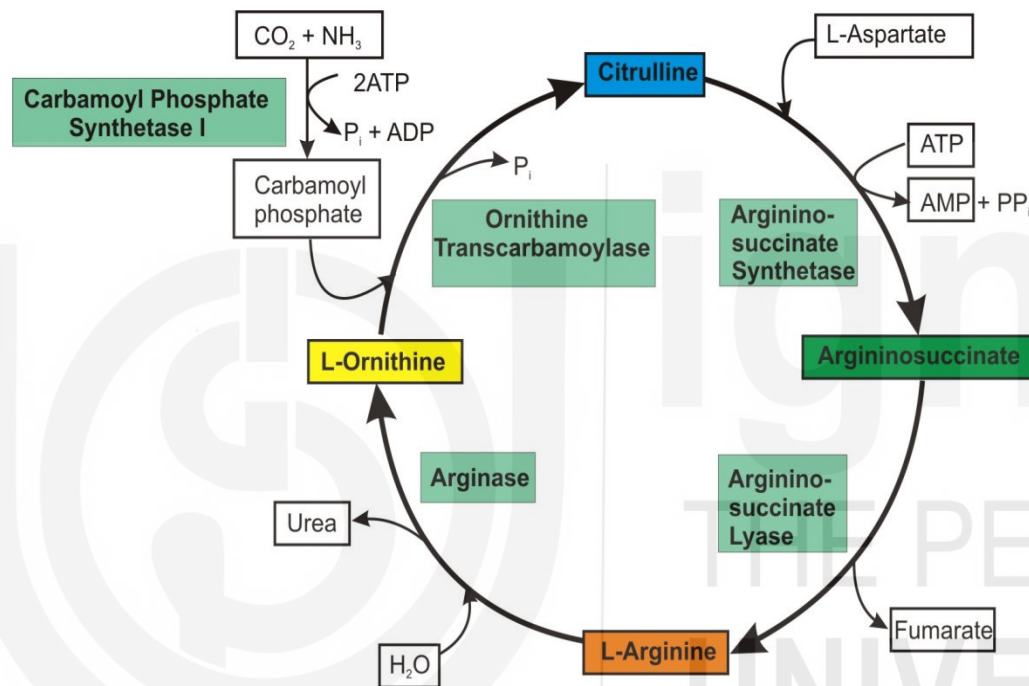


Fig. 17.3: Urea (Krebs - Hensleit) Cycle.

SAQ 2

Choose the correct answer from the parentheses.

- (Creatinine/Urea) is the main nitrogenous compound in urine.
- Transamination reaction in amino acid synthesis is catalysed by enzyme.....(Decarboxylase/Transaminase)
- Urea cycle is also referred to as..... (Krebs-Henseleit/Krebs) cycle.
- In deamination, amino acid is converted into.....(keto acid/ carboxylic acid).
- Process of breakdown of amino acids to α keto acids is called..... (cis-amination/transamination)
- The alpha amino groups of all the amino acids is finally channelised to(glutamate/ alanine).

- g) Oxidative deamination of glutamate is brought about by the enzyme Glutamate dehydrogenase/Glutaminase)
- h) The steps of urea cycle that occur in liver cell are..... (2 in mitochondria and 3 in cytosol/3 in mitochondria and 2 in cytosol).
- i) The total no of ATPs utilised in the formation of urea are (three/four).
- j) The products of the enzyme arginosuccinase are(ornithine and fumarate/arginine and fumarate)
- k) Transaminases require a coenzyme derived from vitamin (B₁₂/B₆).

SAQ 3

Answer in 1-2 lines:

- a) Define deamination.
- b) Give glutamate dehydrogenase reaction (GDH Reaction).
- c) Name the major transport form of ammonia.
- d) Defect/deficiency in which enzyme of the urea cycle causes hyperammonemia?

17.5 OVERVIEW OF AMINO ACID BIOSYNTHESIS

Chirality is a geometrical property. A molecule is called chiral if it cannot be superposed on its mirror image by any combination of rotations.

In living systems, reduced nitrogen is incorporated first into amino acids and then into a variety of other biomolecules, including nucleotides. The key entry point is the amino acid glutamate. Glutamate and glutamine are the nitrogen donors in a wide variety of biosynthetic reactions. If you go back to Equation 17.4 you will recall the involvement of Glutamine Synthetase, which catalyses the formation of glutamine from glutamate. Here you must note that it is a key regulatory enzyme of nitrogen metabolism.

A fundamental problem for biological systems is to obtain nitrogen in an easily usable form. The carbon backbones come from the glycolytic pathway, the pentose phosphate pathway, or the citric acid cycle. In amino acid production, one encounters an important problem in biosynthesis, namely stereochemical control. Because all amino acids except glycine are chiral, biosynthetic pathways must generate the correct isomer with high fidelity. **In each of the 19 pathways for the generation of chiral amino acids, the stereochemistry at the α -carbon atom is established by a transamination reaction that involves pyridoxal phosphate as co-factor.** Almost all the transaminases that catalyse these reactions descend from a common ancestor, illustrating once again that effective solutions to biochemical problems are retained throughout evolution.

Plants and bacteria synthesise all 20 standard amino acids. Mammals can synthesise 10 of the 20; the other 10 are obtained through the diet (essential amino acids). Amino acids are made from intermediates of the citric acid and other major pathways. Among the non-essential amino acids that are synthesised by all organisms including mammals, glutamate is formed by reductive amination of α -ketoglutarate and is the precursor of glutamine, proline and arginine. Alanine and aspartate (and thus asparagine) are formed from pyruvate and oxaloacetate, respectively, by transamination.

The carbon chain of serine is derived from 3-phosphoglycerate. Serine is a precursor of glycine; the β carbon atom of serine is transferred to tetrahydrofolate. Mammals produce serine by a series of reactions requiring S-adenosylmethionine and cystathionine and cysteine from methionine.

Often, amino acids are synthesised as α -ketoacids, and later transaminated with another amino acid, usually glutamate. The enzyme involved in this reaction is transaminase (also called aminotransferase).

α -ketoacid + glutamate $\rightleftharpoons$ amino acid + α -ketoglutarate

Among the essential amino acids (not synthesised by mammals), the aromatic amino acids (phenylalanine, tyrosine and tryptophan) are formed by a pathway in which chorismate occupies a key branch point. Phosphoribosyl pyrophosphate is a precursor of tryptophan and histidine. The pathway to histidine is interconnected with the purine synthetic pathway. Tyrosine can also be formed by hydroxylation of phenylalanine (and thus considered non-essential). The pathways for the other essential amino acids are complex.

Biosynthetic pathways are often highly regulated as such that building-blocks are synthesised only when supplies are low. Very often, a high concentration of the final product of a pathway inhibits the activity of enzymes that function early in the pathway. Generally, **allosteric enzymes present in the system are capable of sensing and responding to changes in the concentrations of regulatory species**. These enzymes are similar in functional properties to aspartate transcarbamoylase and its regulators. The amino acid biosynthetic pathways are subject to allosteric end product inhibition; the regulatory enzyme is usually the first in the sequence. Regulation of the various synthetic pathways is coordinated. Feedback or allosteric mechanisms ensure that all twenty amino acids are maintained in sufficient amounts for protein synthesis and other processes.

SAQ 4

Fill in the blanks:

- The stereochemistry at the α -carbon atom is established by a transamination reaction that involves as cofactor.
- Mammals can synthesise amino acids out of the 20, remaining ones are obtained through the diet and are known as
- Glutamate is the precursor of glutamine, ... and.....
- Alanine is formed from by transamination.

- e) Serine is a precursor of
- f) Phenylalanine, tyrosine and tryptophan are formed by a pathway in which occupies a key branch point.
- g) Phosphoribosyl pyrophosphate is a precursor of and histidine.

Evidently, short term metabolic regulation is at the level of enzyme activity modification.

17.6 REGULATION OF UREA CYCLE

Short term regulation of urea cycle occurs principally at the regulation of carbamoyl phosphate synthetase-1. N-acetylglutamate is allosteric activator of CPS-1. N-acetylglutamate is synthesised from acetyl CoA and glutamate by N-acetylglutamatesynthetase enzyme which is allosterically activated by arginine, again a substance generated in urea cycle. Thus a load of amino acid for breakdown will go to produce arginine which, in turn would activate the enzyme for the synthesis of N-acetyl-glutamate to cause activation of carbomyl phosphate synthesis.

Long term regulation occurs at the gene level with the long term changes in the quantity of dietary protein. For instance, increase in the quantity of dietary proteins over a long period of time leads to a significant increase in the concentration of the enzymes of urea cycle through long term regulation. This is because intake of large amount of protein over a long period of time induces changes at genetic level for example at the level of transcription. Thus, long term regulation operates by the quantitative increase in enzyme levels through their synthesis.

17.7 SUMMARY

Let us sum up what we have learnt so far:

- Nitrogen balance is the relationship between the amount of nitrogen ingested in the form of nitrogenous substances i.e. proteins in the diet and the amount of nitrogen excreted from the body in the form of nitrogenous end products (largely urea in humans). Nitrogen balance is an indicator of the overall protein metabolism of the body.
- Protein metabolism refers to all enzymes catalysed chemical reacting taking place within an organism or its individual cells involved in growth, reproduction, tissue repair and energy production.
- The carbon skeletons of amino acids undergo oxidative degradation to compounds that can enter the tricarboxylic acid cycle for oxidation.
- The amino groups of most of the L-amino acids are removed by transamination catalysed by the transaminase (amino transferase) enzymes.
- Amino acid synthesis is the set of biochemical processes (metabolic pathways) by which the various amino acids are produced from other compounds. The substrates for these processes are various compounds in the organism's diet or growth media.

- In living systems, reduced nitrogen is incorporated first into amino acids and then into a variety of other biomolecules, including nucleotides. The key entry point is the amino acid glutamate.
- Glutamate and glutamine are the nitrogen donors in a wide variety of biosynthetic reactions.
- Glutamine synthetase, which catalyses the formation of glutamine from glutamate, is a key regulatory enzyme of nitrogen metabolism.

17.8 TERMINAL QUESTIONS

1. What is transamination?
2. Describe the reaction catalysed by Glutamate dehydrogenase.
3. Explain the various steps involved in transport of ammonia from extrahepatic tissues to liver/kidney.
4. Describe urea cycle.
5. Write a note on amino acid biosynthesis.
6. How short term regulation of urea cycle differs from long term regulation?

17.9 ANSWERS

Self Assessment Questions

1. (a) False, (b) True, (c) False, (d) False, (e) True, (f) False.
2. (a) Urea, (b) Transaminase, (c) Krebs-Henseleit, (d) keto acid, (e) transamination, (f) Glutamate, (g) Glutamate dehydrogenase, (h) 2 in mitochondria and 3 in cytosol, (i) four, (j) arginine and fumarate, (k) B₆.
3. (a) Deamination is the removal of an amine group from the α -amino acids to form α -keto acid catalysed by *deaminases*, (b) GDH Reaction is a reversible oxidative deamination reaction of glutamic acid by L-glutamate dehydrogenase, a zinc containing enzyme to yield ammonia and α -ketoglutarate, (c) Glutamine, and (d) Hyperammonemia type I occurs due to a defect in carbamoyl phosphate synthetase I and Hyperammonemia type II due to the defect in ornithine transcarbamoylase enzyme.
4. (a) Pyridoxal phosphate, (b) 10, essential amino acids, (c) Proline and arginine, (d) Pyruvate, (e) Glycine, (f) Chorismate, and (g) tryptophan.

Terminal Questions

1. Refer to Section 17.4.1.
2. Refer to Section 17.4.2.
3. Refer to Section 17.4.3.
4. Refer to Section 17.4.4.
5. Refer to Section 17.4.5.
6. Refer to Section 17.4.6.

FURTHER READINGS

1. Outline of Biochemistry 5/E. Conn, EE., Stumpf, George, B., and Doi, RH. Pub. John Wiley & Sons.
2. Harper's Illustrated Biochemistry- a Lange Medical Book. Murray, RK. Granner, DK., Mayes, PA., and Rodwell, VW. Pub. Mc Graw Hill.
3. Lehninger Principles of Biochemistry. Nelson, DL., and Cox, MM., Pub. W. H Freeman.