UNIT 6 CARBOHYDRATE METABOLISM

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6.1 INTRODUCTION

Metabolism comprises of various cellular reactions. These reactions are important for maintaining the structural integrity of the cell. Structural integrity imparts the functional characteristics to the cell. Metabolism provides the energy for the cellular activities. In

the earlier unit we learnt that the chemical nature of the energy in a cell is ATP (Adenosine triphosphate). Glucose is the principal carbohydrate involved in ATP production. Glucose produces ATP by undergoing various structural changes catalyzed by several enzymes. The sequences of enzymatic reactions collectively constitute metabolic pathways wherein the product of one enzyme reaction becomes the substrate to the next reaction in the sequence. These successive products of the reaction are termed as metabolites or metabolic intermediates. In this unit, various metabolic pathways that glucose can take in a cell are described i.e. glycolysis, gluconeogenesis.

Objectives

After going through this unit, you will be able to:

- describe the conversion of various monosaccharides into glucose,
- identify the various metabolic pathways available for glucose in the cell,
- work out the energy (ATP) production when glucose is oxidized in various metabolic pathways, glycolysis, citric acid cycle etc.,
- illustrate the reactions involved when the availability of glucose is more than needed through glycogenesis and when the availability of glucose is less than needed through glycogenolysis,
- find out the interconversion of various monosaccharides with 3 carbon to 7 carbon along with their biochemical significance,
- discuss the synthesis of glucose from non carbohydrate sources (gluconeogenesis),
- elaborate on the role of various hormones in the carbohydrate metabolism,
- describe the regulation of blood glucose levels, and
- explain electron transport chain and oxidative phosphorylation.

6.2 CARBOHYDRATE METABOLISM: AN OVERVIEW

In Unit 5, we studied that carbohydrates are broken down into monosaccharides which are absorbed into the blood stream. In the liver and muscles, most of the glucose is changed into glycogen by the process of *glycogenesis* (anabolism). Glycogen is stored in the liver and muscles until needed at some later time when glucose levels are low. If blood glucose levels are low, then epinephrine and glucagon hormones are secreted to stimulate the conversion of glycogen to glucose. This process is called *glycogenolysis* (catabolism). If glucose is needed immediately upon entering the cells to supply energy, it begins the metabolic process called *glycolysis* (catabolism). The end products of glycolysis are pyruvic acid and ATP. Since glycolysis releases relatively little ATP, further reactions continue to convert pyruvic acid to acetyl CoA and then citric acid in the citric acid cycle. The majority of the ATP is made from oxidations in the citric acid cycle in connection with the electron transport chain.

During strenuous muscular activity, pyruvic acid is converted into lactic acid rather than acetyl CoA. During the resting period, the lactic acid is converted back to pyruvic acid. The pyruvic acid in turn is converted back to glucose by the process called *gluconeogenesis* (anabolism). If the glucose is not needed at that moment, it is converted into glycogen by glycogenesis. These processes are summarized in the Figure 6.1. A detailed discussion on each of these processes is included in the following sections. We begin our study of carbohydrate metabolism with glycolysis.

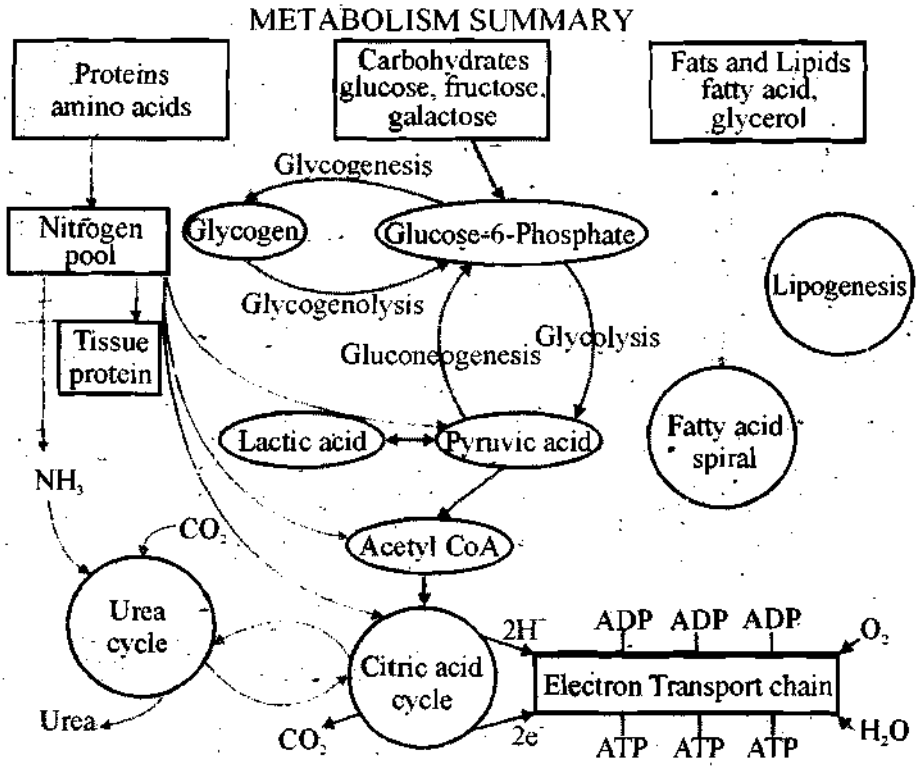

Figure 6.1: Carbohydrate metabolism summary

6.3 GLYCOLYSIS

The glycolytic pathway is also called the *Embden-Meyerhof Pathway (EM Pathway)* and is employed by all tissues for the utilization of glucose to generate energy (in the form of ATP) and intermediates for other metabolic pathways. Pyruvate, as you have read above, is the end product of glycolysis in cells with mitochondria and an adequate supply of oxygen. A series of ten reactions are called *aerobic glycolysis* because oxygen is needed to reoxidise the NADH formed during the oxidation of glyceraldehyde-3-phosphate. In *anaerobic glycolysis,* the glucose is converted to pyruvate, which is reduced by NADH to form lactate and there is no net formation of ATP. Aerobic glycolysis yields net 8 molecules of ATP per molecule of glucose, whereas anaerobic glycolysis results in net 2 molecules of ATP generation from one molecule of glucose. Anaerobic glycolysis allows the continued production of ATP in tissues that lack mitochondria (for e.g. red blood cells) or in cells deprived of sufficient oxygen.

Let us then learn about the glycolytic pathway in greater details.

6.3.1 Glycolytic Pathway

Glycolysis, a series of ten reactions that occur in the cytoplasm, is a process in which one glucose molecule is converted into two molecules of pyruvate. The glycolytic pathway comprises of two stages:

- In the first phase, energy is utilized in the synthesis of phosphorylated form of glucose.
- In the second phase, energy is generated in the form of ATP. 8 molecules of ATP are produced per molecule of glucose metabolized when pyruvate is the end product and only 2 molecules of ATP are produced per molecule of glucose metabolized when lactate is the end product.

The sequence of reactions involved in the entire glycolysis pathway is given in Figure 6.2.

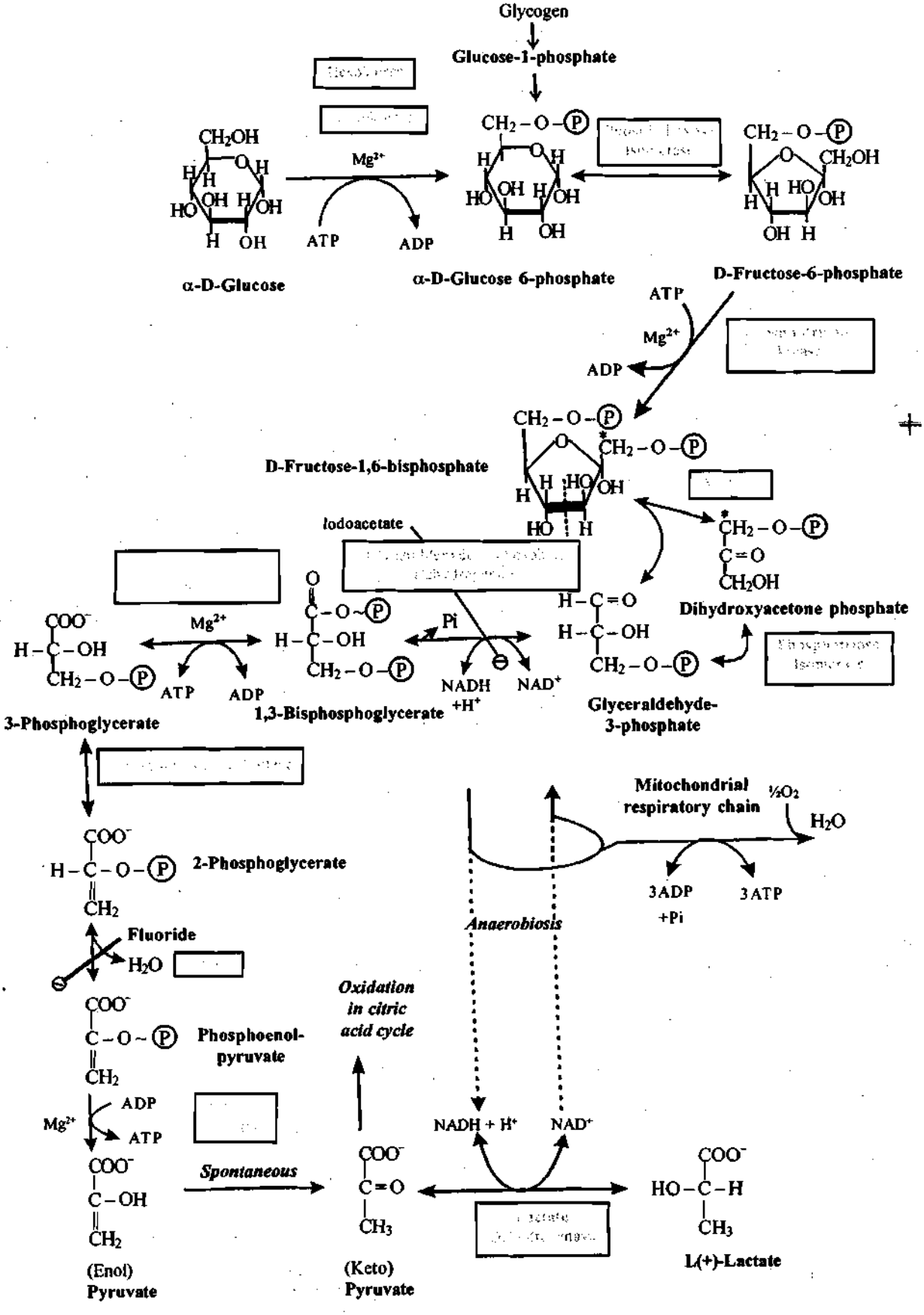

Figure 6.2 : Reactions of Glycolysis

Let us begin our journey into the glycolytic pathway, starting from the first phase.

First phase

As mentioned above, the first step in glycolysis is the synthesis of phosphorylated forms of glucose. This and the other steps are enumerated herewith:

1) *Phosphorylation of glucose:* Glucose is converted to *glucose-6-phosphate* since phosphorylated intermediates do not readily penetrate cell membrane and this commits glucose to further metabolism in the cell. *Hexokinase* catalyses this irreversible reaction in most tissues and in liver; *glucokinase* is the predominant enzyme for the phosphorylation of glucose. Hexokinase is an allosteric enzyme that is strongly inhibited by the product glucose-6-phosphate.

   Hexokinase exists in many isozyme forms and can act upon any aldo- or keto-hexose but has a low K_m (20 μM) (i.e. high affinity) for glucose. Glucokinase has high K_m (12 mM) for glucose, not inhibited by the product glucose-6-phosphate and is an inducible enzyme induced by carbohydrate rich diets and insulin.

2) *Isomerization of glucose-6-phosphate:* This step is catalyzed by *phosphoglucoisomerase* to form fructose-6-phosphate. This is a reversible reaction as can be seen in Figure 6.2.

3) *Phosphorylation of fructose-6-phosphate:* This is an irreversible reaction, catalyzed by *phosphofructokinase*, (PFK-1) a rate-limiting enzyme of glycolysis in most tissues and is the most important regulatory enzymes of glycolysis. PFK-1 is activated by high concentrations of AMP and fructose–2,6-bisphosphate. Inhibitors are citrate and ATP.

4) *Cleavage of fructose-1,6-bisphosphate:* Aldolase cleaves fructose–1, 6-bisphosphate to dihydroxyacetone phosphate and glyceraldehyde-3-phosphate in the reversible reaction shown in Figure 6.2.

5) *Isomerization of dihydroxyacetone phosphate:* Triosephosphate isomerase interconverts dihydroxyacetone phosphate and glyceraldehyde-3-phosphate. Dihydroxyacetone phosphate must be isomerized to glyceraldehyde-3-phosphate for further metabolism in the glycolytic sequence. This isomerization results in the production of two molecules of glyceraldehyde-3-phosphate from the cleavage of fructose-1,6-bisphosphate.

The sequence of reactions within the first phase was enumerated above. Now we move on to the second phase.

Second Phase

This phase is the energy-producing stage from triose phosphate to pyruvate. The reaction starts with the oxidation of glyceraldehyde-3-phosphate. Let us look at the reactions in this phase.

6) *Oxidation of glyceraldehyde-3-phosphate:* The conversion of glceraldehyde-3-phosphate to 1,3-bisphosphoglycerate is catalysed by *glyceraldehyde-3-phosphate dehydrogenase* and the required cofactors are NAD^+ and Pi. The NADH formed is reoxidized either via the respiratory chain or by the NADH linked conversion of pyruvate to lactate. The high-energy phosphate group at carbon 1 of 1,3-bisphosphoglycerate conserves much of the free energy produced by the oxidation of glyceraldehyde-3-phosphate. This is an example of substrate level phosphorylation in which the production of a high-energy phosphate is coupled directly to the oxidation of a substrate instead of resulting from oxidative phosphorylation via the election transport chain.

7) *Formation of ATP from 1,3-bisphosphoglycerate* and *ADP:* The high-energy phosphate group of 1,3-bisphosphoglycerate is used to synthesise ATP from ADP catalysed by *phosphoglycerate kinase* and is a reversible reaction. The reaction

product is 3-phosphoglycerate. Two molecules of ATP are produced since two molecules of 1,3-bisphosphoglycerate are formed from one molecule of glucose.

3-phosphoglycerate may also be formed as follows: 1,3-bisphosphoglycerate is converted to 2,3-bisphosphoglycerate (BPG) by the action of *bisphosphoglycerate mutase* which is present at high concentration in erythrocytes. 2,3-bisphosphoglycerate is hydrolyzed to 3-phosphoglycerate by a *phosphatase*. This constitutes a bypass reaction occurring in erythrocytes and no ATP is formed. However, it serves to provide 2,3-BPG which binds to haemoglobin, decreasing its affinity for oxygen and promoting unloading of oxygen in the tissue.

8) *Shift of the phosphate group from carbon 3 to carbon 2:* This reversible reaction is catalyzed by *phosphoglycerate mutase.*

9) *Dehydration of 2-phosphoglycerate: Enolase* causes dehydration of 2-phosphoglycerate to phosphoenol pyruvate.

10) *Formation of pyruvate: Pyruvate kinase* converts phosphoenolpyruvate to pyruvate in this irreversible reaction with the release of high-energy phosphate to form ATP as can be seen in Figure 6.2. This is the 2nd example of substrate level phosphorylation. This is one of the regulatory sites of glycolysis. *Pyruvate kinase* is an allosteric enzyme activated by fructose-1,6-bisphosphate and inactivated by glucagon via cyclic AMP. Other inhibitors are ATP, alanine, fatty acid and acetyl CoA.

The ten step glycolytic pathway discussed above must have given you a good idea about how glucose is broken down to generate ATP. We will look at the net energy production in glycolysis in sub-section 6.3.3, after studying about the fate of pyruvate in the next sub-section 6.3.2.

6.3.2 Fate of Pyruvate

In the last step of the glycolysis cycle, we saw that *pyruvate kinase* converts phosphoenolpyruvate to pyruvate which is the end-product of glycolysis. In this sub-section we shall look at the fate of pyruvate.

Pyruvate has three different fates. Under aerobic conditions, pyruvate enters mitochondria and is converted to *acetyl CoA* as illustrated in Figure 6.1. The acetyl CoA enters the citric acid cycle. Reducing equivalents produce of the citric acid cycle enter the electron transport chain where oxidation is completed and ATP is synthesized. A second fate of pyruvate is *conversion to lactate*. This takes place in anaerobic microorganisms and in our own bodies when glycolysis occurs faster than the oxygen dependent citric acid cycle and electron transport chain can operate. Some microorganisms convert pyruvate to ethanol, the third fate of pyruvate. In each of these three processes, NAD^+ is regenerated so that glycolysis can continue. We shall learn in greater details about the first two fates of pyruvate now, starting with formation of lactate.

1) *Formation of lactate and its consumption:* If anaerobic conditions prevail, the reoxidation of NADH through the respiratory chain is prevented. Then the regeneration of NAD^+ from NADH is carried out by the action of *lactate dehydrogenase* on pyruvate resulting in lactate and NAD^+ as shown in Figure 6.2 thereby allowing the glycolytic cycle to continue even in the absence of oxygen. Thus tissues that function under hypoxic conditions tend to produce lactate. The advantage of using pyruvate for reoxidation of NADH lies in the fact that pyruvate is the end product of glycolysis and would readily be available in the cells.

In exercising skeletal muscle, there is accumulation of lactate, which is released into the blood and taken up by the liver where it is converted to glucose by the

process called *gluconeogenesis*. In liver and heart, the ratio of NADH/NAD^+ is lower than in exercising muscle. These tissues oxidize lactate obtained from the blood to pyruvate. In liver, pyruvate is either converted to glucose by gluconeogenesis or oxidized in the citric acid cycle as illustrated in the Figure 6.1 and heart muscle oxidizes lactate to CO_2 and H_2O via the citric acid cycle. Glycolysis in erythrocytes, even under aerobic conditions, always terminates in lactate since erythrocytes lack mitochondria.

Next, let us look at the second fate of pyruvate i.e. its conversion to acetyl CoA.

2) *Oxidative decarboxylation of pyruvate:* This is an important step in tissues with high oxidative capacity such as cardiac muscle, whereby pyruvate is converted to acetyl CoA, a major fuel of the citric acid cycle and the building block for fatty acid synthesis about which we will learn in the next unit. Besides oxygen, this pathway requires the participation of mitochondrion with a functional electron transport chain. A detailed discussion on this is presented in section 6.4.

The third fate of pyruvate is specific to microorganisms. The carboxylation of pyruvate by *pyruvate decarboxylase* to form ethanol occurs in yeast and certain micro-organisms but not in humans. Hence, we shall not go into any further details on this aspect now.

Having understood the entire glycolysis cycle, now can you calculate and tell what the net energy production in glycolysis is. Look up the cycle once again and calculate and tally your response with the calculation presented in sub-section 6.3.3.

6.3.3 Energy Production in Glycolysis

We learnt earlier in sub-section 6.3.1 that in the first phase of glycolysis, from one molecule of glucose, 2 molecules of glyceraldeyde-3-phosphate are formed. After that in the reaction of glycolysis, each product yields two molecules as highlighted herewith:

ATP generated reactions	*ATP Formed*
1) Glyceraldehyde-3-phosphate ⟶ 1,3-Bisphosphoglycerate *(respiratory chain oxidation of 2NADH. Oxidation of one NADH by the electron transport chain leads to formation of 3ATP)*	6
2) 1,3-Bisphosphoglycerate ⟶ 3-Phosphoglycerate (phosphorylation)	2
3) Phosphoenolpyruvate ⟶ Enol pyruvate (phosphorylation)	2
ATP utilized reactions	
1) Glucose ⟶ Glucose-6-phosphate	1
2) Fructose-6-phosphate ⟶ Fructose-1,6-bisphosphate	1
Therefore Net ATP generated	**8**

But in anaerobic conditions, the total number of ATP will be only two up to lactate. The regeneration of NAD^+ from NADH formed in the reaction by glyceraldehyde-3-phosphate dehydrogenase step is not via the respiratory chain but utilized by lactate dehydrogenase to form lactate. Thus only two ATP molecules are synthesized in anaerobic glycolysis.

Next, do you know the mechanism involved in regulation of glycolysis?

6.3.4 Regulation of Glycolysis

There are three markedly exergonic reactions in the glycolytic pathway, which are considered physiologically irreversible. These reactions are catalyzed by the enzymes *hexokinase (glucokinase), phosphofructokinase-1* and *pyruvate kinase* and these reactions are the major sites of regulation of glycolysis. The activities of all these enzymes are increased by glucose.

Phosphofructokinase-1 is activated by AMP and inhibited by ATP and citrate. When ATP is utilized in energy requiring process, the concentration of AMP is highly increased. Thus a large increase in AMP acts as a metabolic amplifier of a small change in ATP. This mechanism allows the activity of phosphofructokinase-1 to be highly sensitive to even small changes in energy status of the cell and to control the quantity of carbohydrate undergoing glycolysis prior to its entry into citric acid cycle. The increase in AMP also explains why glycolysis is increased during hypoxia when ATP decreases.

The regulation of glycolysis by allosteric activation or inhibition or the phosphorylation / dephosphorylation of rate limiting enzyme is short term i.e., they exert their action and affect glucose consumption over periods of minutes or hours, whereas, the effect of hormones on the amount of enzyme protein synthesised is more pronounced. The hormonal effects can result in 10 to 20-fold increase in enzyme activity that typically occurs over hours to days.

Consumption of a meal rich in carbohydrate or administration of insulin initiate an increase in the amount of *glucokinase*, *phosphofructokinase-1* and *pyruvate kinase* in liver. These changes reflect an increase in gene transcription resulting in an increased enzyme synthesis. High activity of these 3 enzymes favours the conversion of glucose to pyruvate, a characteristic of the well-fed state. When plasma insulin is low and glucagon is high, the gene transcription and synthesis of these key enzymes are decreased as seen in starvation or diabetes.

We have looked at the three enzymes which play an important role in the regulation of glycolysis above. There are other inhibitors to the glycolysis cycle. These are highlighted next.

Other inhibitors

Iodoacetate is the inhibitor of glyceraldehyde-3-phosphate dehydrogenase.

Arsenite inhibits synthesis of ATP, in the conversion of 1,3-bisphosphoglycerate to 3-phosphoglycerate, by causing uncoupling of oxidation and phosphorylation in the mitochondrial electron transport chain.

Fluoride inhibits enolase enzyme involved in the conversion of 2-phosphoglycerate to phosphoenol pyruvate.

With the study of the reactions and the mechanisms involved in regulating the utilization of glucose to release energy, we come to an end of our study on glycolysis. Next, we shall study in details the fate of pyruvate in terms of its oxidation to acetyl CoA. But first let us recapitulate our understanding so far.

Check Your Progress Exercise 1

1) Name the three irreversible reactions in the glycolytic pathway.

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2) Describe the nature of the enzymes involved in the phosphorylation of glucose.

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3) Name the most important allosteric effector of glycolysis in the liver.

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4) Explain the energy production in glycolysis.

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6.4 OXIDATION OF PYRUVATE TO ACETYL CoA

In Figure 6.1, you would have seen, and subsequently studied that the oxidation of pyruvate to acetyl CoA is the irreversible route from glycolysis to the citric acid cycle. What is citric acid cycle? It is a cycle which stores energy, released by the oxidation of carbohydrates, fats and proteins, in the form of ATP. We will learn about this cycle later in section 6.5.

Before pyruvate can enter the citric acid cycle, it must be transported into the mitochondria via a special pyruvate transporter, which involves a symport mechanism whereby one proton is cotransported. In this case, both pyruvate and H^+ are transported from the cytosol into the mitochondria. Oxidative decarboxylation of pyruvate to acetyl CoA as shown in Figure 6.3 takes place inside the mitochondrion and this is catalyzed by a multi-enzyme complex designated as *pyruvate dehydrogenase complex*, which is located in the mitochondrial matrix. The irreversibility of this reaction precludes the formation of pyruvate from acetyl CoA, and explains why glucose cannot be formed from acetyl CoA in gluconeogenesis.

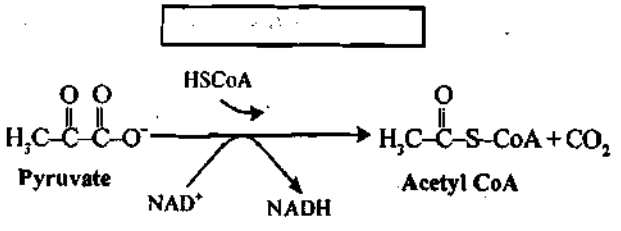


Figure 6.3: Oxidation of pyruvate to acetyl CoA

The pyruvate dehydrogenase (PDH) complex is a multi-molecular aggregate of three enzymes namely, *pyruvate dehydrogenase, dihydrolipoyl transacetylase* and *dihydrolipoyl dehydrogenase*. Each catalyzes a part of the overall reaction. Their physical association links the reactions in proper sequence without the release of intermediate. Table 6.1 presents the components of PDH, with their reactions and prosthetic group.

Table 6.1: Components of pyruvate dehydrogenase (PDH)

Enzyme	Reaction	Prosthetic group
Pyruvate Dehydrogenase (E1)	Oxidative decarboxylation of pyruvate	Thiamine diphosphate TDP
Dihydrolipoyl Transacetylase (E2)	Transfer of acetyl group to CoA	Lipoamide Lipoic acid covalently attached to α amino group of specific lysine residue of the enzyme.
Dihydrolipoyl Dehydrogenase (E3)	Regeneration of oxidized form of lipoamide	FAD

As can be seen from Table 6.1, the complex requires different coenzymes (prosthetic groups), FAD, TDP, lipoic acid etc. The reactions involved in the oxidation of pyruvate are enumerated next.

6.4.1 Reactions Involved in the Oxidation of Pyruvate to Acetyl CoA

Four distinct enzymatic activities are associated with the overall reaction as illustrated in Figure 6.4. Each enzymatic activity requires different substrates and cofactors that comprise the enzyme complex. The steps involved include:

Step 1. Oxidative decarboxylation of pyruvate

a) This reaction is catalysed by the E1 subunit of PDH.

b) The cofactor thiamine diphosphate (TDP) is required.

c) In this step, CO_2 is formed and the α hydroxyethyl group derived from pyruvate becomes covalently bound to TDP.

Step 2. Transfer of the 2 c, unit from E1 to E2

a) E2 requires the coenzyme lipoic acid, which is covalently attached to the lysine residue of the enzyme protein and hence is called lipoamide.

b) In this step, the α hydroxyethyl group is simultaneously oxidized to an acetyl group and transferred to the oxidized form of lipoamide.

c) TDP is regenerated in this step.

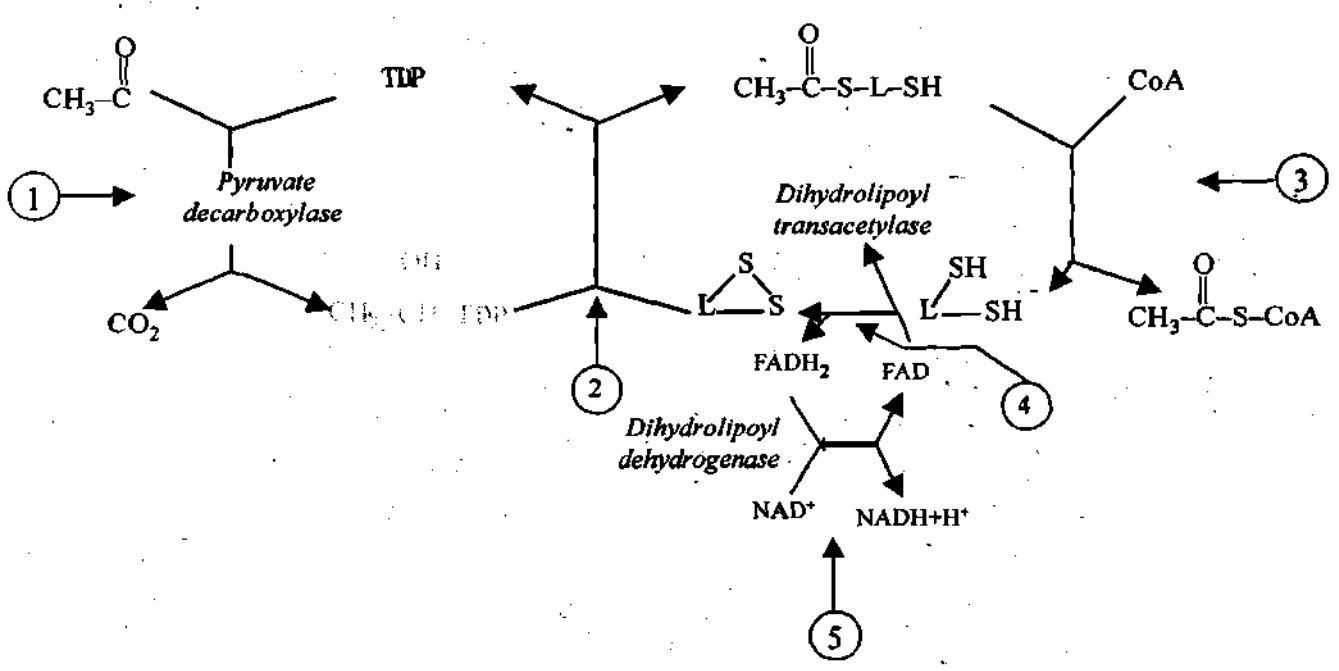


Figure 6.4: Action of Pyruvate Dehydrogenase complex

Step 3: Formation of Acetyl CoA

a) This reaction is catalyzed by the E2 subunit of PDH.

b) The reaction involves transfer of the acetyl group from lipoamide to coenzyme A to form acetyl CoA.

c) Lipoamide is in the reduced state after this transfer.

Step 4: Regeneration of oxidized lipoamide

a) This reaction is catalyzed by E3 subunit of PDH.

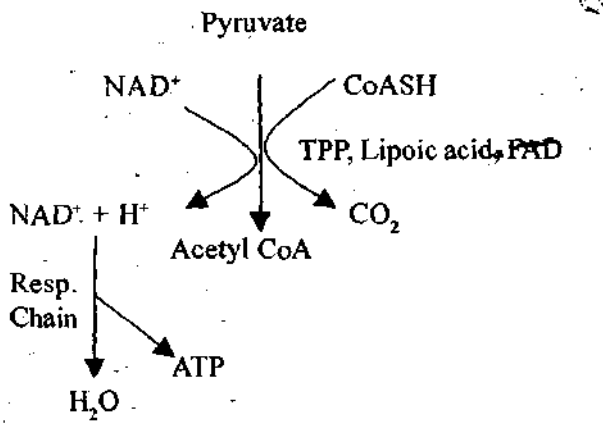
b) NAD^+ and tightly bound FAD are cofactors for E3.

c) FAD reoxidizes lipoamide and is reduced to $FADH_2$.

Step 5: Regeneration of oxidized FAD

$FADH_2$ is reoxidized to FAD^+ by NAD^+. NADH and H^+ are produced. NADH and H^+ are oxidized in the adjacent electron transport chain forming NAD^+ and 3 molecules of ATP. Thus the overall equation for oxidation of pyruvate is:

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



Next, we shall learn about the regulation of pyruvate dehydrogenase.

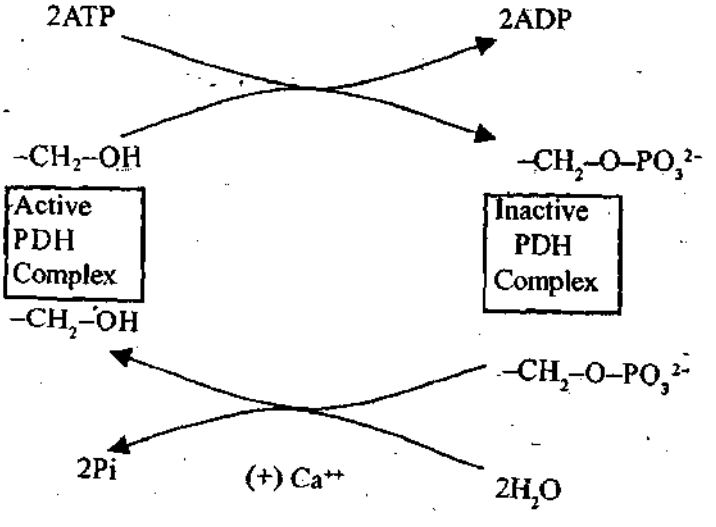
6.4.2 Regulation of Pyruvate Dehydrogenase

The activity of the PDH complex is highly regulated by a variety of factors. These include:

a) *Product inhibition:* Both acetyl CoA and NADH inhibit pyruvate dehydrogenase.

b) *Availability of substrate:* Adequate concentration of the acceptor molecule CoA and NAD^+ must be present for the complex to function.

c) *Covalent modification:* PDH exists in 2 forms
   i) Inactive, phosphorylated
   ii) Active, dephosphorylated



The active form of PDH is phosphorylated by a protein kinase with the help of ATP and Mg^{++} to the inactive form of PDH. Acetyl CoA and NADH are activators for this action and CoA, NAD and pyruvate are inhibitors. The inactive form of PDH is dephosphorylated by phosphoprotein *phosphatase* to the active form in the presence of increased Ca^{++} ion concentration.

The importance of the PDH complex can be further appreciated with the knowledge that certain diseases are associated with the deficiency of PDH complex. These defects are discussed next.

6.4.3 Genetic Defect in Pyruvate Dehydrogenase

A defect in any of the protein subunits of PDH can result in decrease or complete loss of activity. Severe cases are usually fatal. Symptoms of deficiency include:

a) Lactic acidosis, and

b) Neurologic disorders.

Chronic alcoholics suffer from the deficiency of thiamin, which results in the accumulation of pyruvic acid. Excess pyruvate is also reduced to lactate leading to potentially fatal pyruvic and lactic acidosis.

When the defect is in E1, administration of large doses of thiamin may be effective and for the defect in E2, administration of large doses of lipoic acid may be effective. A ketogenic diet, high in fat and low in carbohydrate, helps to lower the level of pyruvate and lactate, which is formed from the excess pyruvate.

Check Your Progress Exercise 2

1) Which enzyme acts on pyruvate in mitochondria and converts it to acetyl CoA? Name its components and cofactors associated with it.

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2) Describe the regulation of pyruvate dehydrogenase through covalent modification.

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6.5 CITRIC ACID CYCLE

The citric acid cycle (also called the krebs cycle or the tricarboxylic acid (TCA) cycle) is a series of enzymatically catalyzed reactions that form a common pathway for the final oxidation of all metabolic fuels (carbohydrates, free fatty acids, ketone bodies and amino acids) which are catabolized to the substrate (acetyl CoA) of the citric acid cycle as you may have noticed in Figure 6.1 earlier. Its central function is the *oxidation of*

acetyl CoA (i.e. acetyl group) to CO_2 and H_2O. This oxidation is the major site of oxygen consumption and ATP production in most animals including humans. The enzymes of citric acid cycle are located in the mitochondrial matrix and are therefore in close proximity to enzymes of the respiratory chain thereby facilitating the transfer of electrons from the reduced coenzymes formed during citric acid cycle to oxygen. Thus it is an aerobic process.

In the citric acid cycle, the oxaloacetate is first condensed with acetyl CoA, and then regenerated as the cycle is completed. But these reactions are not part of a closed circle system and are more similar to a traffic circle with compounds entering and leaving as required. Only a small quantity of oxaloacetate is needed for the oxidation of a large quantity of acetyl CoA, as it is regenerated. Hence, oxaloacetate may be considered to play a catalytic role.

We begin our study of the citric acid cycle, by first learning about its functions.

6.5.1 Functions of Citric Acid Cycle

The citric acid cycle is an amphibolic pathway i.e. it is involved in both anabolic and catabolic processes. Let us see how?

Anabolic reactions: The intermediates of citric acid cycle are used as precursors in the biosynthesis of many compounds like synthesis of glucose from carbon skeletons of amino acids, and providing building blocks for heme synthesis.

Catabolic reactions: The cycle provides a means for the degradation of two carbon acetyl residues which are derived from carbohydrates, fatty acid and amino acids.

Further, the citric acid cycle generates ATP by oxidative phosphorylation when electrons generated in the cycle are transferred to the electron transport chain.

Hence, citric acid cycle is the one which stores energy. Let us get to know the reactions involved in this important cycle next.

6.5.2 Reactions of the Citric Acid Cycle

The citric acid cycle is illustrated in Figure 6.5. Do not get intimidated by the reactions involved in this cycle. While reading the reactions highlighted here in the text, look up the corresponding reactions in the cycle. This will help you understand the sequence of reactions. So here we begin, step by step.

a) *Synthesis of citrate from acetyl CoA* and *oxaloacetate:* Citrate synthase catalyses this aldol condensation reaction with the release of CoA. There are certain inhibitors to this reaction, which include:

   Inhibitors: Citrate synthase is inhibited by ATP, NADH, succinyl CoA and acyl CoA derivative of fatty acids (fatty acyl CoA). The rate of the reaction is also determined by the availability of the substrate.

   What is the role of citrate in this cycle? Let us get to know, next.

   Role of Citrate

   - Citrate in addition to being an intermediate of citric acid cycle provides a source of acetyl CoA for the cytosolic synthesis of fatty acids.
   - Citrate inhibits phosphofructokinase-I, the rate limiting enzyme of glycolysis, and
   - Citrate activates acetyl CoA carboxylase (the rate limiting enzyme of fatty acid synthesis)

b) *Isomerization of citrate:* In this step, as shown in Figure 6.5, citrate is isomerized to isocitrate by *aconitase* which has iron-sulphur centre as its prosthetic group.

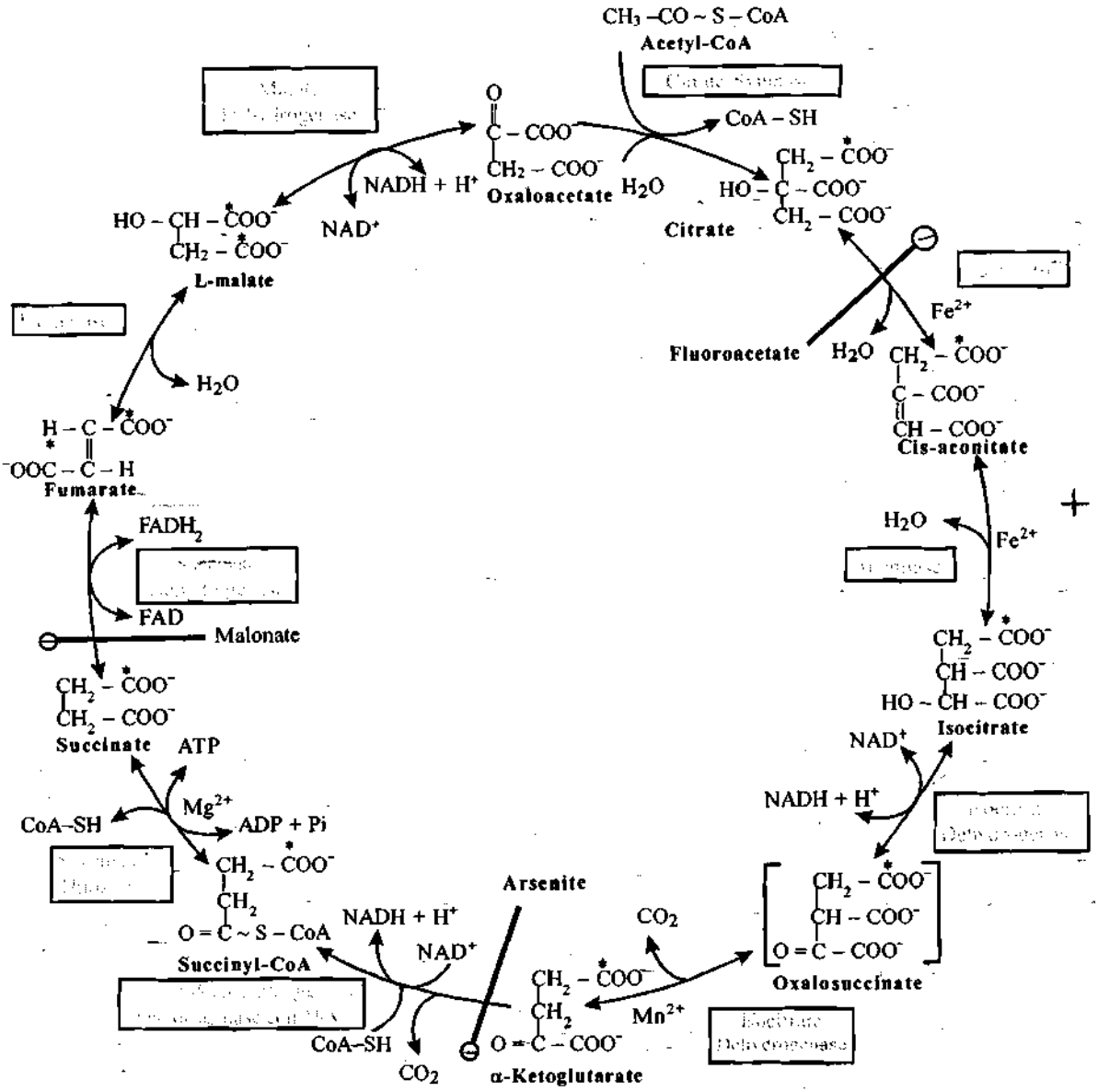


Figure 6.5 : Reaction of citric acid cycle

c) *Oxidation and decarboxylation of isocitrate to α-ketoglutarate: Isocitrate dehydrogenase* catalyses this reaction, yielding the first three NADH molecules produced by the cycle, and the first release of CO_2. Note, the aim of the citric acid cycle is to oxidize acetyl units to 2 molecules of CO_2. In fact, this is one of the *rate limiting steps* of the citric acid cycle. The enzyme *isocitrate dehydrogenase* is activated by ADP and inhibited by ATP and NADH.

d) *Oxidative decarboxylation of α-ketoglutarate to succinyl CoA:* This conversion of α-ketoglutarate to succinyl CoA is catalyzed by the *α-ketoglutarate dehydrogenase* complex and the mechanism of action is similar to that of PDH. This reaction releases the 2nd CO_2 and produces the 2nd NADH of the cycle. The equilibrium of the reaction is far in the direction of succinyl CoA, a high energy thioester similar to acetyl CoA.

The coenzymes required in this reaction are similar to those involved in PDH complex discussed earlier, which include: thiamine diphosphate, lipoic acid, FAD, NAD^+ and CoA.

The enzyme *α-ketoglutarate dehydrogenase* complex is inhibited by ATP, GTP, NADH and succinyl CoA (all indicators of high energy status in the cell) but not

regulated by phosphorylation / dephosphorylation reaction as described for pyruvate dehydrogenase complex.

e) *Cleavage of succinyl CoA to succinate: Succinate thiokinase* (succinyl CoA synthetase) cleaves the high energy thioester linkage in succinyl CoA to release succinate and CoA along with the substrate level phosphorylation of GDP to GTP (GTP and ATP are inter-convertible by nucleoside diphosphate kinase reaction). Succinyl CoA is also used in the biosynthesis of heme. This is the only reaction in citric acid cycle in which ATP is generated by substrate-level phosphorylation.

f) *Oxidation of succinate to fumarate:* This reaction is catalyzed by *succinate dehydrogenase* and FAD^+ is needed as a cofactor. Malonate, a structural analogue of succinate, competitively inhibits succinate dehydrogenase. It is also competitively inhibited by oxaloacetate.

g) *Hydration of fumarate to L-malate: Fumarase* catalyses this reversible reaction.

h) *Oxidation of malate to oxaloacetate:* Malate is oxidized to oxaloacetate by *malate dehydrogenase* and NAD^+ is required as coenzyme. This is the third step of NADH production in the citric acid cycle by the electron transport chain along with the generation of ATP molecules.

So starting with oxaloacetate and acetyl CoA, we move round the circle to once again produce oxaloacetate. The relationship between the reactants and the product of the chemical reactions of the citric acid cycle and the summary is highlighted next.

Stoichiometry of the citric acid cycle

$$\text{Acetyl CoA} + 3NAD^+ + FAD^+ + ADP \text{ (or GDP)} + Pi + 2H_2O \longrightarrow 2\,CO_2\uparrow + 3NADH^+ + FADH_2 + ATP \text{ (or GTP)} + 3H^+ + CoA$$

Summary of Reactions

a) Two carbon atoms enter the cycle as acetyl CoA and leave in the form of CO_2.

b) Four pairs of electrons are released from the substrate; three pairs leave in the form of NADH and one pair leave as $FADH_2$.

c) One high energy phosphate bond is generated in the form of adenosine (or guanosine) triphosphate (ATP or GTP) by substrate level phosphorylation.

d) Although intermediates of the citric cycle may be inter-converted, the cycle does not consume or produce solely from acetyl CoA any intermediate of the cycle.

What is the net energy output of citric acid cycle? The equation of ATP production is presented next.

ATP Production

Oxidation of one NADH by the electron transport chain leads to formation of 3ATP, whereas oxidation of $FADH_2$ yields 2 ATP.

Isocitrate ⟶ α-ketoglutarate (NADH ⟶ NAD^+)	3
α-ketoglutarate ⟶ Succinyl CoA (NADH ⟶ NAD^+)	3
Succinyl CoA ⟶ Succinate (ADP ⟶ ATP or GDP ⟶ GTP)	1
Succinate ⟶ Fumarate ($FADH_2$ ⟶ FAD)	2
Malate ⟶ Oxaloacetate (NADH ⟶ NAD)	3

Thus, *12 molecules of ATP* are produced from oxidation of one molecule of acetyl CoA (using both substrate level and oxidative phosphorylation).

How are the reactions involved in the citric acid cycle regulated? The next sub-section focuses on this aspect.

6.5.3 Regulation of the Citric Acid Cycle

The citric acid cycle is regulated by certain enzymes and by the availability of ADP. These factors are discussed next.

a) *Regulatory enzymes: Citrate synthase, isocitrate dehydrogenase* and *α-ketoglutarate dehydrogenase* complex are the key enzymes which regulate the citric acid cycle. Table 6.2 summarizes the enzymes their inhibitors and activators.

Table 6.2 : Key enzymes which regulate the citric acid cycle

Inhibitor	Activator	Enzyme
ATP, NADH, Succinyl CoA, Acyl CoA derivative	ADP	Citrate synthase
ATP, NADH	ADP	Isocitrate dehydrogenase
ATP, GTP, NADH, Succinyl CoA	ADP	α-ketoglutarate dehydrogenase complex

Besides the enzymes, the availability of ADP also regulates the citric acid cycle. How? Read and find out.

b) *Regulation by the availability of ADP:* When the ADP levels increase due to hydrolysis of ATP in various biosynthetic reactions, the rate of reaction to generate ATP is accelerated and this is mainly by oxidative phosphorylation. There are 4 reactions in which the reducing equivalents are transported to respiratory chain coupled with the generation of ATP in this cycle and thus increase in ADP causes oxidation of acetyl CoA by the citric acid cycle. On the contrary a low level of ADP inhibits the formation of ATP by oxidative phosphorylation. The rate of oxidative phosphorylation is proportional to [ADP] [Pi] / [ATP] which is known as *respiratory control* of energy production. When low ADP levels prevail, the oxidation of NADH and $FADH_2$ also cease and get accumulated because the processes of oxidation and phosphorylation are highly coupled and occur simultaneously. The accumulation of reduced form of coenzymes inhibits the oxidation of acetyl CoA by the citric acid cycle due to lack of oxidized coenzyme forms.

Having studied about the reactions and mechanisms involved in regulating the citric acid cycle, it is clear that 12 ATP molecules are produced from oxidation of one molecule of acetyl CoA.

Now what is the total picture which emerges in terms of the total high energy phosphates formed from one mole of glucose? We have studied about the energy production through glycolysis, next, in oxidation of pyruvate and now in the citric acid cycle. Can you now estimate the total energy production? Go ahead and do the exercise. Tally your answer with the estimation given here in the next sub-section.

6.5.4 Generation of High Energy Phosphates (From Oxidation of Glucose)

The overall high energy phosphate formed starting with one molecule of glucose is estimated herewith. The reactions responsible for the generation of ATP during oxidation of glucose are given in the Table 6.3.

Table 6.3: Reactions responsible for the generation of ATP during oxidation of glucose

Pathway	Reaction catalysed by	Method of ~ P production	No. of ~P formed per mole of glucose
Glycolysis	Glyceraldehyde-3-phosphate dehydrogenase	Respiratory chain oxidation of 2NADH	6
	Phosphoglycerate kinase	Oxidation at substrate level	2
	Pyruvate kinase	Phosphorylation at substrate level	2 10
	Consumption of ATP by reaction	Catalyzed by hexokinase and phosphofructokinase-I Net	-2 8
	Pyruvate dehydrogenase	Respiratory chain oxidation of 2NADH	6
Citric acid cycle	Isocitrate dehydrogenase	Respiratory chain oxidation of 2NADH	6
	α-ketogluatarate dehydrogenase	Respiratory chain oxidation of 2NADH	6
	Succinate thiokinase	Phosphorylation at substrate level	2
	Succinate dehydrogenase	Respiratory chain oxidation of $2NADH_2$	4
	Malate dehydrogenase	Respiratory chain oxidation of $2NADH_2$	6
	Total (net)per mole of glucose under aerobic condition		**38**
	Total (net) per mole of glucose under anaerobic condition		**2**

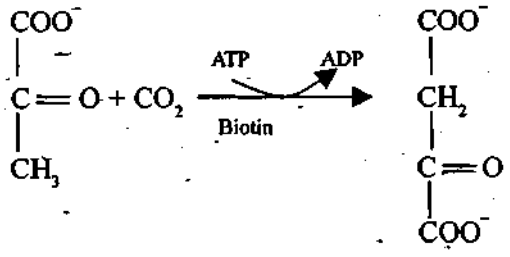
When 1 mole of glucose is combusted in a calorimeter to CO_2 and H_2O, approximately 2870 KJ of energy is liberated as heat. When oxidation occurs in the tissues, some of this energy is not lost immediately as heat but is captured as high energy phosphate. A total of 38 molecules of ATP are generated per molecule of glucose oxidized to CO_2 and H_2O. Assuming each high energy bond to be equivalent to 51.6 KJ (in tissues), the total energy captured in ATP per mole of glucose oxidized is 1961 KJ (38 × 51.6), or approximately 68% of the energy of combustion. Most of the ATP is formed as a consequence of oxidative phosphorylation resulting from the reoxidation of reduced coenzymes by the respiratory chain. The remainder is generated by phosphorylation at the substrate level.

Before we end our study of citric acid cycle, we need to learn about anaplerotic reactions. What are these reactions and what is their significance in citric acid cycle? The last sub-section in this section focuses on this aspect.

6.5.5 Anaplerotic Reactions

Anaplerotic reactions are *reactions that replenish the intermediates of citric acid cycle*. The special enzymatic mechanisms by which the pool of citric acid cycle intermediates can be replenished are called anaplerotic (filling-up) reactions. Anaplerotic reactions can increase the concentration of citric acid cycle intermediates, allowing an increased rate of oxidation of two-carbon units. As more intermediates are available, more moles of acetyl CoA can be processed. The intermediates may also be used for other biosynthetic reactions and need to be replaced. These anaplerotic reactions include:

1) Pyruvate carboxylase which forms oxaloacetate through the following reaction.



Pyruvate **Oxaloacetate**

2) Phosphoenol pyruvate (PEP) + CO_2 + GDP $\xrightarrow{\text{PEP carboxy kinase}}$ Oxaloacetate + GTP

3) Pyruvate + HCO_3^- + NADPH $\xrightarrow{\text{malic enzyme}}$ Malate + $NADP^+$

4) Glutamate dehydrogenase, which also provides α-ketoglutarate.

Glutamate + NAD^+ + H_2O $\longleftrightarrow$ α-ketoglutarate + NH_3 + NADH + H^+

5) Succinyl CoA formation from isoleucine, valine, methionine and threonine

For a reaction to be classified as anaplerotic reaction, net synthesis of citric acid cycle intermediates must occur. Accordingly the reaction catalysed by glutamate-oxaloacetate transaminase as presented herewith is not anaplerotic since formation of oxaloacetate is counterbalanced by utilization of α-ketoglutarate.

α-ketoglutarate + aspartate $\longrightarrow$ glutamate + oxaloacetate

With anaplerotic reactions, we come to an end of our study on citric acid cycle.

Check Your Progress Exercise 3

1) What is the function of the citric acid cycle?

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2) Describe the reactions leading to the generation of ATP in the citric acid cycle.

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3) Name the high-energy complex generated during the conversion of succinyl CoA to succinate in the citric acid cycle.

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4) What are anaplerotic reactions?

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The discussion so far focussed on the reactions involved with the breakdown of glucose. The next section focuses on synthesis of glucose.

6.6 GLUCONEOGENESIS

Gluconeogenesis (i.e synthesis of new glucose) is the synthesis of carbohydrate from non-carbohydrate, source. The major substrates for gluconeogenesis are the glucogenic amino acids, lactate, glycerol and (important in ruminant) propionate. We shall get to know about them later in sub-section 6.6.2. Liver and kidney are the major tissues involved in gluconeogenesis due to the availability of the necessary enzymes.

But, first, what is the significance of gluconeogenesis? Read the next sub-section and find out.

6.6.1 Functions of Gluconeogenesis

The significance of gluconeogenesis include:

1) During starvation or during periods of limited carbohydrate intake, when the levels of liver glycogen are low, gluconeogenesis is important in maintaining adequate blood sugar concentration since a continual supply of glucose is necessary as a source of energy for the nervous system and the erythrocytes.

2) Even when most of the energy requirement of the organism is met by the supply of fat, there is always a certain basal requirement for glucose which is provided by gluconeogenesis.

3) During extended exercise, when high catecholamine levels have mobilized carbohydrate and lipid reserves, the gluconeogenic pathway allows the use of lactate from glycolysis and of glycerol from fat break down.

4) During metabolic acidosis, gluconeogenesis in the kidney allows the excretion of an increased number of protons.

5) Gluconeogenesis also allows the use of dietary protein in carbohydrate pathway after disposal of the amino acid nitrogen as urea.

6) Gluconeogenesis is important to human beings everyday, making it possible for us to make it through the night and from meal to meal without nibbling on a source of carbohydrate continuously.

So, you would have realized that the production of glucose from other substrates is necessary for use as fuel. Hence, it is important for us to learn about these substrates and their reactions in gluconeogenesis. The next sub-section presents a discussion on these substrates.

6.6.2 Gluconeogenesis – Substrates

Earlier in this section, you may recall reading that the major substrates for gluconeogenesis are the glucogenic amino acids, lactate, glycerol etc. Let us get to know about these substrates and their role in gluconeogenesis. We start with lactate as a substrate.

A) *Lactate*

Lactate is transported to the liver in the Cori cycle (lactic acid cycle) and is converted to pyruvate as shown in Figure 6.6. Hepatic gluconeogenesis then converts lactate back to glucose. Glucose is then free to circulate back to peripheral tissue to re-enter anaerobic glycolysis. This is the Cori cycle. It functions to:

- maintain glucose substrate for vital tissues, and
- prevent excessive acidosis due to an excess o lactate.

The process involved in the Cori cycle is enumerated herewith along with the graphic illustration in Figure 6.6. Read the steps given here and at the same time follow the sequence in Figure 6.6.

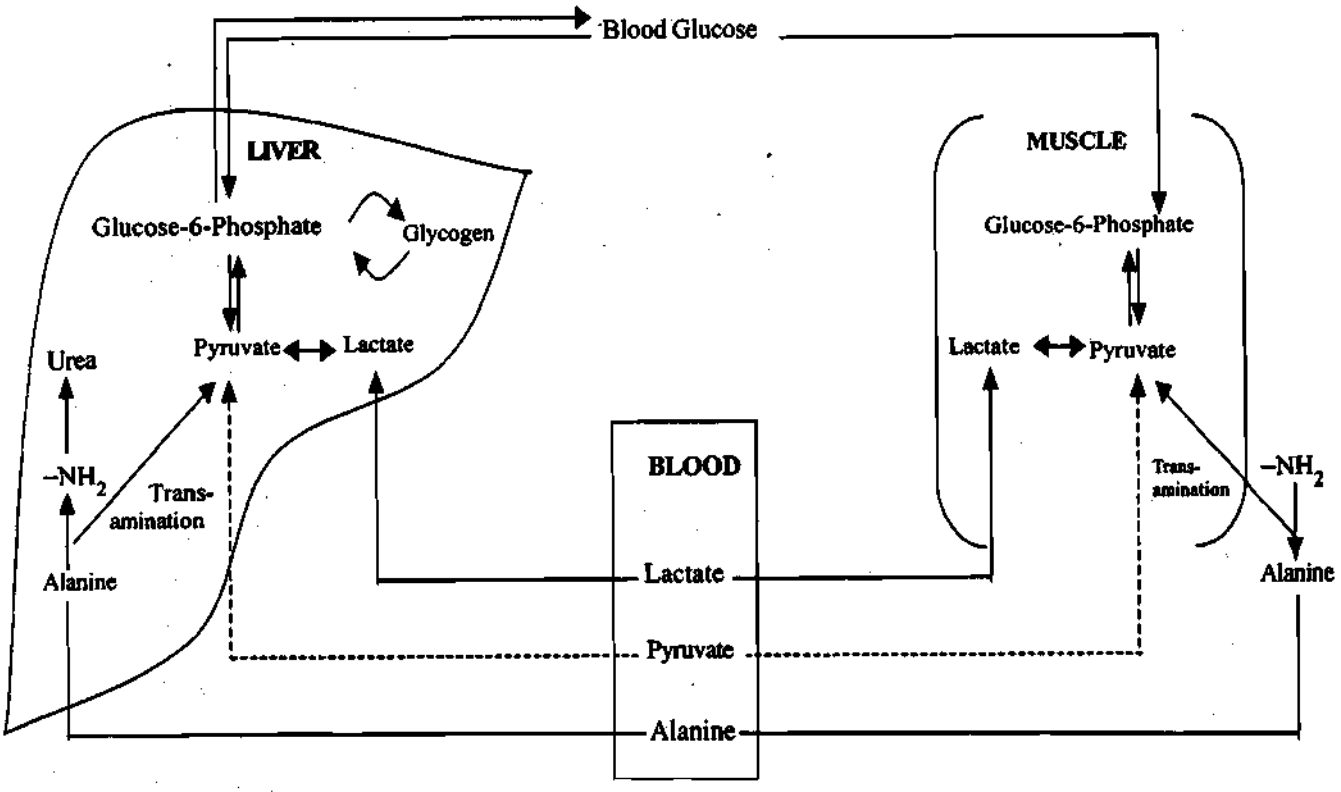

Figure 6.6 : The Cori cycle and Alanine cycle

1) *The Cori cycle*

a) Pyruvate formed from glucose is converted to lactate by *lactate dehydrogenase* in the muscle cell.

b) Lactate is released into the blood and taken up by the liver.

c) Lactate is converted to pyruvate by the isoenzyme of *lactate dehydrogenase* using NAD^+ as cofactor in the liver.

d) Pyruvate is converted to glucose by gluconeogenic mechanism in the liver and released into the blood where it can be used as energy source for muscle and other tissue.

In Figure 6.6, you would have noticed that pyruvate formed in the muscle can be converted to alanine as well. Hence alanine too can function as a substrate for gluconeogenesis, as discussed next.

2) *The Alanine cycle*

Look at Figure 6.6. Follow the alanine link in the alanine cycle. The process goes as under:

1) Pyruvate formed from glycolysis in the muscle is converted to alanine by transamination reaction.

2) Alanine is released by the muscle into the blood and is taken up by the liver.

3) In the liver, alanine is converted back to pyruvate by the reverse of the transamination reaction that occurred in the muscle.

4) Pyruvate is converted to glucose via gluconeogenic pathway.

5) The NH_3 liberated in conversion to urea in the liver.

Next, let us study about the substrate – glycerol.

B) *Glycerol*

The process includes:

1) Glycerol is formed in the adipose tissue by lipolysis of triacylglycerol when metabolic fuel is scarce.

2) Glycerol is released into the blood and taken up by the liver, where it is first converted to glycerol-3-phosphate by *glycerokinase* and ATP.

3) Glycerol-3-phosphate is oxidized to dihydroxyacetone phosphate by *glycerol-3-phosphate dehydrogenase* in presence of NAD^+. Dihydroxyacetone phosphate is then converted to glucose.

C) *Amino acids*

Figure 6.7 illustrates the citric acid cycle intermediates from the amino acids. Here, as you can see:

1) the glucogenic amino acids are converted to the intermediates of citric acid cycle either by transamination or deamination, and

2) these intermediates are converted to oxaloacetate and finally converted to glucose by the enzymes of gluconeogenesis.

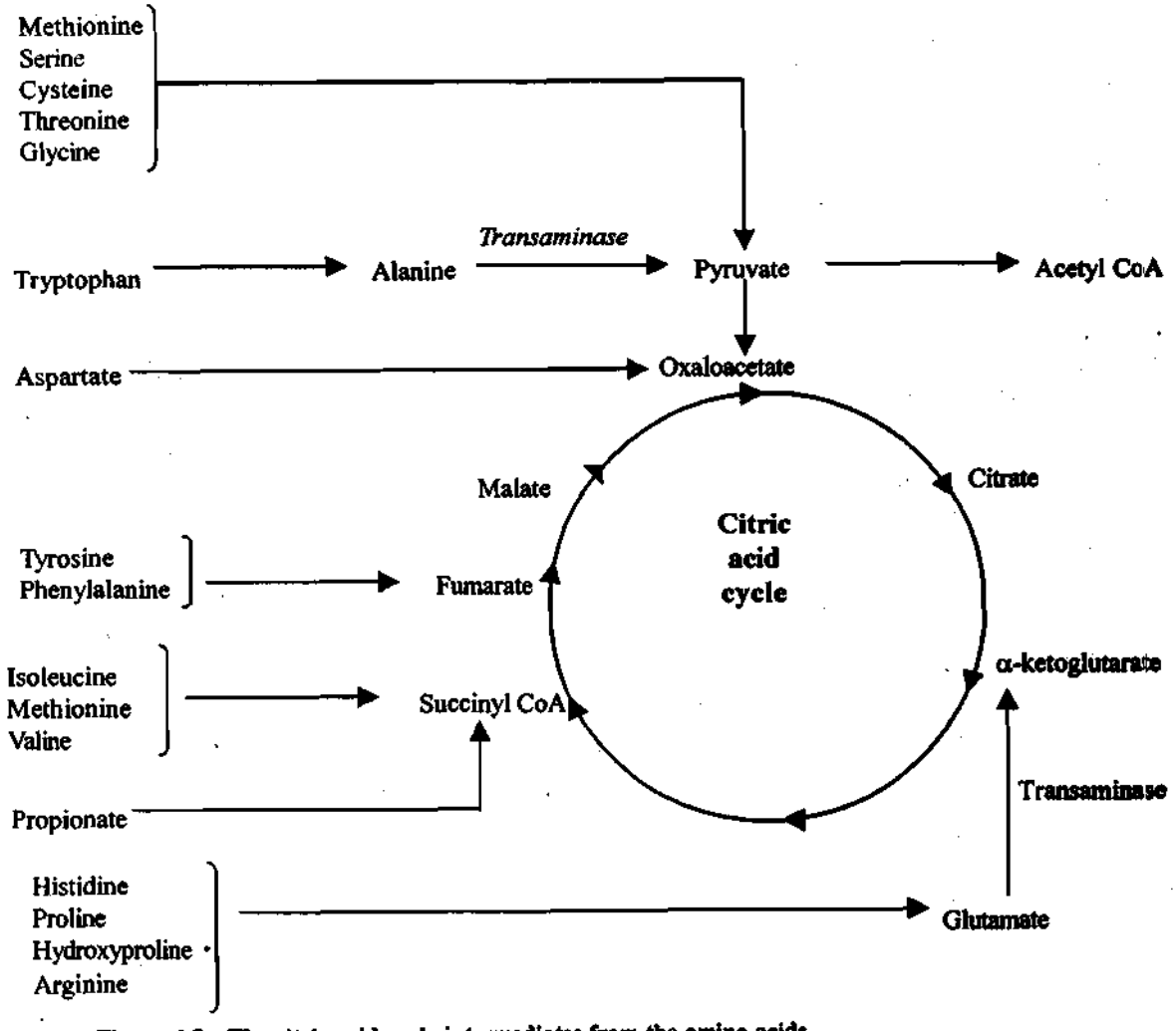

Figure 6.7 : The citric acid cycle intermediates from the amino acids

Another important substrate, particularly in ruminant is propionate. Let us learn about it.

D) *Propionic acid*

Propionic acid is formed as a residual unit (propionyl CoA) in *β-oxidation* of odd-carbon fatty acids. The conversion of propionate to succinyl CoA involves a long process as given herewith and illustrated in Figure 6.8.

1) Propionate is first activated by thiokinase with ATP and CoA to form propionyl CoA.
2) Propionyl CoA undergoes CO_2 fixation reaction to form D-methyl malonyl CoA catalyzed by *propionyl CoA carboxylase* and biotin is required as a coenzyme.
3) D-methyl malonyl CoA is converted to L-methyl malonyl CoA by *methyl malonyl CoA racemase.*
4) L-methyl malonyl CoA is isomerised to succinyl CoA by *methyl malonyl CoA isomerase* which requires vitamin B_{12} as a coenzyme.
5) Succinyl CoA enters citric acid cycle and is converted to oxaloacetate and then further to glucose via gluconeogenic pathway.

Look up Figure 6.7 , which illustrates the substrates for gluconeogenesis. The discussion above on substrate was indeed quite extensive. The idea behind giving the mechanism for each substrate was to help you understand how the substance forms a substrate for gluconeogenesis. Hope you enjoyed reading it. Next, we shall move on to the gluconeogenesis – the pathway involved in the process.

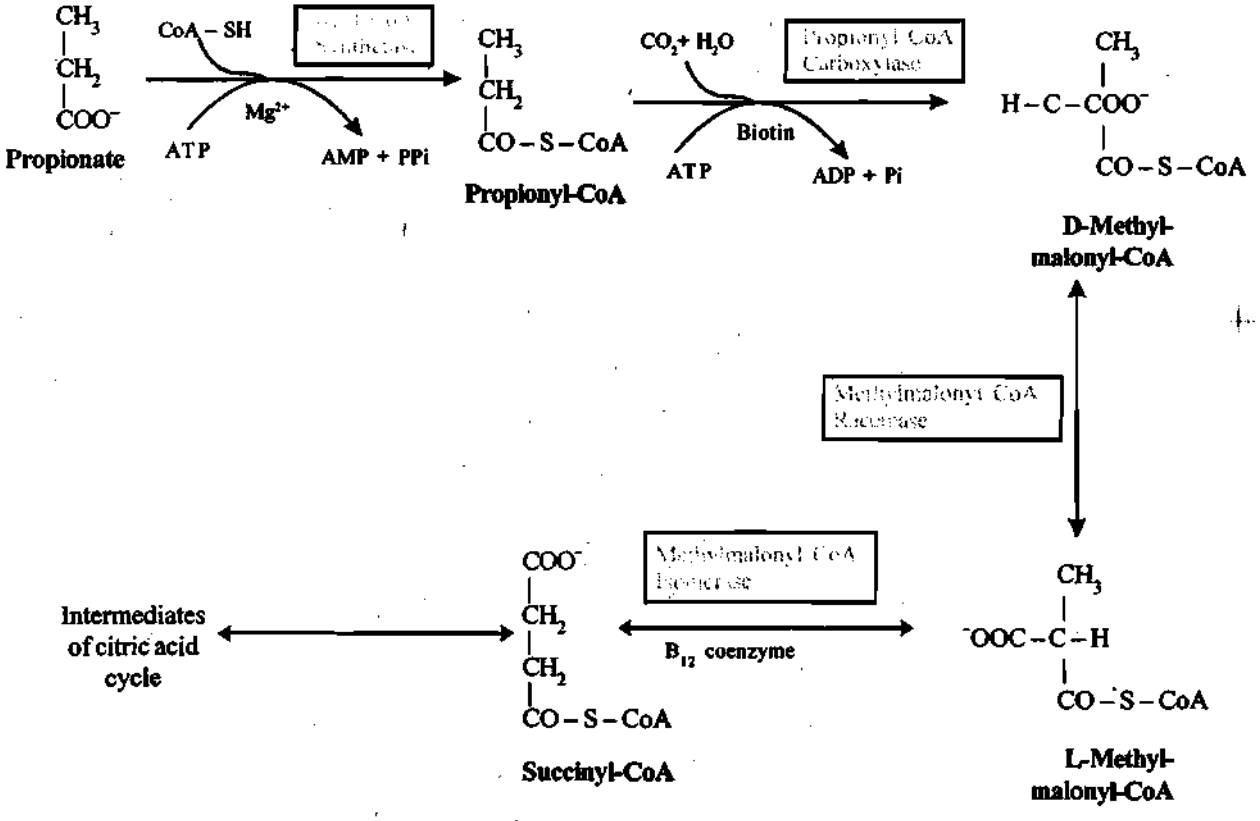

Figure 6.8 : Metabolism of Propionate

6.6.3 Gluconeogenic Pathway

The metabolic pathways in connection with gluconeogenesis are the modification of the EM pathway and citric acid cycle. The synthesis of glucose from substrates is essentially a reversal of glycolysis. However, *Krebs* pointed out that energy barriers obstruct a simple reversal of glycolysis and must be bypassed for gluconeogenesis to be completed. These reactions are:

i) Between pyruvate and phosphoenolpyruvate (PEP)
ii) Between fructose 1,6 bisphosphate and fructose 6-phosphate
iii) Between glucose-6-phosphate and glucose, and
iv) Between glucose-1-phosphate and glycogen.

You may recall reading about these reactions in the glycolysis pathway. To help you understand the process, a summary of the gluconeogenesis pathway with gluconeogenesis enzyme names in red and names of reversible glycolysis enzymes in blue is presented in Figure 6.9. The above mentioned reactions are circumvented by the special reactions highlighted in Figure 6.9 (Under A, B and C) and also discussed herewith.

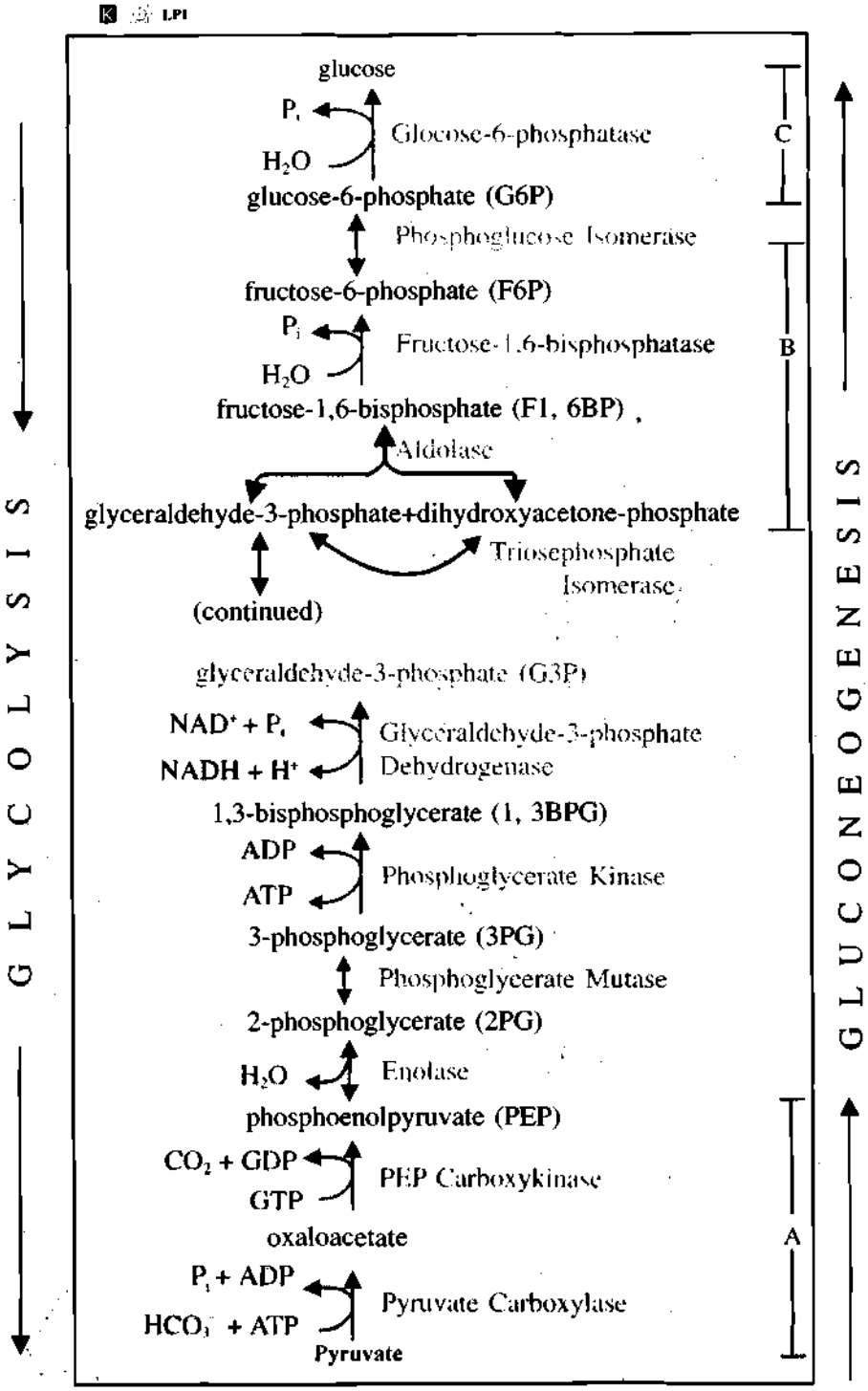

Figure 6.9 : Summary of the gluconeogenesis pathway

A) *Pyruvate* and *Phosphoenolpyruvate* (Look at Figure 6.9, A)

1) *Pyruvate carboxylation:* In this reaction, pyruvate, CO_2 and ATP are converted to oxaloacetate, ADP and Pi catalysed by the enzyme *pyruvate carboxylase* and the cofactors required are biotin and Mg^{++} ions. This reaction occurs in the mitochondrial matrix.

2) *Conversion of oxaloacetate to phosphoenolpyruvate:* In this reaction, oxaloacetate and guanosine triphosphate (GTP) are converted to PEP, CO_2 and guanosine diphosphate (GDP). This reaction is catalyzed by *phosphoenolpyruvate carboxykinase* (PEPCK) which requires Mn^{++} for its activation. In humans, this enzyme is equally distributed between mitochondria and the cytosol.

In mitochondria,

Pyruvate + ATP + Mg^{++}

Pyruvate Carboxylase | Biotin, Mg^{++}

↓

Oxaloacetate + ADP + Pi

In mitochondria or cytosol,

Oxaloacetate + GTP

PEPCK ↓ ↑ Mn^{++}

Phosphoenolpyruvate (PEP) + CO_2 + GDP

With the help of the above 2 enzymes and LDH, lactate can also be converted to PEP.

3) *Oxaloacetate to Malate:* Oxaloacetate cannot permeate mitochondrial membrane well and it must be transported across the membrane in the form of malate.

This reaction is catalyzed by malate dehydrogenase.

- Oxaloacetate + NADH + H^+ ⇌ Malate + NAD^+

a) In the mitochondria, a mitochondrial *malate dehydrogenase* catalyses the above reaction, and

b) In the cytosol, a cytosolic *malate dehydrogenase* catalyses the reverse reaction which regenerates oxaloacetate so it can be converted to PEP. Look at Figure 6.10.

Malate + NAD^+ ⟶ Oxaloacetate + NADH + H^+

c) In this process, malate also serves to transfer reducing equivalents from the mitochondria to the cytosol. The NADH formed is used in gluconeogenesis.

B) *Fructose-1,6-bisphosphate* and *Fructose-6-phosphate:* (Look at Figure 6.9, B)

The conversion of fructose-1,6- bisphosphate to fructose-6-phosphate is catalysed by *fructose-1,6-bisphosphatase* which is the major regulatory enzyme in gluconeogenesis. This enzyme is present in liver, kidney and striated muscle but absent from adipose tissue, heart muscle and smooth muscle. Fructose-1,6 bisphosphatase is an allosteric enzyme, activated by citrate and inhibited by AMP and fructose-1,6-bisphosphate.

These allosteric effects are exactly the opposite of those observed with phosphofructokinase, the regulatory enzyme in glycolysis. This is an example of reciprocal control of opposing metabolic pathways.

C) *Glucose-6-phosphate to Glucose:* (Look at Figure 6.9, C)

Glucose-6-phosphate is converted to glucose by *glucose-6-phosphatase* which is present in intestine, liver and kidney but absent from muscle and adipose tissue.

D) *Glucose-1-phosphate to Glycogen*

The conversion of glucose-1-phosphate to glycogen is through UDPG and glycogen synthase. We shall learn about this later in section under glycogen synthesis.

The conversion of 2 moles of pyruvate and 1 mole of glucose uses 4 moles of ATP and 2 moles of GTP, the total equivalent of 6 moles of high energy phosphate since GTP, like ATP, possesses high energy phosphate bonds.

Overall, each pathway i.e. glycolysis and gluconeogenesis may be summarized as follows:

Glycolysis:

$$\text{glucose} + 2NAD^+ + 2ADP + 2P_i \longrightarrow 2 \text{ pyruvate} + 2NADH + 2ATP$$

Gluconeogenesis:

$$2 \text{ pyruvate} + 2 NADH + 4ATP + 2GTP \longrightarrow \text{glucose} + 2NAD^+ + 4ADP + 2GDP + 6P_i$$

Having studied the gluconeogenic pathway, we also need to learn about the factors which regulate the pathway. A brief discussion follows in sub-section 6.6.4.

6.6.4 Regulation of Gluconeogenesis

Gluconeogenesis and glycolysis are reciprocally regulated. Figure 6.10 illustrates the regulation of gluconeogenesis and glycolysis. All factors increasing gluconeogenesis, simultaneously decrease glycolysis. Similarly decrease in gluconeogenesis is accompanied by increase in glycolysis.

The key enzymes of gluconeogenesis, as we studied earlier, include:

a) Pyruvate carboxylase

b) Phosphoenol pyruvate carboxykinase

c) Fructose-1,6-bisphosphatase, and

d) Glucose-6-phosphatase.

Important aspects related to regulation include:

1) The hormones glucagon and glucocorticoids which are secreted during starvation stimulate glucose-6-phosphatase to enhance gluconeogenesis.

2) During starvation, the increased level of glucagon also stimulates the enzyme phosphoenolpyruvate carboxykinase and thus increases gluconeogenesis.

3) During starvation, increased fatty acid oxidation provides more acetyl CoA which allosterically activates the enzyme pyruvate carboxylase, thereby forming oxaloacetate and enhancing gluconeogenesis.

4) The released glucagon also stimulates gluconeogenesis by decreasing the concentration of fructose-2,6-bisphosphate which in turn cannot activate phosphofructokinase-1 but activates the enzyme fructose-1,6-bisphosphatase.

5) High carbohydrate diets increase the insulin / glucagon ratio and thus minimizes the gluconeogenic mechanism by reducing the activity of key enzymes.

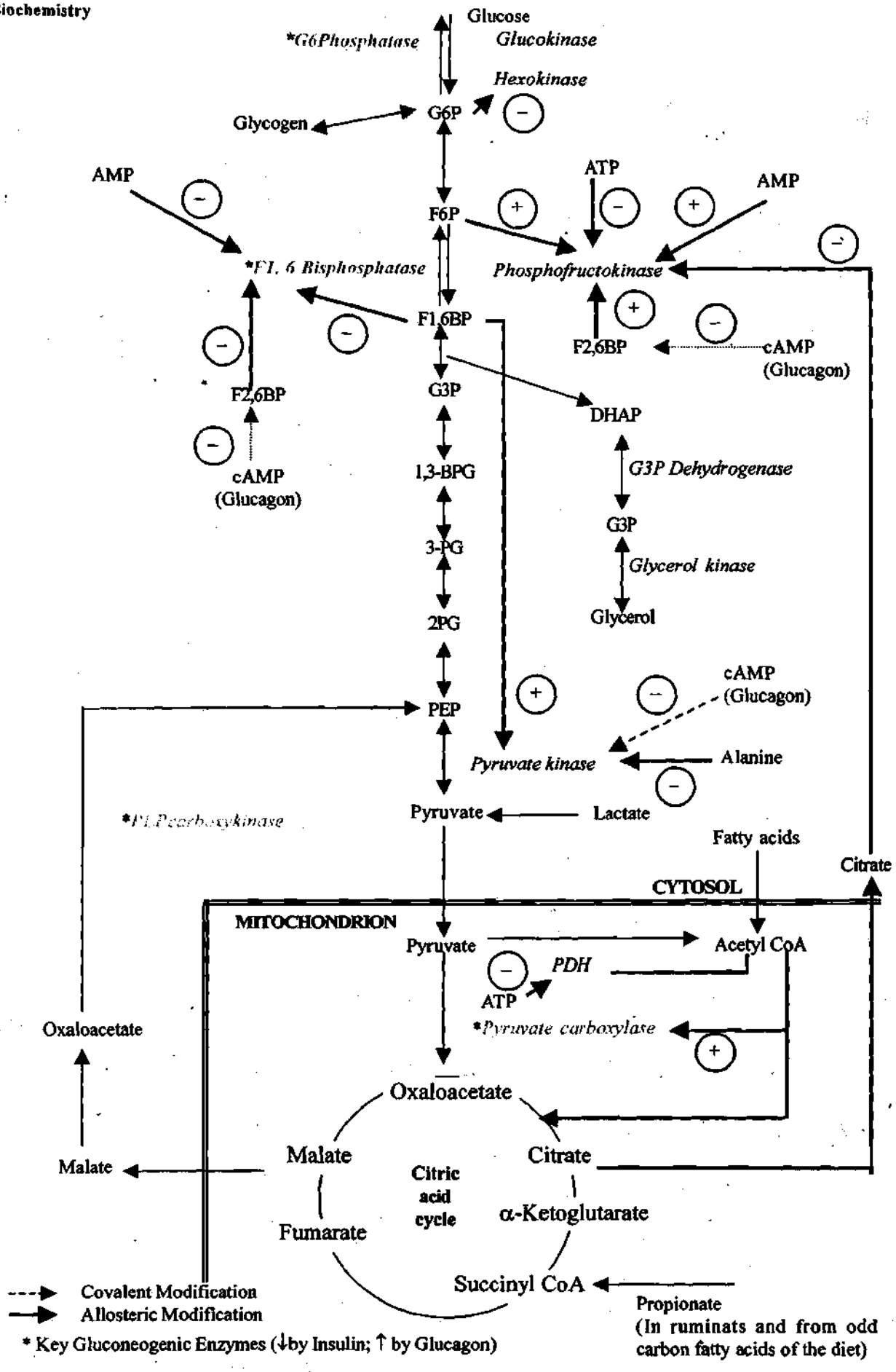

Figure 6.10 : Regulation of Gluconeogenesis and Glycolysis

6) ATP and citrate, as can be seen in Figure 6.10, are the activators of fructose-1,6 bisphosphatase and hence gluconeogenesis is increased. But high level of AMP in liver cells inhibits fructose-1,6-bisphosphatase activity and thus reduces gluconeogenesis.

7) Increased ADP allosterically inhibits pyruvate carboxylase and thus reduces gluconeogenesis.

8) The hormones glucagon, epinephrine and glucocorticoids stimulate the synthesis of pyruvate carboxylase and thus enhances gluconeogenesis. But the hormone insulin depresses the enzyme pyruvate carboxylase and thus reduces gluconeogenesis.

Earlier in the section on glycolysis we studied about the regulation of glycolysis at the PFK-1 reaction. The fructose-1,6-bisphosphate (F1,6BPase) reaction is a major point of control of gluconeogenesis. The regulatory role of fructose-2,6-bisphosphate is discussed herewith and highlighted in Figure 6.11.

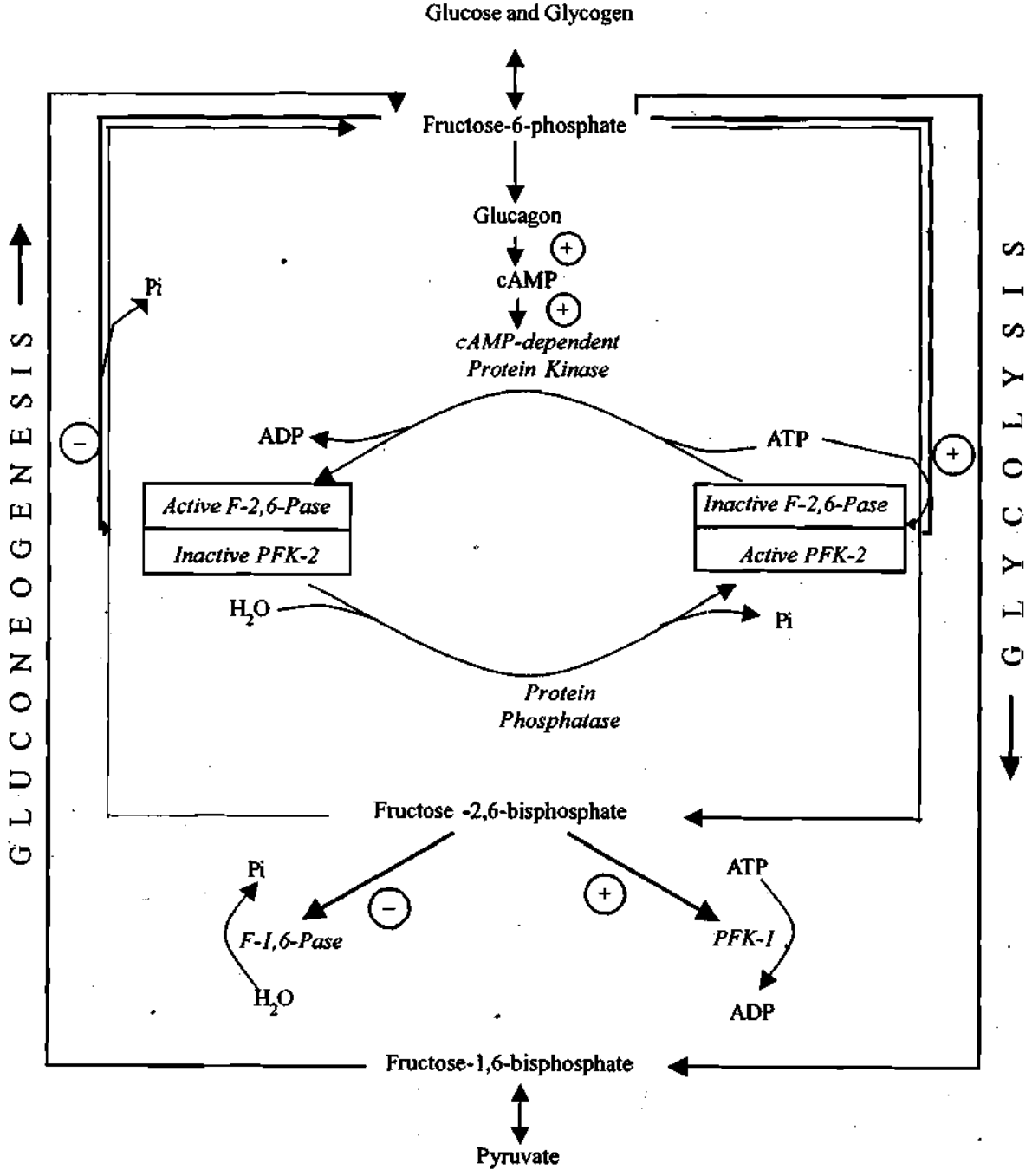

Figure 6.11 : Role of Fructose-2,6-Bisphosphate

Regulatory Role of Fructose-2,6-Bisphosphate

1) Fructose-2,6-bisphosphate is the most potent allosteric effector of phosphofructokinase-1 and inhibitor of fructose-1,6-bisphosphatase in liver.

2) It relieves inhibition of phosphofructokinase-1 by ATP and increases affinity for fructose-6-phosphate.

3) It inhibits fructose-1,6-bisphosphatase by increasing the k_m for fructose-1,6-bisphosphate.

4) Fructose-2,6-bisphosphate is formed from fructose-6-phosphate by the enzyme phosphofructokinase-2 (PFK-2) (a bifunctional enzyme). Thus the same enzyme also causes its breakdown by possessing fructose-2,6-bisphosphatase activity. It is allosterically controlled by fructose-6-phosphate.

5) When glucose is less, glucagon stimulates the production of cAMP which inactivates phosphofructokinase-2 and activates fructose-2,6-bisphosphatase by phosphorylation.

6) When there is abundance of glucose, the concentration of fructose-2,6-bisphosphate stimulates glycolysis by activating phosphofructokinase-1 and inhibiting fructose 1,6-bisphosphatase.

7) In glucose shortage, gluconeogenesis is stimulated by a decrease in the concentration of fructose-2,6-bisphosphate, which deactivates phosphofructokinase-1 and deinhibits fructose-1,6-bisphosphatase. This mechanism also shows that glucagon stimulation of glycogenolysis in liver results in glucose release rather than glycolysis.

8) Recently, it has been indicated that glucose-1,6-bisphosphate plays a similar role in some extrahepatic tissue.

Next, we shall learn about the metabolism of glycogen. Glycogen, we know, is the storage form of glucose. How is glycogen synthesized in the body? How is the stored glycogen degraded? We shall learn about these aspects in section 6.7.

Check Your Progress Exercise 4

1) What is gluconeogenesis? Highlight its significance giving any two examples.

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2) Enumerate the major substrates for gluconeogenesis.

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3) Describe how the following function in the body:

a) Alanine cycle

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b) The Cori cycle

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4) List the reactions that need to be circumvented by the special reactions in gluconeogenesis.

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6.7 METABOLISM OF GLYCOGEN

Glycogen is a highly branched, very large polymer of glucose molecules, linked along its main line by α-1,4 glycosidic linkages, branches arise by α-1,6 glycosidic bonds at about every 10^{th} residue.

The storage form of glucose is *glycogen* and the major storage sites are *liver* and *muscle*. Although the concentration of glycogen is higher in the liver, the much greater mass of skeletal muscle stores a greater total amount of glycogen. The function of muscle glycogen is to act as a readily available source of hexose units for glycolysis within muscle itself and muscle glycogen is significantly depleted after prolonged exercise. Liver glycogen is largely concerned with storage and export of hexose units for maintenance of the blood glucose, particularly between meals and after 12-18 hours of fasting, the liver glycogen is completely depleted.

After a meal, when there is a rise in blood glucose level, the synthesis of glycogen in liver and muscle is initiated. *This process is called glycogenesis*. This not only prevents excessive rise in blood glucose level, but also helps to store glycogen for future use.

In the liver, glycogen is metabolized to glucose and then released into the circulation in a fasting person. In the muscle, although glycogen cannot be converted into glucose it can still be used for obtaining energy during muscle contraction. This breakdown of glycogen in the liver (glycogen ⟶ glucose) and muscle (glycogen ⟶ glucose-1-phosphate) is called *glycogenolysis*.

Glycogen synthesis (glycogenesis) and glycogen usage (glycogenolysis) occur in separate pathways, which are discussed next.

6.7.1 Glycogenesis

The synthesis of glycogen in liver and muscle, we learnt earlier, is called *glycogenesis*. What does this process involve? Figure 6.12 illustrates the synthesis of glycogen.

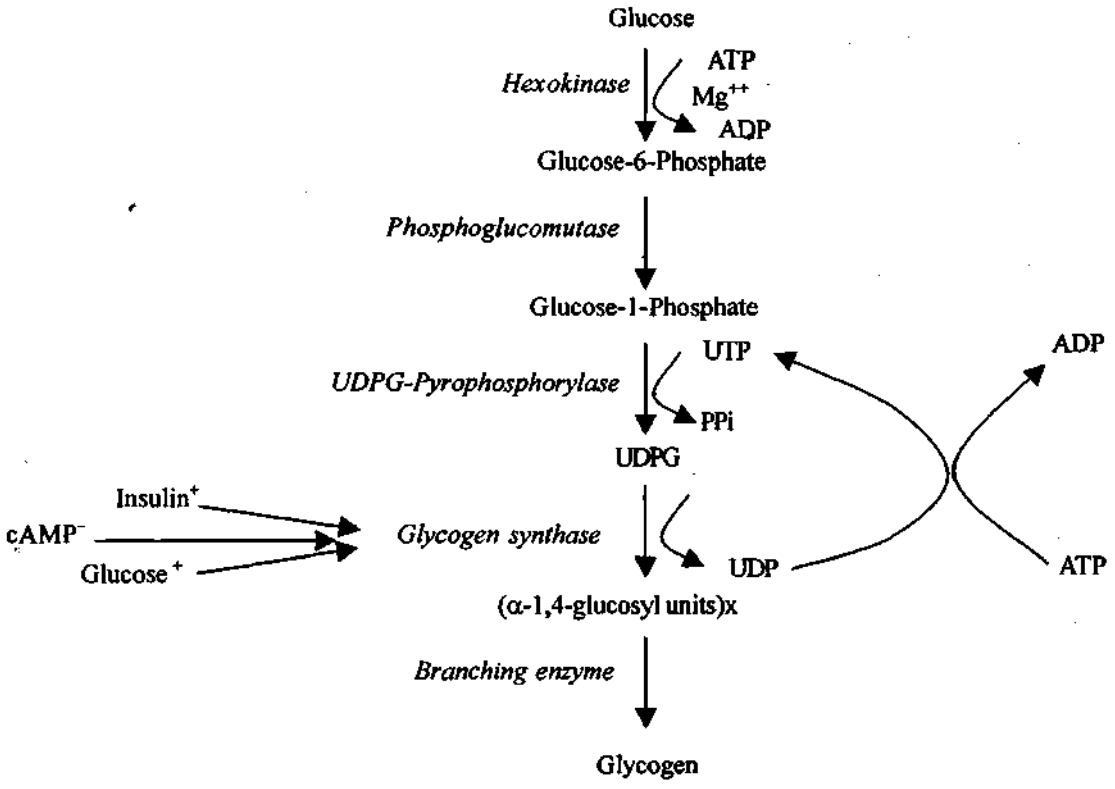

Figure 6.12 : Glycogenesis

The process is enumerated herewith:

1) Glucose is phosphorylated to glucose-6-phosphate by *hexokinase* (glucokinase in liver) in the presence of ATP and Mg^{++} ions. The action of glucokinase in liver is to remove glucose from the blood following a meal.

2) Glucose-6-phosphate is acted upon by *phosphoglucomutase* to form glucose-1-phosphate. This is a reversible reaction.

3) Glucose-1-phosphate reacts with uridine triphosphate (UTP) to form the active nucleotide uridine diphosphate glucose (UDPG) catalyzed by the enzyme *UDPG pyrophosphorylase*. The released pyrophosphate (PPi) is rapidly broken down by pyrophosphorylase to 2 inorganic phosphate (Pi) molecules, thereby rendering the reaction essentially irreversible.

4) The synthesis of new glycogen requires the presence of a glycogen primer (i.e. a preformed molecule) and α- glucosyl residues from UDP glucose. The residues are successively transferred to the C-4 terminus (non-reducing end) of an existing glycogen chain in α-1,4 glycosidic linkage. This process is repeated till about 10 to 12 molecules have been added. This reaction, which is the rate limiting step in glycogen synthesis, is catalyzed by *glycogen synthase* (glycogen synthetase).

5) After the chain has been lengthened to a minimum of 11 glucose residues, the branching enzyme (amylo [1→4]→[1→6] transglucosidase transfers a part of the 1→4 chain (minimum length of 6 glucose residue) to a neighbouring chain to form 1→6 linkage, thus, establishing a branch point in the molecule. The branches grow by further addition of 1→4 glucosyl units and further branching.

Perhaps as a revision exercise, you could write the pathway of glycogenesis giving the chemical structure of all the intermediates.

Next, let us look at the factors which regulate glycogenesis.

6.7.2 Regulation of Glycogenesis

Glycogen synthase, the key enzyme in glycogenesis, is activated by insulin and glucose and inhibited by cAMP as shown in Figure 6.13.

Glycogen synthase exists in two forms: the phosphorylated form designated as 'D' form is the inactive one and the dephoshorylated form designated as 'I' form is the active one. The 'D' form is an allosteric enzyme, activated by high concentration of 5'–AMP and the 'I' form does not require 5'–AMP for its action. Hence the names D and I forms – D for dependent on 5'–AMP activity and I for independent of 5'–AMP concentration. The interconversion of D and I form is catalyzed by cAMP dependent protein kinase. The level of cAMP is in turn regulated by adenylate cyclase enzyme, which is activated by glucagon and epinephrine as shown in Figure 6.13.

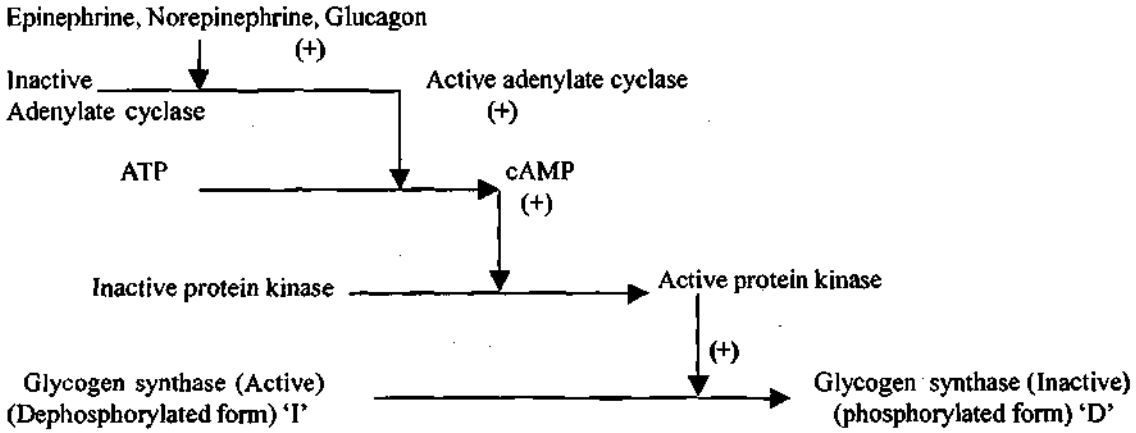

Figure 6.13 : Control of Glycogen Synthase

Having studied about glycogen synthesis and regulation, next it is the turn of glycogenolysis i.e. the breakdown of glycogen.

6.7.3 Glycogenolysis

Unlike glycogenesis, glycogenolysis is the breakdown of glycogen. Glycogen is broken down in the liver and muscle catalysed by the enzyme *glycogen phosphorylase*. Inorganic phosphate (Pi) is used for the lysis and hence is called phosphorolysis. Phosphorylase specifically acts upon α 1→4 linkage of glycogen to produce glucose-1-phosphate. The removal of α1,4 glucosyl residues continues until about 4 glucose residues remain on either side of α-1,6 branch, then the debranching enzyme (amylo α-1,6 glucosidase) causes the hydrolytic splitting of α 1,6 linkages. Here free glucose is formed (since no phosphate is used for lysis). However, since α-1,6 linkages are very few compared to α 1→4 linkages, the major end product of glycogenolysis is glucose with small amounts of glucose-1-phosphate. By the combined action of both the enzymes, glycogen is catabolized. The reversible reaction of *phosphoglucomutase* causes the conversion of glucose-1-phosphate to glucose-6-phosphate. In liver and kidney (but not in muscle), there is a specific enzyme glucose-6 phosphatase, which acts upon glucose-6-phosphate to release free glucose from the cell to the extracellular compartment as illustrated in Figure 6.14.

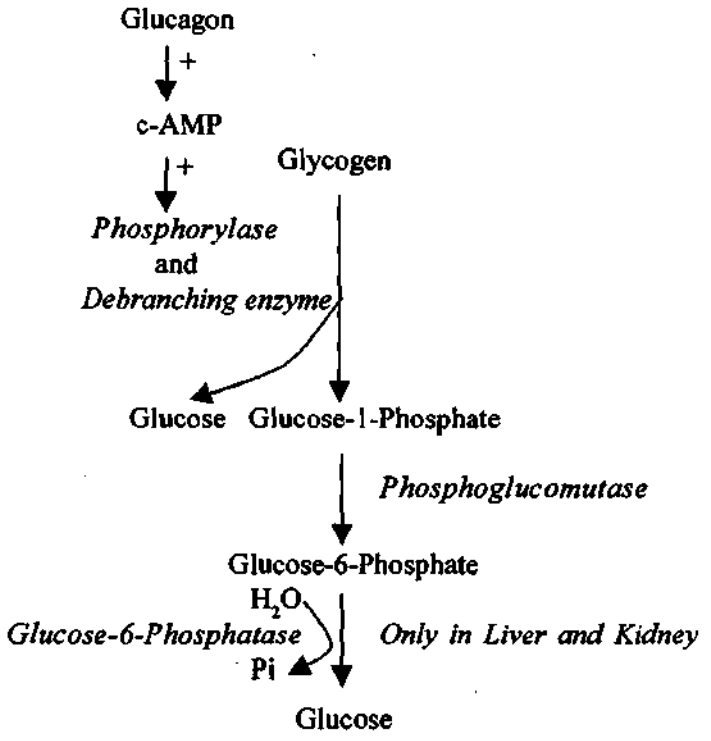

Figure 6.14 : Glycogenolysis

Glycogen phosphorylase is a dimeric (2 polypeptide) enzyme that utilizes pyridoxal phosphate as a prosthetic group. Different isozymes of glycogen phosphorylase are present in different tissues. Phosphorylase from liver is activated by glucagon stimulated cAMP levels whereas muscle phosphorylase is activated only by epinephrine via cAMP. In lysosomes, another enzyme α-1,4 glucosidase is involved in debranching.

Having understood the glycogenolysis process, let us now learn about its regulation.

6.7.4 Regulation of Glycogenolysis

Figure 6.15 illustrates the cascade regulation of glycogen phosphorylase activity, which we learnt above, is the major enzyme involved with breakdown of glycogen, and hence also plays a main role in its regulation. Glycogen phosphorylase exists in two distinct states – *phosphorylase a*, the active state and *phosphorylase b*, the inactive state. The regulation mechanism involves:

a) The hormones catecholamines (epinephrine, norepinephrine) and glucagon cause the increase in cAMP levels in cells. This cAMP activates protein kinase, which stimulates the key enzyme phosphorylase for glycogenolysis. Briefly, phosphorylase b is phosphorylated, and rendered highly active, by phosphorylase kinase.

b) Immediately after the onset of muscle contraction, glycogenolysis is highly increased in muscle by the rapid activation of phosphorylase due to the activation of phosphorylase kinase by Ca^{++} ions.

c) Calmodulin (a Ca^{++} dependent regulatory protein) causes further activation of phosphorylase kinase for glycogenolysis.

So having looked at the mechanism involved, you may have realized that the regulation of glycogen phosphorylase is complex. Its regulation ensures that glucose remains stored as glycogen until it is mobilized from liver for maintaining blood glucose homeostasis, or to supply energy to the muscle cell. This enzyme is phosphorylated in response to hormone signals in a cascade. The enzyme that directly catalyses the phosphorylation of glycogen phosphorylase is *phosphorylase kinase*, which itself can be activated either by

phosphorylation or allosterically by calcium. The cascade is initiated by binding of hormone (glucagon or epinephrine) to its specific receptor. Hormone binding activates adenylyl cyclase. Adenylyl cyclase produces cyclic AMP that activates protein kinase. To accelerate glycogenolysis, protein kinase (active) catalyses the phosphorylation of phosphorylase kinase converting it from its inactive b form to its active a form. Subsequently, phosphorylase kinase-a (active form) catalyses the phosphorylation of the tense (inactive) glycogen phosphorylase-b to generate the relaxed (active) glycogen phosphorylase-a form. Thus glycogen degradation is triggered.

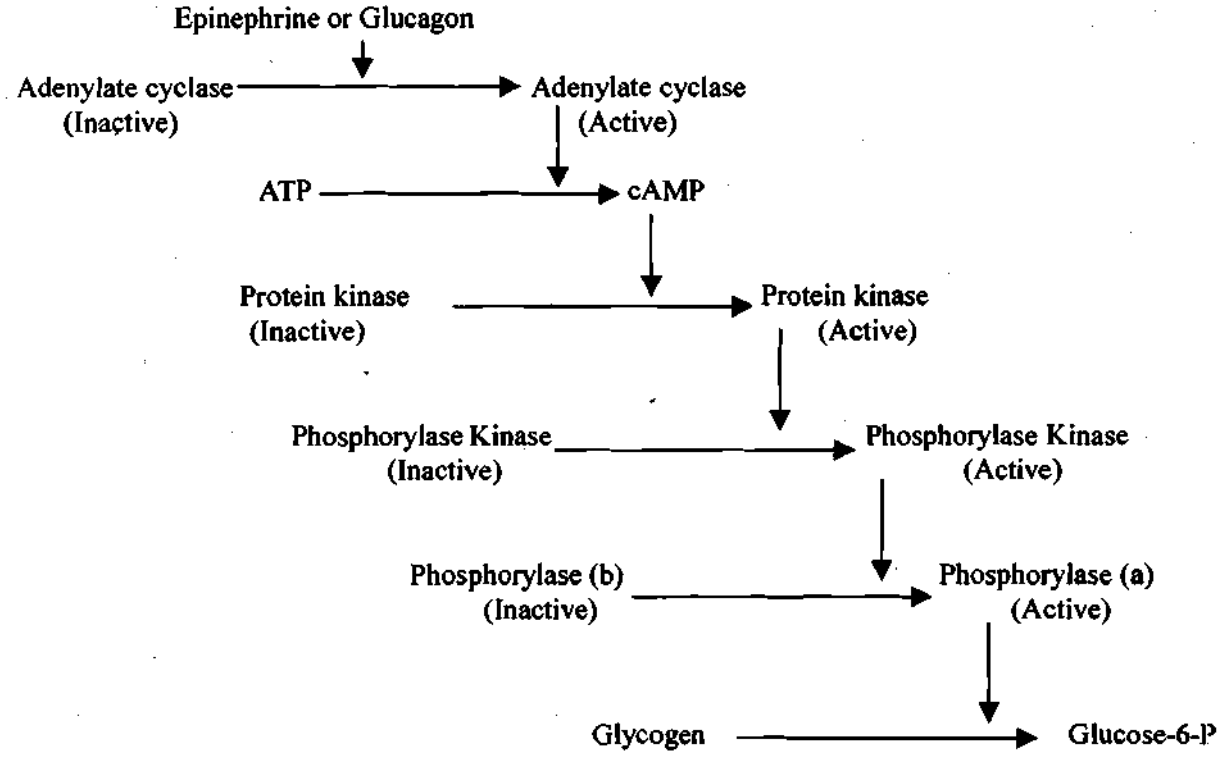

Figure 6.15 : Cascade regulation of glycogen phosphorylase activity

In the sub-sections above, we have studied about glycogenesis and gluconeogenesis. Next we shall learn about regulation of glycogen metabolism.

6.7.5 Regulation of Glycogen Metabolism

It is important for you to understand that glycogenesis and gluconeogenesis are regulated reciprocally. There is a *hormonal regulation* system functioning at the muscle and liver level, which regulates the glycogen metabolism. We have studied about the phosphorylation cascade involving the hormones epinephrine and glucagon above. Epinephrine promotes glycogenolysis and inhibits glycogenesis. It stimulates the formation of cAMP by activating adenylate cyclase in the muscle. Insulin, another hormone, increases glycogenesis and decreases glycogenolysis in the muscle. It heightens the entry of glucose unit in the muscle cells. It reduces cAMP levels by speeding up the destruction of cAMP by phosphodiesterase.

Glucagon activates adenylate cyclase in the liver cell membrane and thus turns on glycogenolysis and reduces glycogenesis. Insulin increases glycogenesis in the liver by increasing the activity of glycogen synthase. The glucagon: insulin ratio appears to be more important than the absolute level of either hormone since glycogen metabolism is strongly influenced by the predominant hormone.

In addition to the hormonal regulation of glycogen metabolism, *the role of covalent modification* in regulation is also important. With separate systems for the synthesis and degradation of glycogen and with glucose-1-phosphate acting as a

common intermediate, the possibility of a futile cycle of glycogen must be considered. What is a *futile cycle*? If two reactions occur uncontrolled, the situation would represent a waste of ATP without any metabolic work being done. Futile cycles or substrate cycles occurs at several points in the pathway that interconnect glycogen and pyruvate. Other such pairs include glucokinase and glucose-6-phosphatase, pyruvate kinase and pyruvate carboxylase plus phosphoenolpyruvate carboxykinase. However, this does not occur extensively, due to the various control mechanisms, which ensure that one reaction is inhibited as the other is stimulated. At the same time allowing some futile cycling is physiologically advantageous. It helps in 'fine tuning' of metabolic control. The heat generated also helps in maintaining body temperature.

The futile cycle is avoided because covalent modification, by phosphorylation, has opposite effects on the enzymes concerned with the synthesis and degradation of glycogen. Look at the following reactions :

i) An adequate level of cAMP stimulates formation of the inactive 'D' form of glycogen synthase and the active form of phosphorylase. Thus, glycogenesis is limited and glycogenolysis is increased.

ii) With a low level of cAMP, the active I form of glycogen synthase predominates and the active form of phosphorylase kinase is low because the common phosphorylating enzyme, cAMP dependent protein kinase, is inactive. There, glycogenesis is increased and glycogenolysis is decreased.

Having reviewed the processes of glycogenesis and glycogenolysis, we have more or less completed our study of glycogen metabolism. However, before we end our study on this topic, we must also study about the disorders linked with glycogen metabolism. This aspect is discussed next.

6.7.6 Glycogen Storage Diseases

Glycogen storage diseases are caused by genetic defects that result in deficiencies in certain enzymes of glycogen metabolism. These deficiencies lead to excessive accumulation of glycogen and / or the inability to use that glycogen as a fuel source. The structure of glycogen may also be abnormal. A summary of the various diseases, defective enzyme and the tissue affected by the disorder is presented in Table 6.3.

Table 6.3: Glycogen storage diseases

Type	Disease Name	Defective enzyme	Glycogen levels	Glycogen structure	Principal tissue affected
I	Von Gierke's disease	Glucose-6-phosphatase (G6pase)	High	Normal	Liver, kidney
II	Pompe's disease	α-1,4 Glucosidase	Very high	Normal	All organs
III	Cori's Forbes' disease	Debranching enzyme	High	Short outer branches	Liver, Heart, Muscle
IV	Andersen's disease	Branching enzyme	Normal	Long outer branches	Liver, Spleen, Muscle
V	McArdle's disease	Muscle Phosphorylase	High	Normal	Muscle
VI	Hers' disease	Liver Phosphorylase	High	Normal	Liver
VII	Tarui's disease	Phosphofructokinase	High	Normal	Muscle
VIII	Hepatic phosphorylase kinase deficiency	Phosphorylase kinase	High	Normal	Liver

Check Your Progress Exercise 5

1) Differentiate between glycogenesis and glycogenolysis.

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2) Mention the two regulation systems of glycogen metabolism.

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3) Write short note on glycogen storage diseases.

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4) Which hormones stimulate glycogenolysis?

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In this unit, so far, we have studied about the glucose and glycogen metabolism. You would be interested to know that besides the oxidative pathway for glucose metabolism about which we have already earlier in section 6.3, there is an alternate oxidative pathway for the metabolism of glucose in the body, which is called the hexose monophosphate pathway. Let us get to know about this pathway next.

6.8 HEXOSE MONOPHOSPHATE PATHWAY

The hexose monophosphate pathway (HMP also called the pentose phosphate pathway, or phoshogluconate pathway) consists of two irreversible oxidative reactions, followed by a series of reversible sugar phosphate interconversions. This is an alternate oxidative pathway for the metabolism of glucose in the liver, lactating mammary gland and adipose tissue in addition to *Embden–Meyerhof* pathway for glycolysis.

In this pathway, 3 molecules of glucose-6-phosphate yield 3 molecules of CO_2 and 3 molecules of five carbon residues (pentose sugar). The latter are converted ultimately to 2 molecules of glucose-6-phosphate and one molecule of glyceraldehyde-3-phosphate. NADP serves as a hydrogen acceptor in this pathway.

Unlike glycolysis or the citric acid cycle in which the direction of the reactions is well defined, the interconversion reactions of the HMP pathway can function in several different directions. The rate and direction of the reactions at any given time are determined by the supply of and demand for intermediates in the cycle. The HMP pathway like glycolysis occurs in the cytosol of the cell. However, CO_2 which is not produced in glycolysis, is a characteristic product in HMP pathway. Further, in this pathway no ATP is generated, which you know, is the major product of glycolysis. Again oxidation uses $NADP^+$ unlike NAD^+ in glycolysis. It would be a useful exercise for you to list the similarities and differences in glycolysis and HMP pathway, later after having gone through the section of HMP pathway here. So let's get moving and get to know the metabolic reactions in the HMP pathway.

6.8.1 Metabolic Reactions in the HMP Pathway

The hexose monophosphate pathway is responsible for the generation of a substantial fraction of the cytoplasmic NADPH required for biosynthetic reactions, and for the generation of ribose-5-phosphate for nucleotide synthesis. Hence, there are the following two phases of HMP pathway:

1) In the oxidation pathway, glucose-6-phosphate is converted to ribulose-5-phosphate by dehydrogenation and decarboxylation reactions.

2) In the nonoxidative phase, ribulose-5-phosphate is converted back to glucose-6-phosphate by a series of reactions involving transketolase and transaldolase.

Let us get to know more about these phases. Figure 6.16 illustrates the HMP pathway.

1) ***The oxidative phase generates NADPH***

The oxidative branch of the pathway generates NADPH and pentose-5-phosphate, through the following reactions:

i) Glucose-6-phosphate is dehydrogenated to 6-phosphogluconate via 6-phosphoglucano-lactone by *glucose-6-phosphate dehydrogenase* in presence of $NADP^+$ and the cofactors Mg^{++}, Mn^{++} or Ca^{++}. Glucose-6-phosphate dehydrogenase deficiency is an inherited disease characterized by haemolytic anaemia if the patient is treated with an oxidant drug (such as primaquine and sulphonamide) or ingests fava beans.

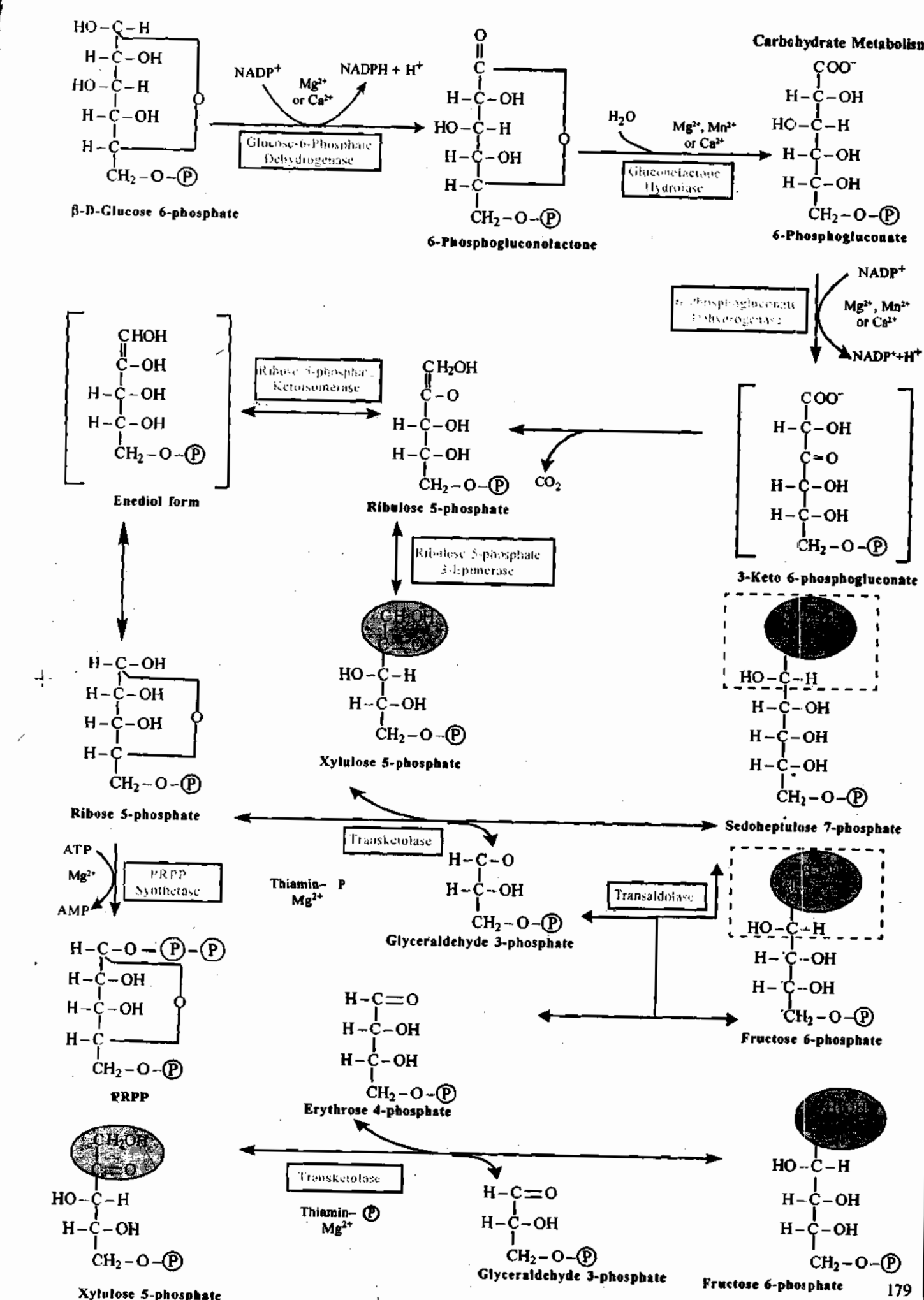

Figure 6.16 : Pentose Phosphate Pathway (HMP Shunt)

ii) 6-phosphogluconate is oxidized by *6-phosphogluconate dehydrogenase* in the presence of coenzyme $NADP^+$ and cofactors Mg^{++}, Mn^{++} or Ca^{++} to 3-keto 6 phosphogluconate which is decarboxylated to form ribulose-5 phosphate.

2) ***The non-oxidative phase generates ribose precursors***

The non-oxidative phase of the pathway, including the following reactions, converts pentose-5-phosphate to other sugars.

i) Ribulose-5-phosphate is acted on by ribulose-5-phosphate *epimerase*, as shown in Figure 6.16, which changes the configuration at carbon 3 forming xylulose-5-phosphate, and also by the enzyme ribose-5-phosphate *ketoisomerase*, which converts ribulose-5-phosphate to ribose-5-phosphate.

ii) The next step involves the action of the enzyme *transketolase*. Transketolase with the help of TDP and Mg^{++} transfers carbons 1and 2 of xylulose-5-phosphate to ribose-5-phosphate forming sedoheptulose-7-phosphate and glyceraldehyde-3-phosphate.

iii) The next step involves the action of the enzyme *transaldolase*. Transaldolase allows the transfer of a 3-carbon moiety from sedoheptulose-7-phosphate to glyceraldehyde-3-phosphate to form fructose-6-phosphate and erythrose-4-phosphate.

iv) Transketolase with the help of TPP and Mg^{++} is required again. This time it transfers carbon 1+2 from xylulose-5-phosphate to erythrose-4-phosphate forming fructose-6-phosphate and glyceraldehyde-3-phosphate.

Thus HMP shunt is not an isolated repetitive cycle, but is integrated with glycolysis. The overall reaction of the HMP shunt is as follows:

3 Glucose-6-phosphate + $6NADP^+$

↓

$3CO_2$ + 6NADPH + $6H^+$ + glyceraldehyde-3-phosphate + 2 glucose-6-phosphate

In order to oxidize glucose completely to CO_2 via the HMP pathway, tissues must have the enzymes for converting glyceraldehyde-3-phosphate to glucose-6-phosphate. This involves reversal of glycolysis and the gluconeogenic enzyme fructose-1,6-bisphosphatase. In tissues that lack this enzyme, glyceraldehyde-3-phosphate follows the normal pathway of glycolysis to pyruvate.

Passage around the cycle oxidises only C-1 of glucose so that six passages around are necessary for the complete oxidation of a molecule of glucose. The pentose phosphates are converted into fructose-6-phosphate, which is isomerized to glucose-6-phosphate to begin the cycle all over again.

How is the HMP pathway regulated? Let's find out next.

6.8.2 Regulation of HMP Pathway

The following factors play an important role in regulation of HMP pathway:

i) The first reaction of this pathway catalysed by glucose-6-phosphate dehydrogenase is the "rate limiting" step. This is mainly regulated by the cytoplasmic levels of $NADP^+$ and NADPH.

ii) High carbohydrate content in the diet accelerates the rate of the pathway by activating both the dehydrogenases whereas diabetes mellitus and starvation reverses these reactions.

iii) The HMP shunt is activated by the increase in $NADP^+$ in the cytoplasm, which in turn, is due to the oxidation (utilization) of NADPH by the synthesis of fatty acids and steroids.

iv) HMP shunt is accelerated due to the stimulation of dehydrogenases by insulin.

v) Thyroid hormone acts in the same way by stimulating glucose-6-phosphate dehydrogenase.

Finally, what is the metabolic significance of the HMP pathway? Let us find out.

6.8.3 Metabolic Significance of HMP Pathway

Having gone through the HMP pathway, you would have got some idea about the significance of this alternative oxidative pathway for the metabolism of glucose. Let us enumerate the significance one by one:

We have seen that CO_2 is the characteristic product in the HMP pathway, which is not produced in the Embden-Meyerhof pathway. CO_2 produced in this pathway is used for the synthesis of fatty acids and purine bases.

The reduced form of NADP (NADPH) is utilized for the synthesis of fatty acids, cholesterol, steroids and also in the synthesis of amino acids via glutamate dehydrogenase outside the mitochondria. In fact tissues specializing in active lipogenesis – liver, adipose tissue and the lactating mammary glands – also posses an active HMP pathway.

The pentose sugars produced in HMP shunt are utilized for the synthesis of nucleic acids and nucleotides.

Skeletal muscle has low activity of glucose-6-phosphate dehydogenase. Yet, like most other tissues it can synthesize ribose-5-phosphate. This is probably accomplished by a reversal of the shunt pathway utilizing fructose-6-phosphate and glyceraldehyde-3-phosphate and the enzymes transketolase and transaldolase. Both fructose-6-phosphate and glyceraldehyde-3-phosphate are utilized in Embden-Meyerhof pathway for glycolysis. Hence it is not necessary to have a completely functioning HMP pathway for a tissue to synthesize ribose-5-phosphate.

The fragility of erythrocytes is impaired in the absence of NADPH generation due to the deficiency of glucose-6-phosphate dehydrogenase thereby causing haemolytic anaemia when the red blood cells are subjected to certain drugs such as primaquine and sulphonamide. An inverse correlation has been found between the activity of glucose-6-phosphate dehydrogenase and the fragility of red cells (i.e. susceptibility to haemolysis).

HMP shunt in erythrocytes is of importance due to the generation of NADPH, which maintains the glutathione (G-SH) in the reduced state by glutathione reductase, a flavoprotein containing FAD. Glutathione is a tripeptide (glycine-glutamate-cysteine), which, in the reduced state takes part in redox reactions in cells. In this process, two glutathione molecules combine to give the oxidized form (G-S-S-G). The reduced glutathione then removes H_2O_2 from the erythrocytes by glutathione peroxidase, an enzyme containing selenium as shown in Figure 6.17. This reaction is important because accumulation of H_2O_2 may decrease the life-span of erythrocytes by increasing the rate of oxidation of haemoglobin to methaemoglobin.

Glutathione peroxidase is a natural antioxidant present in many tissues. Together, with vitamin E it is part of the body's defense against lipid peroxidation. An association between the incidence of some cancers and low level of blood selenium and glutathione peroxidase activity has been reported.

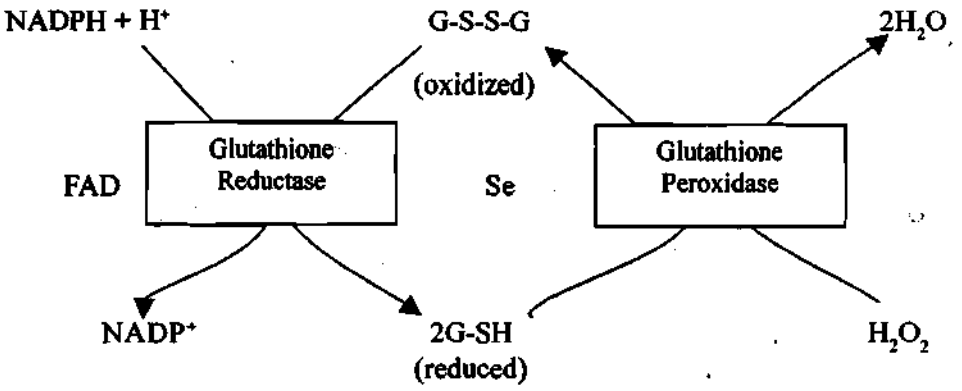

Figure 6.17: Role of NADPH in erythrocytes

A supply of NADPH is critical for the liver microsomal cytochrome P-450 mono oxygenase system which serves to detoxify drugs and foreign compounds by converting them into soluble forms more readily excreted through the kidney as illustrated in Figure 6.18. HMP pathway becomes an important source of NADPH for this reaction.

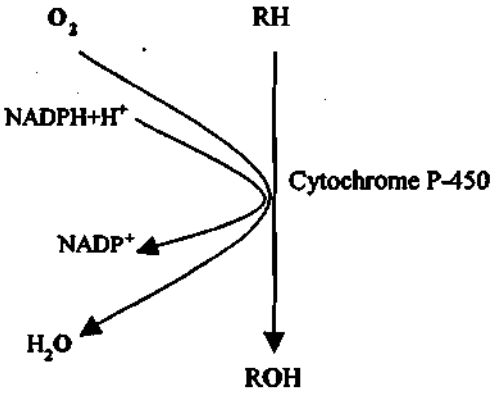

Figure 6.18: Microsomal cytochrome P-450 mono oxygenase system

Having gone through the discussion above, you would have got a very good idea about the significance of HMP pathway. The HMP pathway or the so-called pentose phosphate pathway, you can now understand is a multifunctional pathway.

HMP pathway, other than the glycolytic pathway, is an alternate oxidative pathway for the metabolism of glucose. Besides glucose, the digestion of foodstuffs and the utilization of endogenous metabolites can supply a variety of carbohydrates for glycolysis. What are these carbohydrates? What is their fate in the body? This aspect is covered in the next section here in this unit.

6.9 ENTRY OF OTHER SUGARS INTO GLYCOLYTIC PATHWAY

The digestion of foodstuffs and the utilization of endogenous metabolites can supply a variety of carbohydrates for glycolysis. You may already know that the digestion of dietary carbohydrates results in the absorption of monosaccharides such as galactose, fructose and mannose, in addition to glucose. Dietary galactose, fructose and mannose can be converted into glycolytic intermediates and fed into the glycolytic pathway. The metabolic routes for utilizing substrates other than glucose in glycolysis are summarized in Figure 6.19.

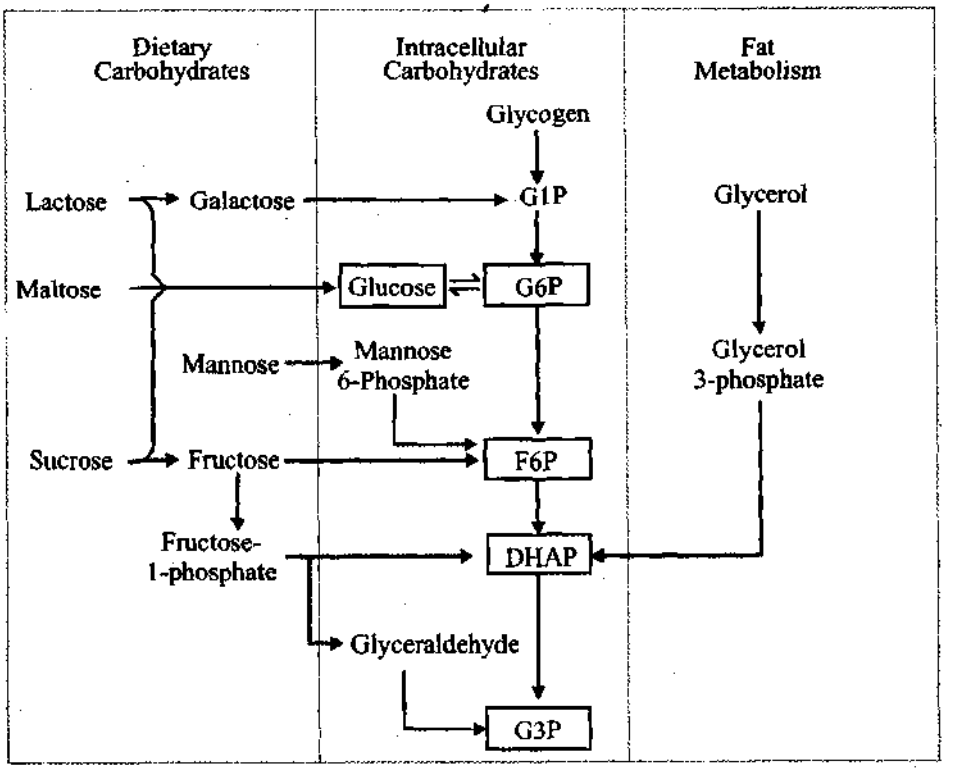

Figure 6.19: Routes for utilizing substrates other than glucose in glycolysis

Let us look at the metabolism of galactose, fructose and mannose in greater details.

Galactose Metabolism

Galactose goes to the liver via portal blood and is phosphorylated by *galactokinase* to galactose-1-phosphate (GIP) using ATP as a phosphate donor as shown in Figure 6.20. Galactose-1-phosphate reacts with uridine diphosphate glucose to form uridine diphosphate galactose catalyzed by *galactose-1-phosphate uridyl transferase*. It is then converted to UDP-glucose catalyzed by *UDP-galactose 4-epimerase*. Since this would be the well-fed state, the glucose would be incorporated into the glycogen chain through the action of glycogen synthase.

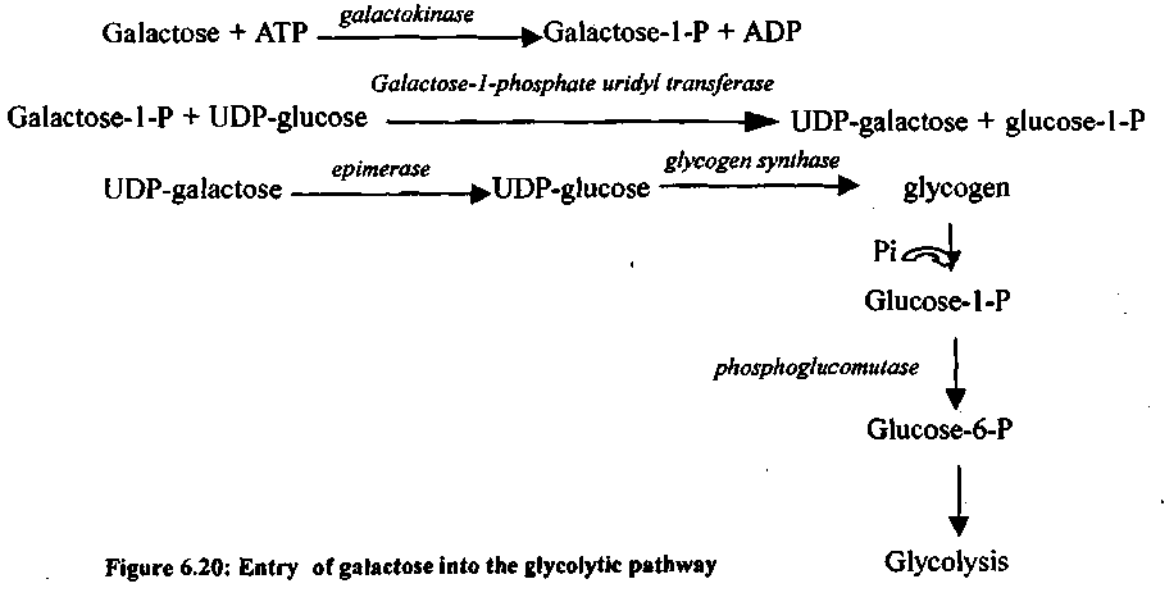

Figure 6.20: Entry of galactose into the glycolytic pathway

Fructose metabolism

The entry of fructose into the glycolytic pathway is illustrated in Figure 6.21. The process starts when fructose absorbed from the diet is taken to the liver. A specific kinase,

fructokinase, phosphorylates fructose to fructose-1-phosphate. This is cleaved by *Aldolase B*, which is found only in the liver, with the formation of dihydroxyacetone phosphate and glyceraldehyde. Glyceraldehyde is phosphorylated by *triokinase* to glyceraldehyde-3-phosphate which can enter glycolysis. Dihydroxyacetone phosphate can be isomerized to glyceraldehyde-3-phosphate and enter glycolysis. However, being the well-fed state, the more likely pathway would be for dihydroxyacetone phosphate and glyceraldehyde-3-phosphate to be converted to fructose-1,6-bisphosphate by Aldolase A or Aldolase B. Aldolase A, unlike Aldolase B is also found in extrahepatic tissues. Fructose-1,6-bisphosphate through the rest of the gluconeogenic enzyme, ultimately gets incorporated into glycogen chain. Alternatively, if there is a need, glyceraldehyde-3-phosphate can enter glycolysis.

A small portion of dietary fructose can also be phosphorylated by hexokinase forming fructose-6-phosphate which in all probability would be incorporated into glycogen chain.

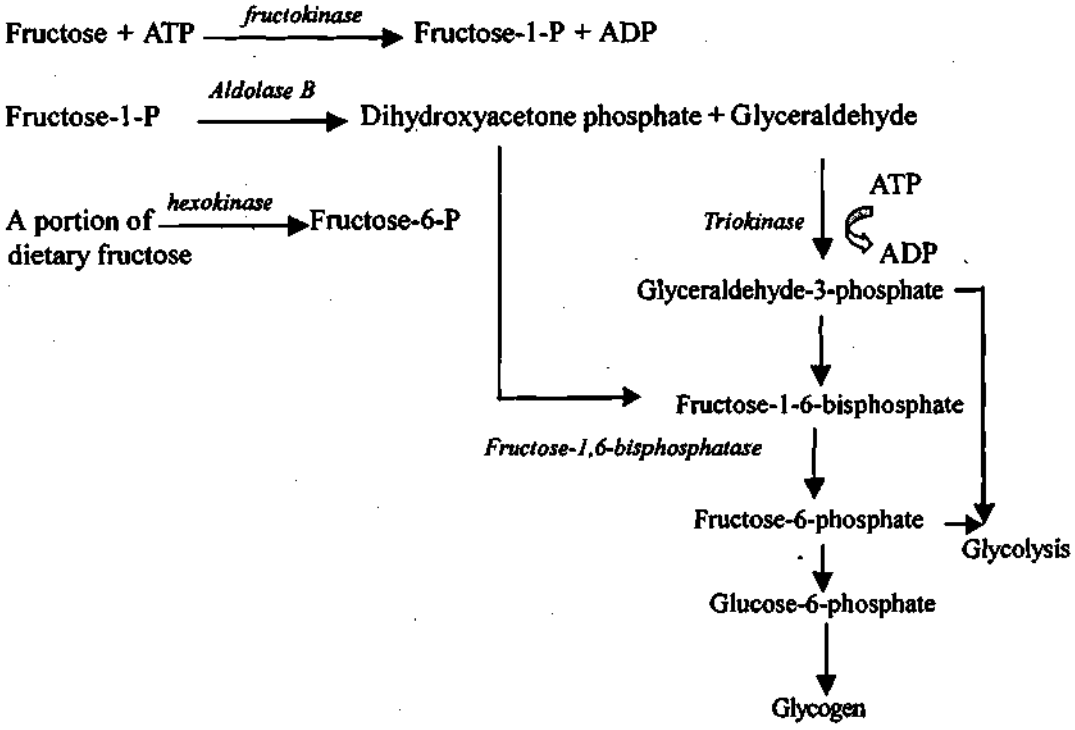

Figure 6.21: Entry of fructose into the glycolytic pathway

Mannose Metabolism

Mannose is phosphorylated by *hexokinase* forming mannose-6-phosphate. This is isomerized by phosphomannose isomerase to form fructose-6-phosphate which can be incorporated into glycogen chain as shown in Figure 6.22.

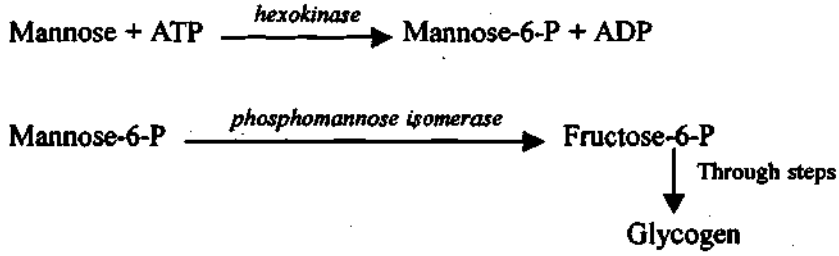

Figure 6.22: Entry of mannose into the glycolytic pathway

To help you recapitulate what you have learnt so far, we suggest, you practice all these reactions with the chemical structures of the intermediates.

Before we end our discussion on this topic, we need to understand that some time the monosaccharides can accumulate in the body and can cause biochemical defects. One such common defect is *galactosemia*. Let us get to know about this next.

Galactosemia

Inability of conversion of galactose to glucose results in the accumulation of galactose in the blood – known as *galactosemia*. The biochemical defect usually found in galactosemia is the deficiency of the enzyme *galactose-1-phosphate uridyl transferase*. Initially, galactose accumulates in the tissues, then in the blood. The major organ damaged by galactose accumulation is liver. Galactose is reduced to the corresponding alcohol called *galactitol* in the eye which causes cataract.

So it is clear that glucose is the main fuel for the body. Other monosaccharides from the diet are converted to glucose. The level of glucose in the blood is maintained within a specific range in our body. What is this range? How is the blood glucose level regulated in the body? We will learn about this in the next section.

6.10 REGULATION OF BLOOD GLUCOSE LEVEL

Various levels of regulation are exerted at substrate level, hormonal level, enzymatic level and at organ level on carbohydrate metabolism so as to maintain the blood glucose level at the optimal range between 4.5-5.5 mmol/litre. Events leading to such maintenance include:

a) In the fed state, clearance of blood glucose is mainly by liver via *glucokinase*. Glucokinase, which is an inducible enzyme, removes most of the blood glucose from circulation after a carbohydrate meal. Further, uptake of glucose also takes place in the extra-hepatic tissues (such as muscle, adipose tissue etc.) favoured by insulin. These two mechanisms regulate the blood glucose level in the fed state.

b) In the fasting state, glucose release from the liver increases due to the action of glucagon and in the muscle by epinephrine. Glucagon also enhances gluconeogenesis from amino acids and lactate. Both hepatic glycogenolysis and glyconeogenesis contribute to the hyperglycaemic effect of glucagon. Through these mechanisms, the blood glucose level is maintained in the fasting state.

c) The anterior pituitary gland secretes hormones that elevate the blood glucose and therefore antagonize the action of glucose. Two of these major hormones include growth hormone and ACTH (corticotropin).

d) The adrenal cortex secretes the *glucocorticoids* which increase gluconeogenesis by increasing hepatic intake of amino acids accompanied by increased activity of enzymes of gluconeogenesis, as well as, of transaminases. Glucocorticoids also inhibit utilization of glucose in extra hepatic tissues. The net effect is one of increasing blood sugar level.

From the above discussion, it can be seen that insulin is the only hormone decreasing blood glucose level, while all other hormones increase the blood glucose level. You may already be aware of the consequences of elevated blood glucose level. Yes, diabetes mellitus is the resulting metabolic disorder. A brief review follows.

Diabetes mellitus

Diabetes mellitus is a complex metabolic disorder characterized by the absolute or relative deficiency of insulin and /or defects in insulin action. This anabolic hormone exerts its action on key glycolytic enzymes thus leading to the conversion of glucose to pyruvate as explained under the regulation of glycolysis. On the other hand, insulin suppresses the action of all key gluconeogenic enzymes as shown in Figure 6.7. With respect to glycogen metabolism, the excess glucose is converted to glycogen by activating glycogen synthase thus leading to glycogenesis and inhibiting glycogenolysis. In this metabolic disease-diabetes mellitus, the lack of insulin reverses these actions and the antagonistic hormones (like glucagon, epinephrine, catecholamines, thyroxine etc.) by their concerted efforts on various pathways bring about the hyperglycemic condition.

Check Your Progress Exercise 6

1) What is HMP pathway? Give any two points of its significance.

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2) With the help of reaction briefly explain the oxidative phase of the pentose phosphate pathway.

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3) Discuss the role of NADPH in erythrocytes.

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4) Discuss how the blood sugar level is maintained at a stable level in the well fed and fasting state.

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6.11 ELECTRON TRANSPORT CHAIN

All processes require energy. In living cells, we constantly use energy for a number of biochemical reactions e.g. muscular movements, synthesis of new components, transport of ions, secretion and excretion etc. We have already learnt earlier in this unit that ATP is the molecule that supplies the energy for all these processes. Therefore, there is a need for the continuous synthesis of ATP. This section deals with the synthesis of ATP in the mitochondria. You may recall studying the structure of mitochondria in the Applied Physiology Course, Unit 2. Look up the structure once again now as you read through

the following section. This will help you to understand the electron transport chain more systematically. So let's get started.

6.11.1 Mitochondrial Electron Transport Chain

Mitochondria houses the electron transport chain (ETC) and the reactions of oxidative phosphorylation (OP). Hence the mitochondria is called as the *power house* of the cell.

Metabolism of carbohydrates, lipids and amino acids yields reducing equivalents such as NADH, $FMNH_2$, $FADH_2$ which undergoes reoxidation. These reduced coenzymes in turn each donate a pair of electrons to a specialized set of electron transport chain. Ultimately these electrons are transferred to O_2. As electrons pass through this chain, they lose much of their free energy. Part of this free energy is captured and stored by the formation of ATP from ADP and inorganic phosphate. This process is called as *oxidative phosphorylation.* We will learn about this later in this section after studying about the components of ETC.

How does the transfer of electron take place? Having read through the carbohydrate metabolism in this unit, surely you should be able to answer this. Yes, a wide variety of enzymes and coenzymes are involved. Read and find out.

6.11.2 Transfer of Electrons

Electron transfer reactions are carried out by a wide variety of enzymes, coenzymes [NAD^+, FMN, FAD, coenzyme Q (ubiquinone)], non-heme iron-sulphur centres, cytochromes and metal ions such as Cu and Fe. The mitochondrial electron transport chain consists of four enzyme complexes embedded in the inner mitochondrial membrane plus two free electron carriers. Electrons enter the chain from carrier to carrier arranged in order of increasing redox potential, finally reducing oxygen to water, as you shall see in the next sub-section.

What are the components of the electron transport chain? Let's get to know next.

6.11.3 Components of Electron Transport Chain

The various components of the electron transport chain are illustrated in Figure 6.23.

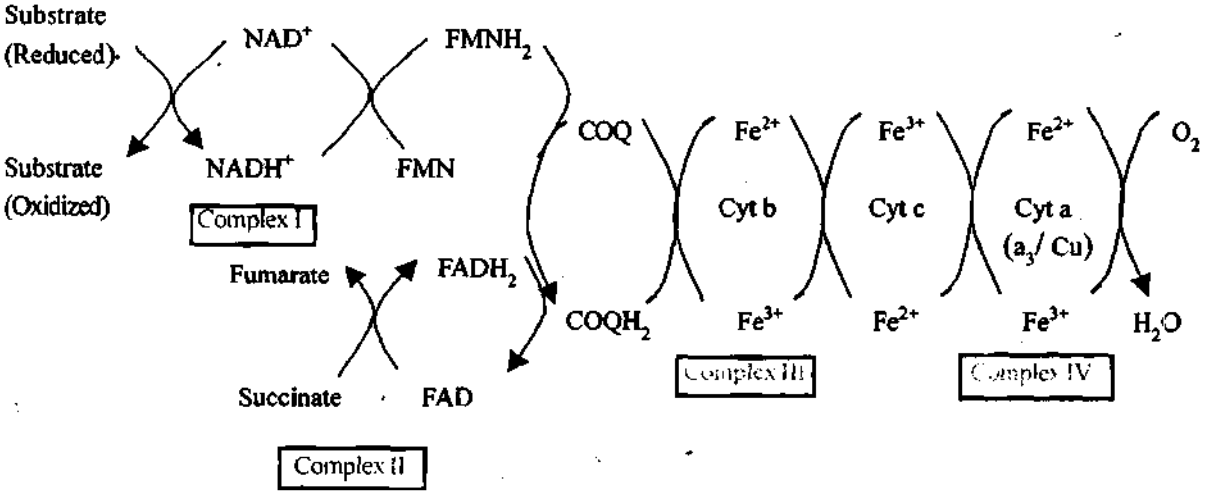

Figure 6.23 : Components of ETC

These components include:

Complex I: NADH ⟶ coenzyme Q (NADH coenzyme Q reductase) contains bound FMN in association with NADH dehydrogenases and Fe-S proteins. The two electrons from NADH first reduce FMN to $FMNH_2$, the Fe-S proteins are reduced next and finally coenzyme Q is reduced to the ubiquinol form.

Complex II: $FADH_2$ ⟶ coenzyme Q

Not all substrates are linked to the respiratory chain through the NAD-specific dehydrogenases. Some e.g. succinate/fumarate are linked directly to flavoprotein dehydrogenases, which in turn are linked to the cytochromes of the respiratory chain.

Succinate dehydrogenase and an Fe-S protein make up complex II (succinate:ubiquinone oxidoreductase). The $FADH_2$ component of succinate dehydrogenase reduces the Fe-S centre, which in turn reduces coenzyme Q to ubiquinol.

Complex III: Coenzyme Q ⟶ cytochrome C (cytochrome reductase)

This complex consists of cytochrome b, an Fe-S protein and cytochrome C (ubiquinone:ferricytochrome C reductase). Electrons are transferred one at a time from CoQ with the inner mitochondrial membrane. Coenzyme Q functions as a mediator between the two electron carriers (complex I and II) and one electron carrier (complex III and IV). Thus coenzyme Q links the flavoproteins to cytochrome b, the member of the cytochrome chain of lowest redox potential. Q exist in the oxidized quinine or reduced quinol form under aerobic and anaerobic conditions.

An additional component is the iron-sulphur protein (Fe-S; nonheme iron). It is associated with the flavoproteins and with cytochrome b. The sulphur and iron are thought to take part in the oxidoreduction mechanism between flavin and Q, which involved only a single electron change, the iron atom undergoing oxidoreduction between Fe^{2+} and Fe^{3+}.

Complex IV: cytochrome C ⟶ O_2 (cytochrome oxidase)

The cytochrome C oxidase complex is the last component in the electron transport chain (ferrocytochrome C:oxygen oxidoreductase). It accepts electrons from cytochrome C and catalyzes the four electron reduction of molecular oxygen to water.

$$4e^- + 4H^+ + O_2 \longrightarrow H_2O$$

Electrons flow from Q through the series of cytochrome in order of increasing redox potential to molecular oxygen. The terminal reducing cytochrome (cytochrome oxidase) responsible for the final combination of reducing equivalents with molecular oxygen has a very high affinity for oxygen, allowing the respiratory chain to function at maximum rate until the tissue becomes depleted of O_2. This is the only irreversible reaction in the chain and gives direction to the whole process.

Interestingly, there are a few inhibitors of the electron transport. These are discussed next.

6.11.4 Electron Transport Inhibitors

Several compounds, including specific drugs, chemicals and antibiotics have been known to inhibit the electron transfer reactions at specific sites of the electron transport chain, thereby making the ETC non functional. Some of these inhibitors are amytal, antimycin A and cyanide. The sites of inhibition are shown in Figure 6.24.

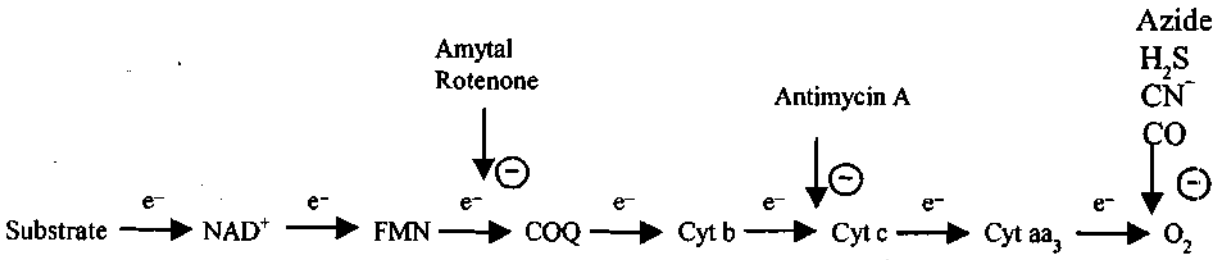

Figure 6.24 : Site specific inhibition of ETC

6.11.5 Oxidative Phoshorylation

Oxidative phosphorylation, you already know, is the process by which ADP is phosphorylated by Pi to ATP in the respiratory chain. Oxidative phosphorylation is coupled to oxidation. The phosphorylation reaction is associated with complex V which synthesises ATP utilizing the entry of the proton gradient generated by the electron transport chain from complexes I, III and IV. These complexes (I, III and IV) are also called as phosphorylation sites I, II and III, respectively as illustrated in Figure 6.25. Two electrons are required to reduce one atom of oxygen to H_2O. Therefore the oxidation of one molecule of NADH or $FADH_2$ corresponds to the synthesis of three or two ATPs, respectively and to the reduction of atom of oxygen. It is usually stated, the oxidation of NADH or $FADH_2$ occurs with P/O ratio of 3 and 2 respectively.

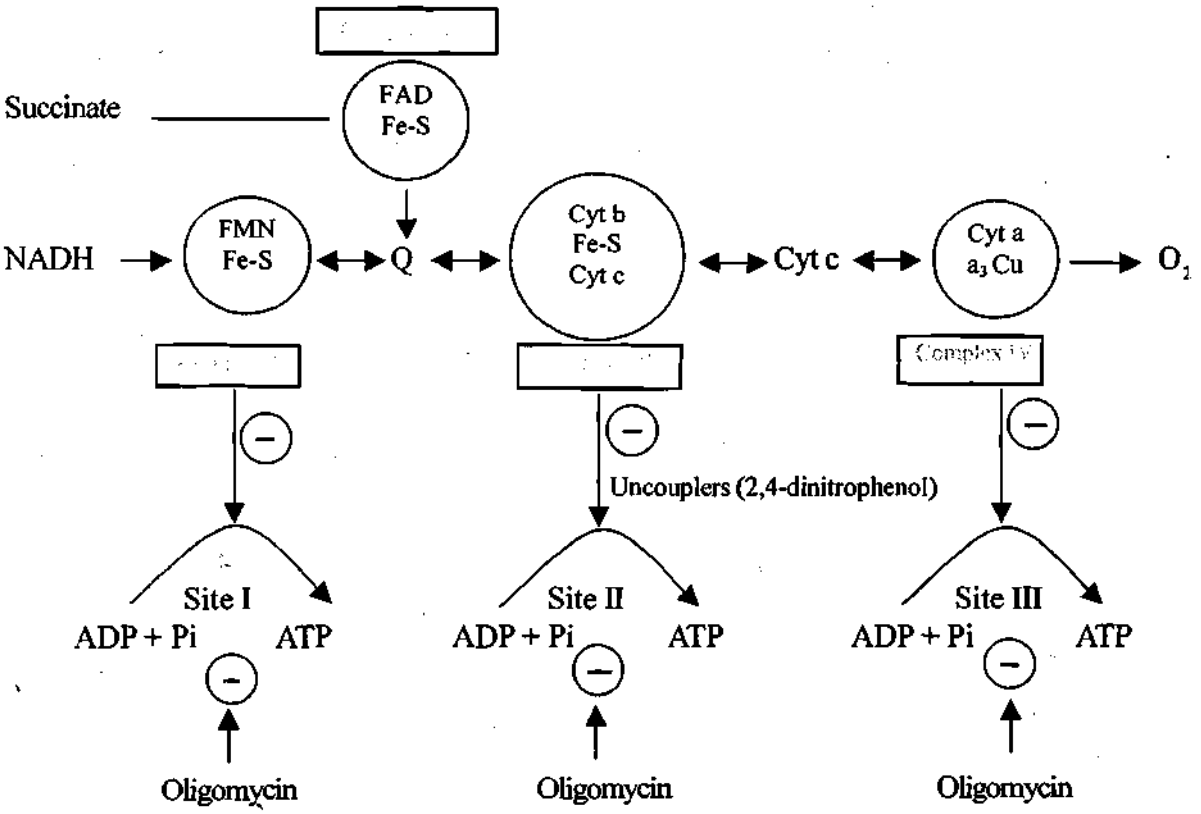

Figure 6.25 : Oxidative phosphorylation sites and action of various inhibitors

Check Your Progress Exercise 7

1) Name the subcellular particle where the electron transport chain is located.

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2) List the components of electron transport chain.

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3) What is oxidative phosphorylation? The oxidation of one molecule of NADH or $FADH_2$ corresponds to the synthesis of how many ATPs?

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6.12 LET US SUM UP

In this unit we studied about carbohydrate metabolism. We learnt that glucose is the principal carbohydrate fuel for the body. Other monosaccharides, such as fructose, galactose, and mannose from the diet are converted to glucose. Glucose in the cell enters any one of the pathways such as glycolysis, glycogenesis, glycogenolysis etc., depending on the cellular requirement of glucose. Excess glucose is converted to fat and stored in adipose tissue. Further, various monosaccharides with atoms ranging from 3 carbons to 7 carbons are all converted to glycolytic pathway intermediates via the HMP shunt pathway. Blood glucose level is maintained within the normal range both during fasting and post-prandial state through the concerted action of several key enzymes and hormones. Several glycogen storage diseases have been characterized due to the deficiency of key enzymes of glycogen metabolism.

The last part of the unit focussed on the mitochondrial electron transport. The mitochondrial electron transport chain, we learnt, mediates the reduction of molecular oxygen to water by NADH and $FADH_2$. The transport of electrons through the electron transport chain makes available a significant quantity of free energy which is used in the synthesis of ATP in the process of oxidative phosphorylation.

6.13 GLOSSARY

Glycolysis : degradation of glucose.

Gluconeogenesis : synthesis of glucose from non-carbohydrate source.

Glycogenesis : synthesis of glycogen.

Glycogenolysis : degradation of glycogen.

Electron transport chain : transport of high-energy electrons through a series of carriers in mitochondria.

Oxidative phosphorylation : synthesis of ATP from ADP and Pi during the passage of electrons in the respiratory chain.

6.14 ANSWERS TO CHECK YOUR PROGRESS EXERCISES

Check Your Progress Exercise 1

1) The three irreversible reactions in the glycolytic pathway are :

   Glucose → Glucose-6-phosphate

   Fructose → Fructose-1,6-bisphosphate

   Phosphoenol pyruvate → Pyruvate

2) Phosphorylation of glucose: Glucose is converted to glucose-6-phosphate since phosphorylated intermediates do not readily penetrate cell membrane and this commits glucose to further metabolism in the cell. Hexokinase catalyses this irreversible reaction in most tissues and in liver glucokinase is the predominant enzyme for the phosphorylation of glucose. Hexokinase is an allosteric enzyme that is strongly inhibited by the product, glucose-6-phosphate.

3) Fructose-2,6-bisphosphate is the most important allosteric effector of glycolysis in the liver.

4) The energy production in glycolysis can be explained as followed :

1)	Glyceraldehyde-3-phosphate ⟶ 1,3-Bisphosphoglycerate	6
2)	1,3-Bisphosphoglycerate ⟶ 3-Phosphoglycerate (phosphorylation)	2
(3)	Phosphoenol pyruvate ⟶ Enol pyruvate (phosphorylation)	2

ATP utilized reactions

1)	Glucose ⟶ Glucose-6-phosphate	1
2)	Fructose-6-phosphate ⟶ Fructose-1,6-bisphosphate	1
Therefore Net ATP generated		**8**

Check Your Progress Exercise 2

1) Pyruvate dehydrogenase complex acts on pyruvate in mitochondria and converts it to acetyl CoA. Pyruvate dehydrogenase, dihydrolipoyl transacetylase and dihydrolipoyl dehydrogenase are its components. The cofactors associated with it are NAD, FAD, TDP, CoA and lipoic acid.

2) PDH exists in 2 forms : Inactive, phosphorylated and Active, dephosphorylated. The active form of PDH is phosphorylated by a protein kinase with the help of ATP and Mg^{++} to the inactive form of PDH. Acetyl CoA and NADH are activators for this action and CoA, NAD^+, and pyruvate are inhibitors. The inactive form of PDH is dephosphorylated by phosphoprotein phosphatase to the active form in the presence of increased Ca^{++} ion concentration.

Check Your Progress Exercise 3

1) The function of the citric acid cycle can be discussed as follows. The intermediates of citric acid cycle are used as precursors in the biosynthesis of many compounds like synthesis of glucose from carbon skeletons of amino acids, and providing building blocks for heme synthesis. The cycle provides a means for the degradation of two carbon acetyl residues which are derived from carbohydrates, fatty acid and amino acids. Further, the TCA cycle generates ATP by oxidative phosphorylation when electrons generated in the cycle are transferred to the electron transport chain.

2) Oxidation of one NADH + H^+ by the electron transport chain leads to formation of 3ATP, whereas oxidation of $FADH_2$ yields 2 ATP.

Isocitrate ⟶ α-ketoglutarate (NADH⟶NAD^+)	3
α-ketoglutarate⟶ Succinyl CoA (NADH ⟶NAD^+)	3
Succinyl CoA⟶Succinate (ADP⟶ATP or GDP ⟶ GTP)	1
Succinate⟶Fumarate ($FADH_2$ ⟶FAD)	2
Malate⟶Oxaloacetate (NADH⟶NAD)	3

Thus, 12 molecules of ATP are produced from oxidation of one molecule of acetyl CoA (using both substrate level and oxidative phosphorylation).

3) GTP or ATP is the high-energy complex generated during the conversion of succinyl Co[illegible]cinate in the citric acid cycle.

4) Anaplerotic r[illegible] are reactions that replenish the intermediates of citric acid cycle.

Check Your Progress Exercise 4

1) Gluconeogenesis (i.e synthesis of new glucose) is the synthesis of carbohydrate from non-carbohydrate source. The significance of gluconeogenesis include: (any two of the following)

- During starvation or during periods of limited carbohydrate intake, when the levels of liver glycogen are low, gluconeogenesis is important in maintaining adequate blood sugar concentration since a continual supply of glucose in necessary as a source of energy for the nervous system and the erythrocytes.
- Even when most of the energy requirement of the organism is met by the supply of fat, there is always a certain basal requirement for glucose which is provided by gluconeogenesis.
- During extended exercise, when high catecholamine levels have mobilized carbohydrate and lipid reserves, the gluconeogenic pathway allows the use of lactate from glycolysis and of glycerol from fat break down.
- During metabolic acidosis, gluconeogenesis in the kidney allows the excretion of an increased number of protons.
- Gluconeogenesis also allows the use of dietary protein in carbohydrate pathway after disposal of the amino acid nitrogen as urea.
- Gluconeogenesis is important to human beings everyday, making it possible for us to make it through the night and from meal to meal without nibbling on a source of carbohydrate continuously.

2) The major substrates for gluconeogenesis are the glucogenic amino acids, lactate glycerol and (important in ruminant) propionate.

3) a) Alanine Cycle : In the alanine cycle the pyruvate formed from glycolysis in the muscle is converted to alanine by transamination reaction. Alanine is released by the muscle into the blood and is taken up by the liver. In the liver, alanine is converted back to pyruvate by the reverse of the transamination reaction that occurred in the muscle. Pyruvate is converted to glucose via gluconeogenic pathway.

b) The Cori cycle : In Cori cycle, the pyruvate formed from glucose is converted to lactate by *lactate dehydrogenase* in the muscle cell. Lactate is released into the blood and taken up by the liver. Lactate is converted to pyruvate by the isoenzyme of *lactate dehydrogenase*. Pyruvate is converted to glucose by gluconeogenic mechanism in the liver and released into the blood where it can be used as energy source for muscle and other tissue.

4) The reactions that are circumvented include:

- Between pyruvate and phosphoenolpyruvate (PEP)
- Between fructose 1,6 bisphosphate and fructose 6-phosphate
- Between glucose-6-phosphate and glucose, and
- Between glucose-1-phosphate and glycogen.

Check Your Progress Exercise 5

1) The synthesis of glycogen in liver and muscle is called glycogenesis. The breakdown of glycogen in the liver (glycogen $\longrightarrow$ glucose) and muscle (glycogen $\longrightarrow$ glucose-1-phosphate) is called glycogenolysis.

2) There is a hormonal regulation system functioning in the muscle and liver which regulates the glycogen metabolism. In addition to the hormonal regulation of glycogen metabolism, the role of covalent modification in regulation is also important.

3) Glycogen storage diseases are caused by genetic defects that result in deficiencies in certain enzymes of glycogen metabolism. These deficiencies lead to excessive accumulation of glycogen and / or the inability to use that glycogen as a fuel source. The structure of glycogen may also be abnormal.

4) Glucagon in liver and epinephrine in liver and muscle stimulate glycogenolysis.

Check Your Progress Exercise 6

1) The HMP is an alternate oxidative pathway for the metabolism of glucose. The significance of the pathway is that NADPH is utilized for the synthesis of fatty acids, cholesterol, steroids and also in the synthesis of amino acids via glutamate dehydrogenase outside the mitochondria. Lipogenesis also posses the pentose sugars produced in HMP shunt are utilized for the synthesis of nucleic acids and nucleotides, and CO_2, produced in this pathway, is used for the synthesis of fatty acids and purine bases.

2) The oxidative branch of the pathway generates NADPH and pentose-5 phosphate, through the following reactions:

i) Glucose-6-phosphate is dehydrogenated to 6-phosphogluconate via 6-phosphoglucaono-lactone by glucose-6-phosphate dehydrogenase in presence of $NADP^+$ and the cofactors Mg^{++}, Mn^{++} or Ca^{++}.

ii) 6-phosphogluconate is oxidized by 6-phosphogluconate dehydrogenase in the presence of coenzyme $NADP^+$ and cofactors Mg^{++}, Mn^{++} or Ca^{++} to 3-keto 6 phosphogluconate which is decarboxylated to form ribulose-5-phosphate.

3) The fragility of erythrocytes is impaired in the absence of NADPH generation due to the deficiency of glucose-6-phosphate dehydrogenase thereby causing hemolytic anaemia when the red blood cells are subjected to certain drugs such as primaquine and sulphonamide. An inverse correlation has been found between the activity of glucose-6-phosphate dehydrogenase and the fragility of red cells (i.e. susceptibility to hemolysis).

4) In the fed state, clearance of blood glucose is mainly by liver via glucokinase. Further, uptake of glucose also takes place in the extra-hepatic tissues (such as muscle, adipose tissue etc) favoured by insulin. These two mechanisms regulate the blood glucose level in the fed state. While in the fasting state, glucose release from the liver increases due to the action of glucagon and in the muscle by epinephrine. Glucagon also enhances gluconeogenesis from amino acids and lactate. Both hepatic glycogenolysis and glyconeogenesis contribute to the hyperglycaemic effect of glucagon. Through these mechanisms, the blood glucose level is maintained in the fasting state.

Check Your Progress Exercise 7

1) Mitochondria is the subcellular particle where the electron transport chain is located.

2) The components of electron transport chain includes

Complex I : NADH ⟶ coenzyme Q (NADH coenzyme Q reductase) contains bound FMN in association with NADH dehydrogenases and Fe-S proteins.

Complex II : $FADH_2$ ⟶ coenzyme Q succinate dehydrogenase and an Fe-S protein make up complex II (succinate:ubiquinone oxidoreductase). The $FADH_2$ component of succinate dehydrogenase reduces the Fe-S centre, which in turn reduces coenzyme Q to ubiquinol.

Complex III: Coenzyme Q ⟶ cytochrome C (cytochrome reductase)

This complex consists of cytochrome b, an Fe-S protein and cytochrome C (ubiquinone:ferricytochrome C reductase). An additional component is the iron-sulphur protein (Fe-S; nonheme iron)). It is associated with the flavoproteins and with cytochrome b.

Complex IV: cytochrome C ⟶ O_2 (cytochrome oxidase)

The cytochrome C oxidase complex is the last component in the electron transport chain (ferrocytochrome C:oxygen oxidoreductase).

3) Oxidative phosphorylation is the process by which ADP is phosphorylated by Pi to ATP in the respiratory chain. Two electrons are required to reduce one atom of oxygen to H_2O. Therefore the oxidation of one molecule of NADH or $FADH_2$ corresponds to the synthesis of three or two ATPs, respectively and to the reduction of atom of oxygen. The oxidation of NADH or $FADH_2$ occurs with P/O ratio of 3 and 2 respectively.