UNIT 3

CARBOHYDRATE METABOLISM

Gustav Embden, Otto Meyerhof, and Jakub Karol Parnas.

Gustav Embden (German physiological chemist), Otto Meyerhof (German physician and biochemist), and Jakub Karol Parnas (Jewish-Polish–Soviet biochemist) studied the carbohydrate metabolism and fully explained the reactions of glycolysis. They have combined results of many smaller experiments carried out by other scientists to explain the pathway as a whole. It took almost a century to fully discover the glycolytic pathway. Otto Meyerhof was awarded the Nobel Prize in Physiology and Medicine, with Archibald Vivian Hill, for his work on muscle metabolism, including glycolysis.

5UMM37

Learning Objectives

After studying this unit the students will be able to

- Describe the metabolic processes catabolism and alnabolism.
- Describe the reactions of glycolysis and gluconeogenesis.
- Describe the reactions of Hexose mono-phosphate shuttle pathway and TCA Cycle.
- Synthesis of glucose from non – carbohydrate sources
- Discuss the types, symptoms and cases of diabetes.
- Explain the protocol to determine blood glucose level.

INTRODUCTION

3.1 OVERVIEW OF METABOLISM

We have already learnt in XI standard about the structures and functions of various biomolecules such as proteins, amino acids, carbohydrates etc... We know that biomolecules are important for the normal functioning of any living system. These biomolecules can be obtained either directly from the diet or synthesised in cells. In the next few chapters, we will study the synthesis of various biomolecules and their degradation. These biochemical studies are termed as metabolism. Metabolism consists of series of connected enzymatic reactions that produce specific products. The different species involved in these reactions are referred to as metabolites. Photosynthesis is an example of carbohydrate metabolism in which a series of chemical reactions convert carbon dioxide and water to glucose. In the next few chapters, we will study about the metabolism of various biomolecules such as proteins, lipds etc. Figure 3.1 gives the overview of the various metabolic processes.

We learnt in the previous unit, that digestion of carbohydrates produce three major products namely glucose, galactose and fructose. These monosaccharides are absorbed into the blood stream. After their absorption, monosaccharides are transported to the liver, where fructose and galactose are rapidly converted into glucose.

In this unit we will study the metabolism of carbohydrates. Carbohydrates are simpler when compared to other biomolecules such as nucleic acids, amino acids etc... and their metabolism supplies more than half of the energy requirements of the body. In fact, our brain mainly depends upon carbohydrate metabolism as a source of energy.

3.1.1 Catabolism and anabolism

Every cell contains thousands of reactions comprising many metabolic pathways. There are two types of metabolic reactions namely anabolism and catabolism. The process of synthesising the required larger biomolecules from the small molecules is called anabolism. These reactions use up energy for example, in photosynthesis, small molecules CO_2 and water are converted into a six carbon carbohydrate. In this reaction sun light provides the energy.

$$6CO_2 + 6H_2O + 2800kJ \xrightarrow[Chlorophyll\&Enzymes]{Sunlight} C_6H_{12}O_6 + 6O_2$$

Catabolism is a reverse process of anabolism, in which bigger biomolecules are broken into simple molecules. Let us consider the oxidation of glucose,

$$C_6H_{12}O_6 + 6O_2 \rightarrow 6CO_2 + 6H_2O + 2800kJ$$

The above reaction is the reversal of photosynthesis reaction in which large (6C glucose) molecules are broken into small $CO_2$and H_2O molecules. If the glucose is burnt in the lab we get CO_2 & water and large amount of energy is released. Whereas, in living cell, the oxidation of glucose proceeds through a series of enzyme-catalysed intermediate reactions and the part of the energy produced in these reactions is stored in the chemical bonds of ATP (Adenosine triphosphate). When energy is needed, for example during the muscle contraction, ATP is utilised.

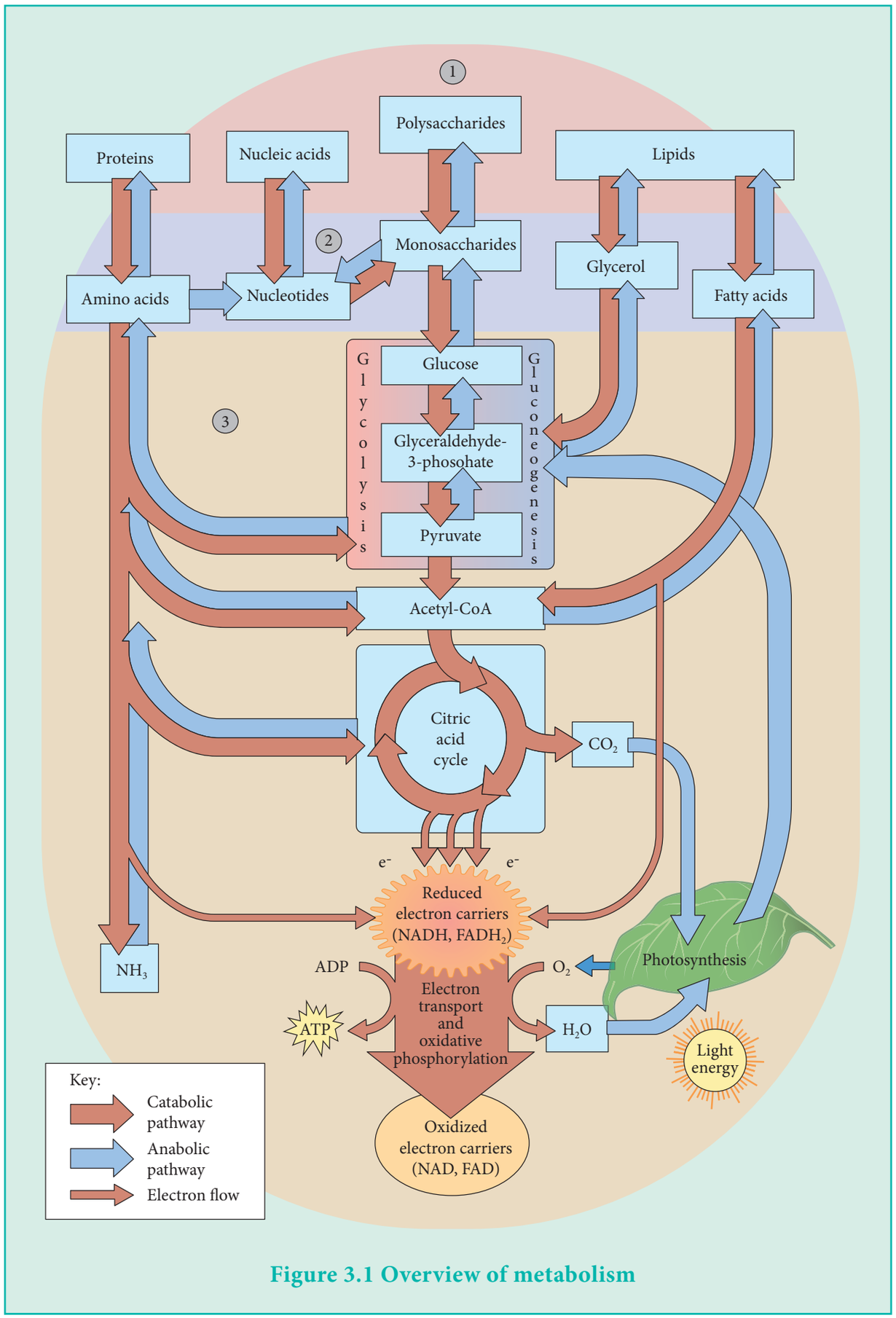
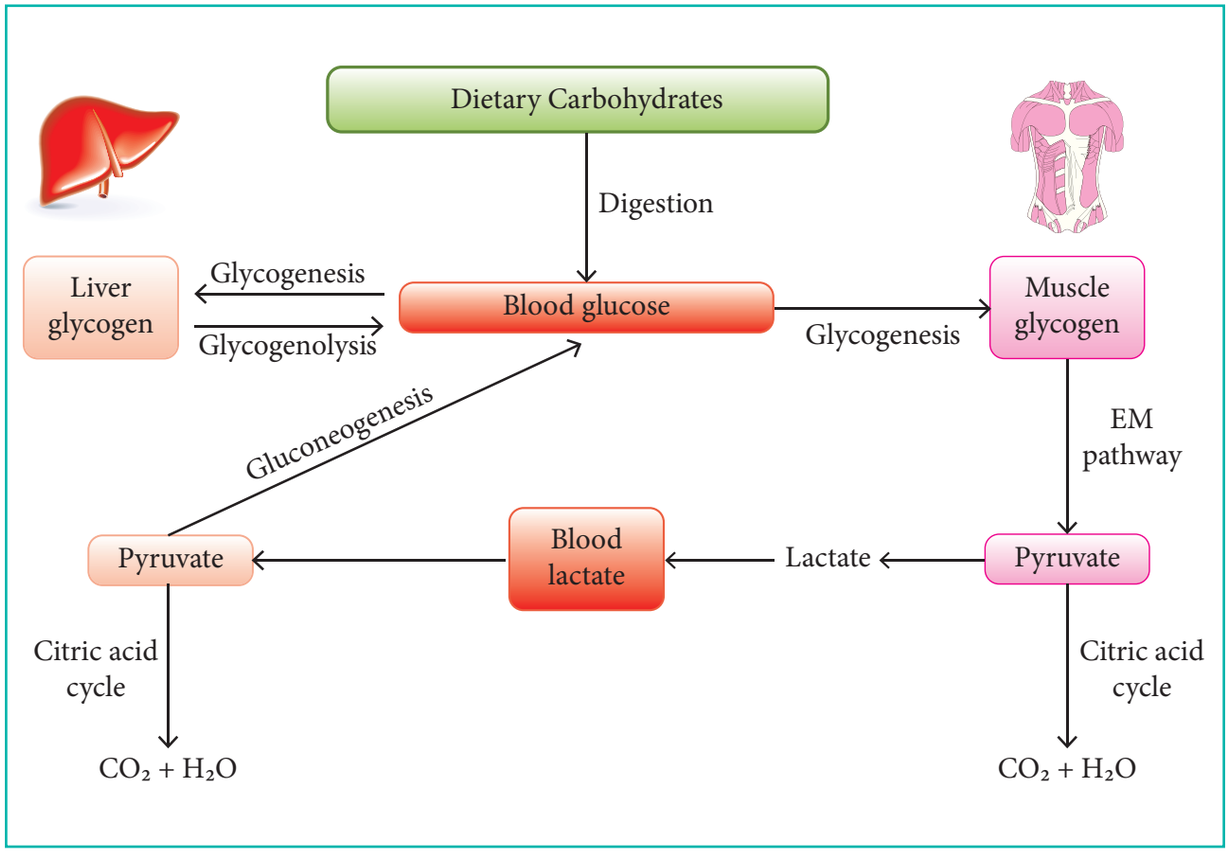


Figure 3.1 Overview of metabolism

3.2 CARBOHYDRATE AS A SOURCE OF ENERGY

The major function of carbohydrate is to serve as a fuel. The catabolic enzymatic oxidation of glucose provides energy and the metabolic intermediates are used in various biosynthetic reactions. We require energy to perform any work such as playing. Reading, etc., Carbohydrate metabolism provides us the required energy. Glucose is oxidized via EMP pathway (Embden - Meyerhodf - Parnas) also known as glycolysis. During glycolysis, glucose is converted into pyruvate. Pyruvate is then converted into acetyl CoA which is fully oxidised to carbon dioxide via the citric acid cycle in the presence of oxygen (aerobic conditions). Under oxygen limiting conditions the pyruvate is converted into lactate. Lactate is then transported to liver where it is metabolised further. Glucose can be synthesised from pyruvate by gluconeogenesis pathway which will be discussed later in detail.



The liver maintains the glucose level in the blood. When blood glucose is in excess, it is converted to glycogen in the liver and muscle tissue. The synthesis of glycogen form glucose is called **glycogenesis**. In addition, glycolytic pathway is also accelerated.

When the glucose level is low, the glycogen is hydrolysed to produce glucose. The hydrolysis of glycogen to glucose is called **glycogenolysis**. The glucose level is also increased by activating the gluconeogenesis pathway.

3.3. GLYCOLYSIS

Glycolysis is an oxidative metabolic process in which one mole of glucose is converted into two moles of pyruvate with the generation of chemical energy in the form of ATP in a series of ten enzyme catalysed reactions. In this process the oxidising agent is coenzyme NAD^+. No molecular oxygen is utilised in glycolysis and hence it is an anaerobic pathway.

Under aerobic conditions, the pyruvate formed by glycolysis is further oxidized to CO_2 in the citric acid cycle. Whereas, in anaerobic conditions, the pyruvate is converted to lactate. This process occurs in the cytoplasm of all the cells.

3.3.1. Reactions of glycolysis pathway

There are two stages in the glycolysis namely preparatory stage and pay off stage.

Preparatory Stage:

It consists of first five reactions in which one molecule of glucose is converted into two molecules of glyceraldehyde-3-phosphate. This stage is an energy consuming stage in which two ATP molecules are utilized.

Pay off Stage:

It consists of the remaining five reactions in which the glyceraldehyde-3-phosphoate is converted into pyruvate. This is an energy producing stage in which two molecules of ATP are produced from ADP, per glyceraldehyde-3-phosphate. i.e. for two glyceraldehyde-3-phosphate molecule, totally four ATP molecules are produced.

In the overall glycolysis process there is a net gain of two ATP molecules for conversion of every glucose molecules into pyruvate. The overall process can be represented by the following equation.

$$\text{Glucose} + 2NAD^+ + 2ADP + 2P_i \rightarrow 2 \text{ pyruvate} + 2NADH + 2\ ATP + 2H^+ + 2H_2O$$

Reactions of Preparatory stage:

Step 1: Glucose is phosphorylated by the transfer of a phosphate group from ATP molecule to form glucose-6-phosphate and ADP. This step is energy consuming step and the required energy is provided by the breakdown of the ATP molecule. Glucose-6-phosphate is more reactive than glucose and also cannot cross the cell membrane. Hence it is trapped inside the cell. This step is catalysed by hexakinase enzyme.

Step 2: Glucose-6-phosphate is isomerised to form fructose-6-phosphate. This step is catalysed by the enzyme phosphogluco isomerase

Step 3: Like the first step the fructose-6-phosphate is phosphorylated again by ATP to form fructose-1,6-bisphosphate. This step is catalyzed by the enzyme phosphofructokinase.

Step 4: In this step, the six carbon, fructose-1,6-bisphosphate splits to form two three-carbon sugars namely dihydroxyacetone phosphate (DHAP) and glyceraldehyde-3-phosphate. They are isomers of each other. This step is catalysed by the enzyme aldolase.

Step 5: The interconversion of DHAP and glyceraldehyde-3-phosphate is catalysed by triose phosphate isomerase enzyme. The two molecules exist in equilibrium, but the equilibrium shifted towards the formation of glyceraldehyde-3-phosphate as it is consumed in subsequent steps of glycolysis. Eventually as the glycolysis continues, all of the DHAP is converted into glyceraldehyde-3-phosphate(Figure 3.3).

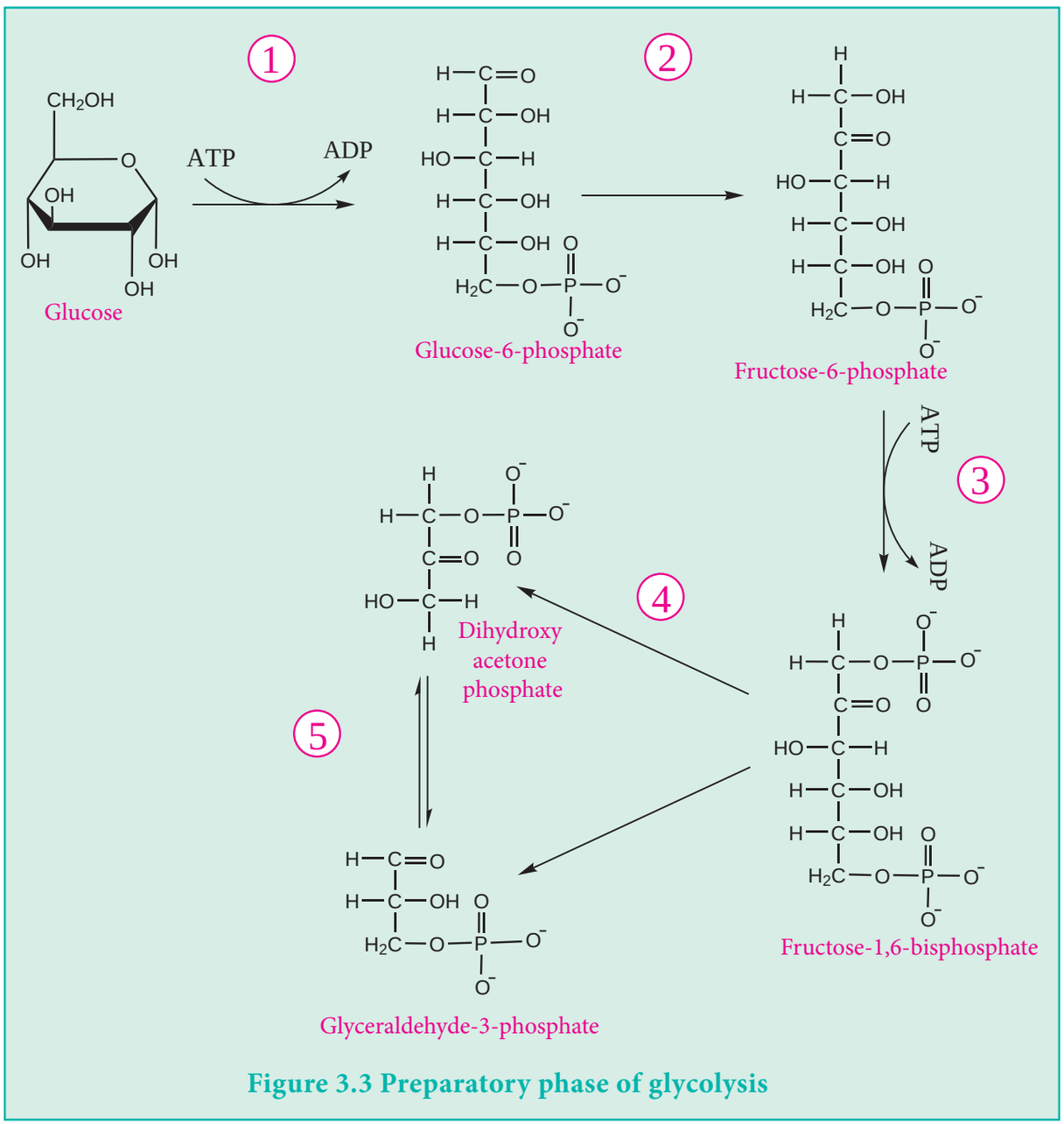


Figure 3.3 Preparatory phase of glycolysis

Reactions of Payoff phase:

Step 6: Glyceraldehyde-3-phosphate is oxidized by NAD^+ which gets reduced to NADH and H^+. This reaction is exergonic, releasing energy which is utilised to phosphorylate the Glyceraldehyde-3-phosphate to form high energy molecule 1,3-bisphosphoglycerate. Here the inorganic phosphate (P_i) is used for phosphorylation. Glyceraldehyde-3-phosphate dehydrogenase catalyses this step.

Step 7: The 1,3-bisphosphoglycerate is converted into 3-phosphoglycerate transfering one of its phosphate group to ADP to form ATP. The enzyme involved in this process is phosphoglycero kinase

Step 8: 3-Phosphoglycerate is isomerised to 2-phosphoglycerate by the enzyme phosphoglycerate mutase

Step 9: 2-Phosphoglycerate loses a molecule of water, becoming phosphoenolpyruvate (PEP). This step is catalysed by enolase enzyme. PEP is an unstable high energy molecule, poised to lose its phosphate group in the final step of glycolysis.

Step 10: In the final step, PEP readily loses its phosphate group to ADP and forms pyruvate, the end product of glycolysis. In this reaction one molecule of ATP is formed. This reaction is catalysed by pyruvate kinase (Figure 3.4).

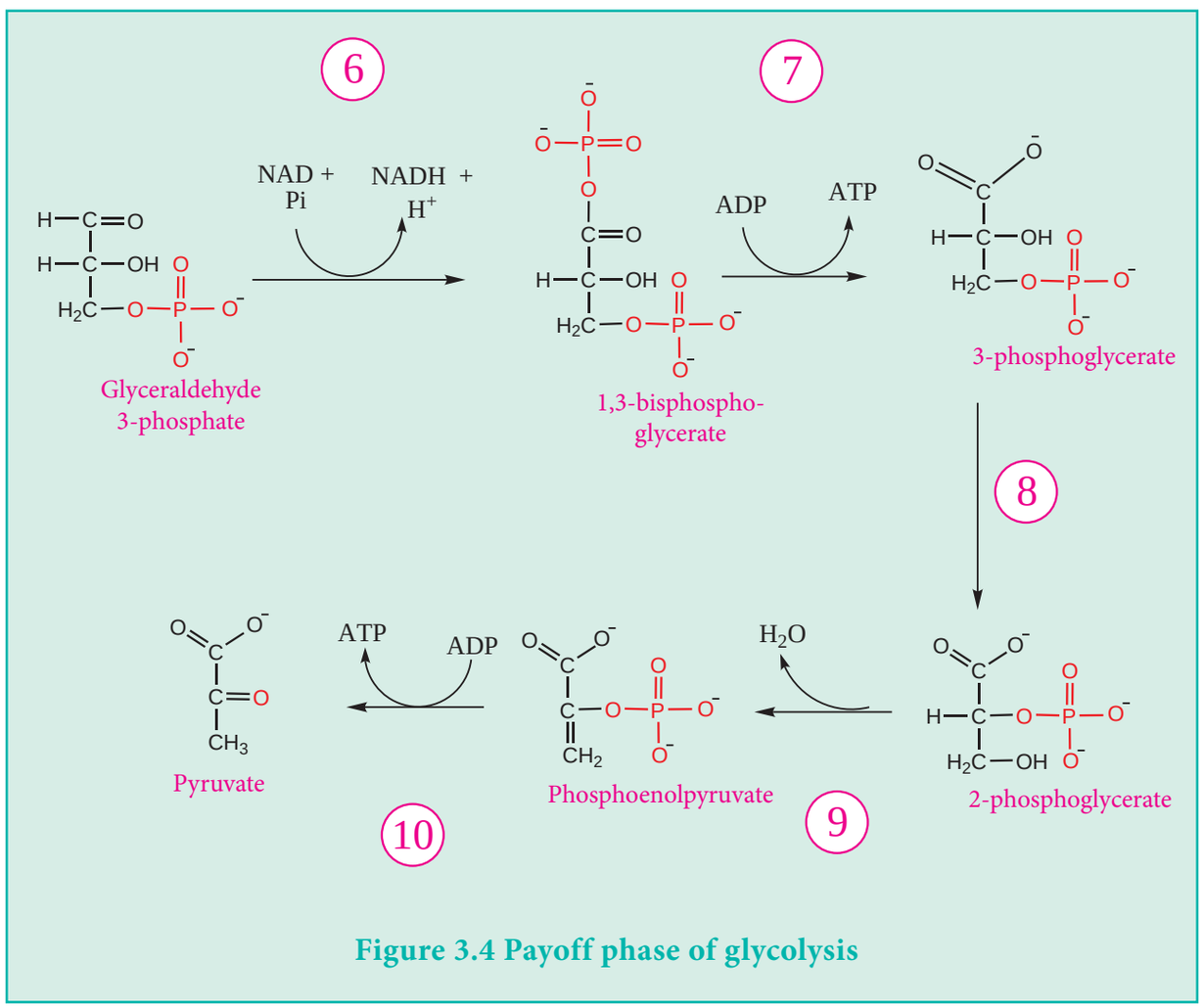
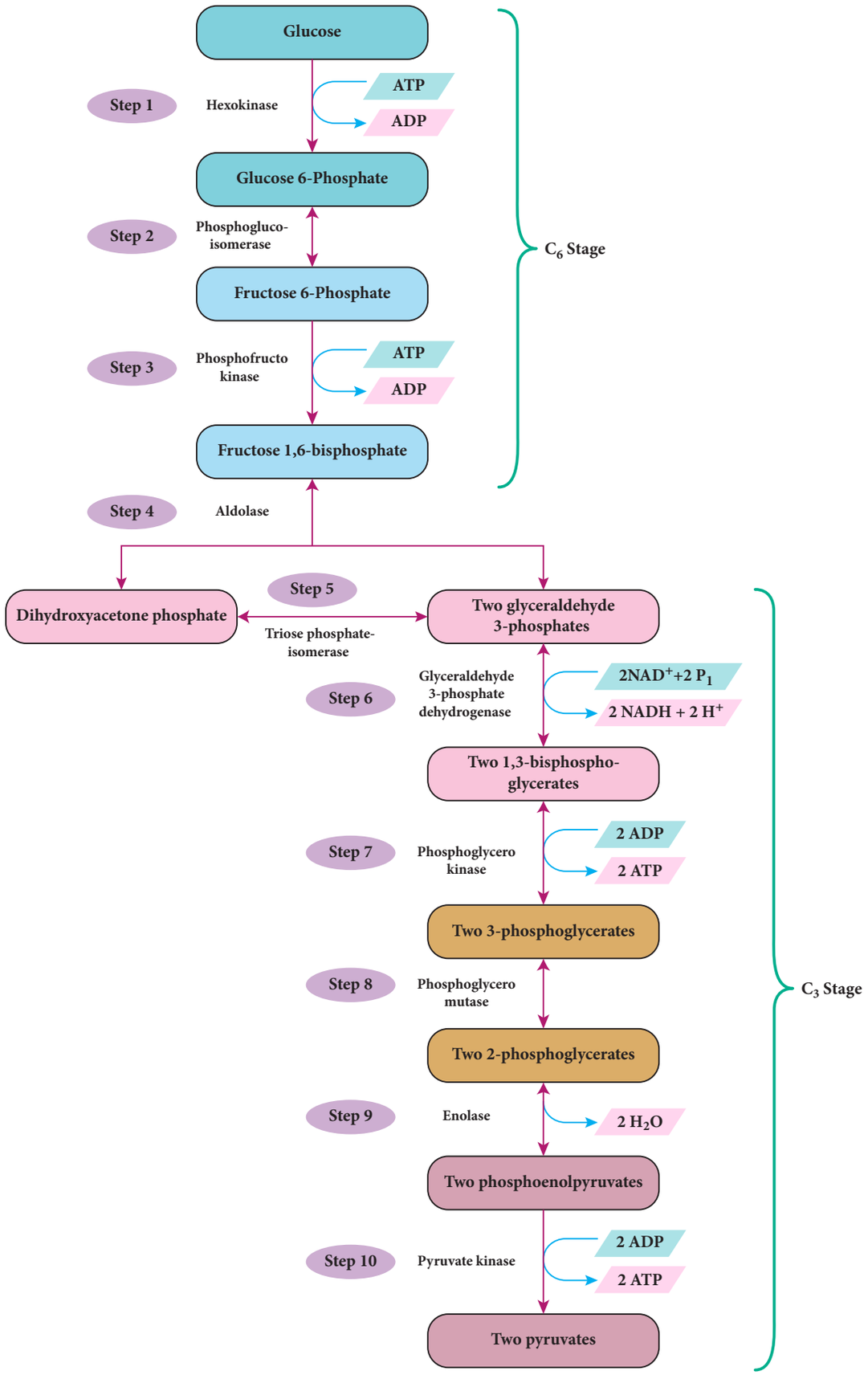


Figure 3.4 Payoff phase of glycolysis

Glycolysis



3.3.2. Summary of glycolysis

At the end of glycolysis, one glucose molecule is converted into two pyruvate molecules. In addition we also have two ATP, two NADH molecules formed from ADP and NAD^+ molecules respectively. To regenerate the coenzyme NAD^+, NADH must be re-oxidised. If oxygen is available, the pyruvate is oxidized all the way to carbon dioxide in cellular respiration, regenerates NAD^+. During this process more ATP molecules are produced. Under anaerobic conditions it is converted to lactate by lactate dehydrogenase enzyme and regenerates NAD^+. Here no further ATP molecules are produced.

3.3.3. Energetics of glycolysis

During the glycolysis of one molecule of glucose, two molecules of ATP and two molecules of NADH are produced. The ATP molecules serves as energy source. The NADH molecules are oxidised in mitochondria producing more number of ATP molecules (approximately 3 ATPs per NADH molecule).

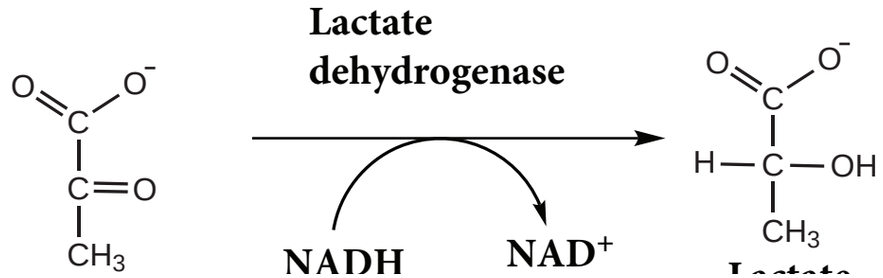
Step No.	Description	Number of ATP/ NADH formed/ consumed
6	Formation of 1,3-bisphospho glycerate from glyceraldehyde 3-phosphate	2 NADH
7	Formation of 3 phosphoglycerate from 1,3 bisphospho glycerate	2 ATP
10	Formation of pyruvate from phosphoenol pyruvate	2 ATP
1,3	Formation of Glucose-6-phosphate & Fructose-1,6-bisphosphate	2 ATP (consumed)
Number of net ATP molecules generated per one molecule of glucose		2 ATP
Number of net NADH molecules generated one molecule of glucose		2 NADH

3.3.4. Glycolysis under anaerobic condition:

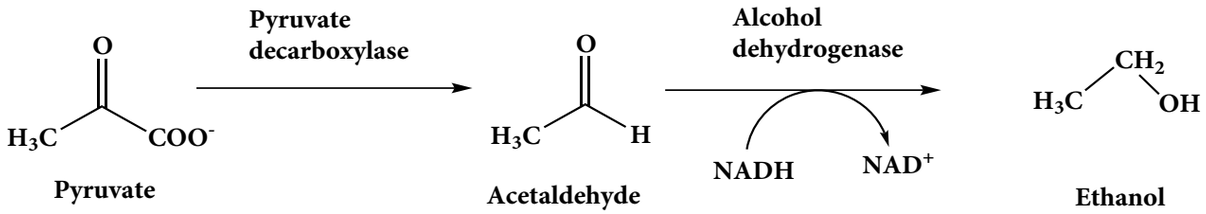
Some organisms are able to continually convert energy without the presence of oxygen. They undergo glycolysis, followed by the anaerobic process to produce ATP.

As discussed earlier anaerobic glycolysis is the transformation of glucose to lactate when limited amounts of oxygen (O_2) exist.

Muscle cells can continue to produce ATP when oxygen runs low using lactic acid fermentation. However, this often results in muscle fatigue and pain due to accumulation of lactate. When sufficient oxygen is not present in the muscle cells, the pyruvate, the terminal electron acceptor is converted to lactate by the enzyme lactate dehydrogenase, regenerating NAD^+.



Many yeasts convert glucose to ethanol and CO_2 rather than pyruvate using alcoholic fermentation process. In this process, pyruvate is first converted to acetaldehyde by enzyme pyruvate decarboxylase in the presence of thiamine pyrophosphate and Mg^{2+}. Carbon dioxide is released during this reaction. Acetaldehyde is then converted to ethanol by the enzyme alcohol dehydrogenase during which the NADH is oxidized to NAD^+.



3.4. TRICARBOXYLIC ACID CYCLE:

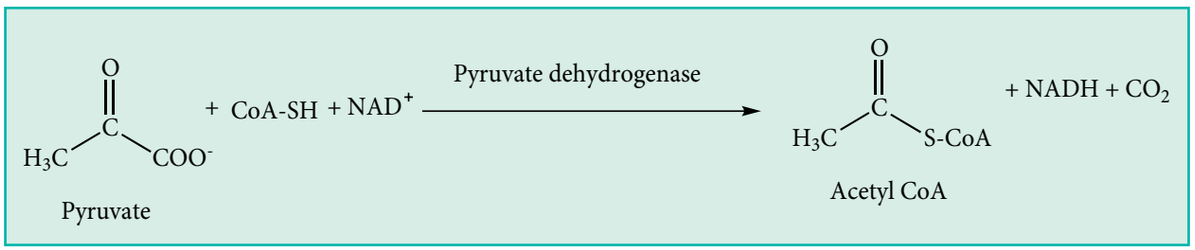
Citric acid cycle is the common mode of oxidative degradation in eukaryotes and prokaryotes. This process occurs in the mitochondria. This cycle, which is also known as the tricarboxylic acid (TCA) cycle or Krebs cycle, is the central driver of cellular respiration. It accounts for the major part of oxidation of carbohydrate, fatty acid & amino acid and it also generates many biosynthetic precursors. The citric acid cycle is amphibolic, i.e. it operates both catabolically and anabolically.

Unlike glycolysis, the citric acid cycle is a cyclic process. i.e. the last reaction of the pathway regenerates the compound utilised in the first reaction. The citric acid cycle produces only one GTP molecule (ATP equivalent) directly and does not directly consume oxygen.

3.4.1. Reactions of tricarboxylic acid cycle

TCA cycle takes acetyl CoA produced by the oxidation of pyruvate (derived from glucose and other catabolic processes) as its starting material and in a series of reactions, harvests much of its bond energy in the form NADH, $FADH_2$ and GTP molecules. The reduced electron carriers NADH and $FADH_2$ generated in the TCA cycle, will pass their electrons into the electron transport chain resulting in oxidative phosphorylation, which will generate most of the ATP produced in cellular respiration.

Prior to the start of the first step, a transitional phase occurs during which pyruvate is converted to acetyl CoA. This process occurs in the mitochondrial matrix and provides a link between the glycolysis and the TCA Cycle which consists the following eight steps.



Step 1: In the first step of the cycle, the acetyl CoA (two-carbon) reacts with an oxaloacetate molecule (four-carbon) to form citrate (six-carbon) in a condensation reaction. This reaction is catalysed by the enzyme citrate synthase. The CoA is bound to a thiol group (-SH) and diffuses away to eventually combine with another acetyl group. This step is irreversible because it is highly exergonic. The rate of this reaction is controlled by negative feedback and the amount of ATP available. If ATP levels increase, the rate of this reaction decreases. If ATP is in short supply, the rate increases.

Step 2: In second step, citrate is converted into its isomer, isocitrate, by the enzyme aconitase.

Step 3: In the third step, the isocitrate is oxidized, to produce a five-carbon molecule, α-ketoglutarate releasing a molecule of CO_2. In this process two electrons are also released which reduce a NAD^+ molecule to NADH. This step is catalysed by isocitrate dehydrogenase.

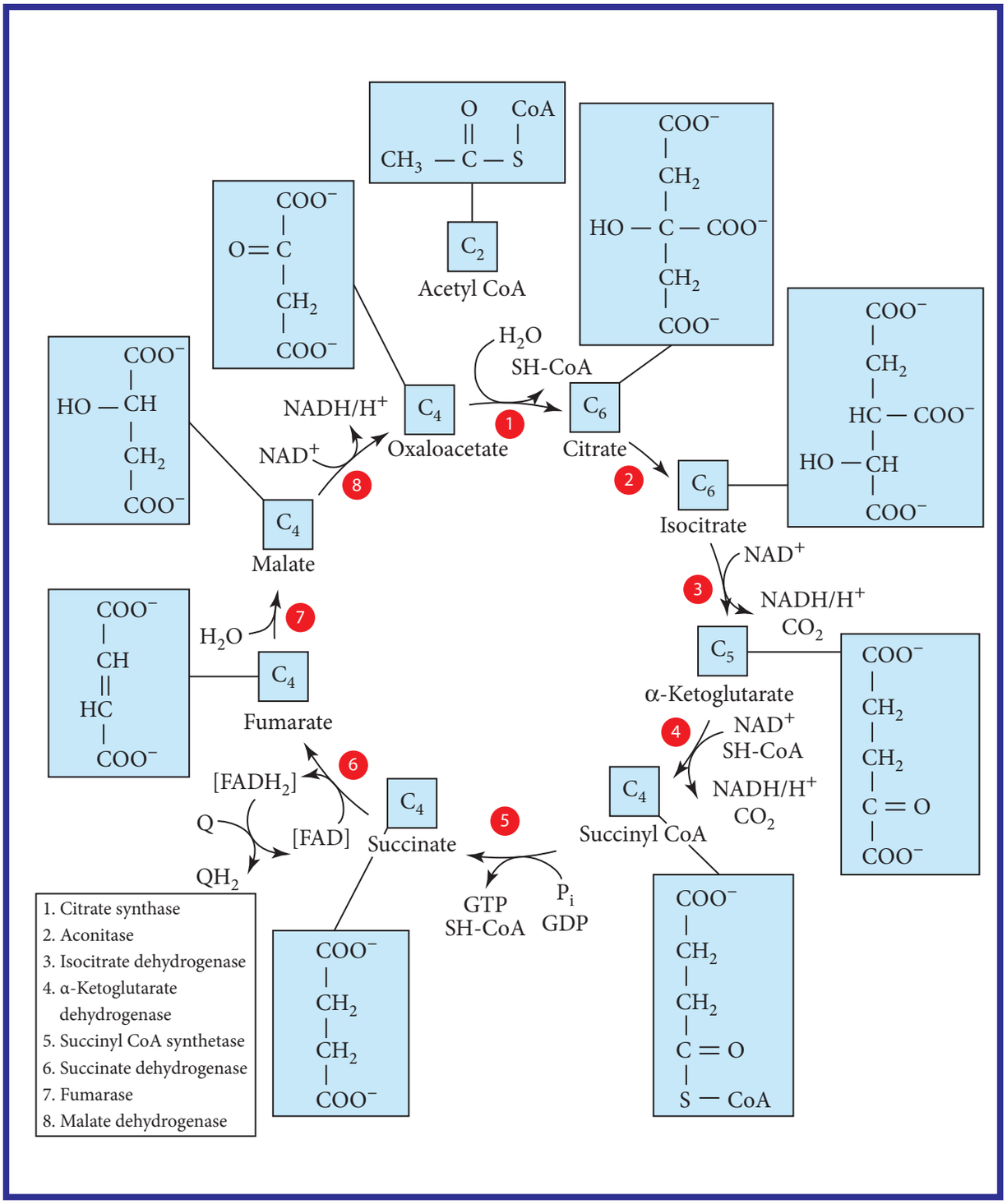
Step 4: In the step four, α-ketoglutarate is oxidised to succinyl CoA a four carbon molecule in the prescence of Coenzyme A. In this step also a NAD^+ molecule is reduced to NADH. This reaction is catalysed by α-ketoglutarate dehydrogenase enzyme.

Step 5: In step five, the succinyl CoA is converted into succinate with the release of large amount of energy. This energy is utilised for the phosphorylation of a guanosine diphosphate (GDP) to guanosine triphosphate (GTP) by the addition of inorganic phosphate. GTP is energetically equivalent to ATP; it is primarily used in protein synthesis, however, its use is more restricted. This reaction is catalysed by the enyme, succinyl-CoA synthetase (succinate thiokinase).

Step 6: Step six is a dehydration process that converts succinate into fumarate. Two hydrogen atoms are transferred to FAD, producing $FADH_2$. Unlike NADH, this carrier remains attached to the enzyme succinate dehydrogenase which catalyses this reaction, and transfers the electrons to the electron transport chain directly.

Step 7: Water is added to fumarate in step seven, and malate is produced. This reaction is catalysed by fumarase.

Step 8: The last step of the citric acid cycle regenerates oxaloacetate by oxidizing malate. Another molecule of NADH is produced in this reaction, which is catalysed by malate dehydrogenase.



3.4.2. Energetics of Tricarboxylic acid cycle

In a single turn of the cycle which consumes one acetyl CoA molecule,

- Two molecules of carbon dioxide are released
- Three molecules of NADH and one molecule of $FADH_2$ is generated
- One molecule of GTP is produced

High energy molecule	Total number formed during TCA	No. of ATP generated per molecule	Total number of ATPs
NADH	3	3	9
$FADH_2$	1	2	2
GTP	1	1	1
Total no. of ATP per acetyl coA molecule			12

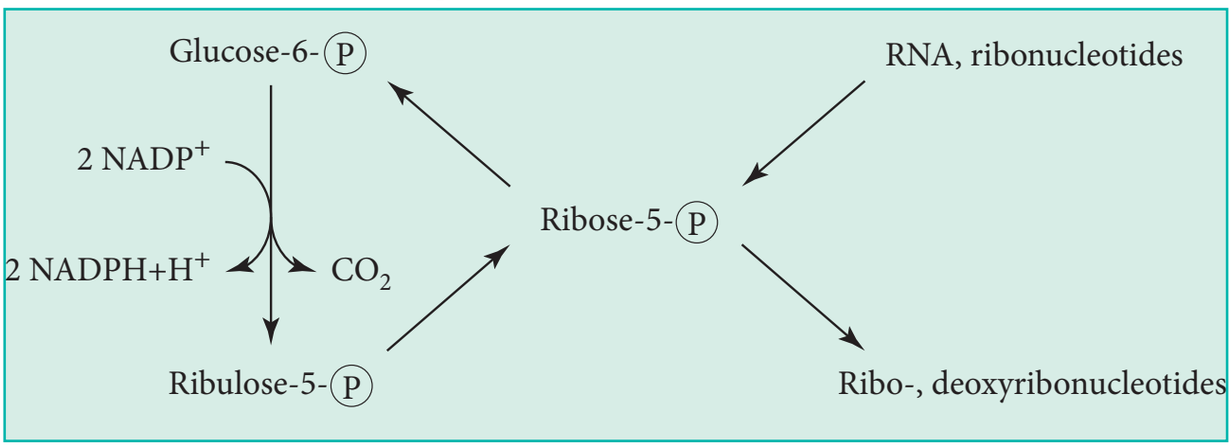
3.5. HEXOSE MONOPHOSPHATE SHUNT

So far we have learnt that glucose is oxidised by glycolysis & citric acid cycle, to generate energy and produce the necessary precursor metabolites. However, a number of alternative pathways have also been discovered. Hexose Monophosphate Shunt Pathway (HMP shunt). is one such important pathway. This occurs in the extra mitochondrial soluble portion of the cells.

This pathway, starts with glucose-6-phosphate as a key metabolite. Since, this pathway comprises both pentoses and hexoses, it is also referred to as the pentose phosphate pathway. This pathway consists of two phases namely the oxidative phase and the regenerative phase (non oxidative phase).

The oxidative phase, in which glucose-6-phosphate is oxidized and decarboxylated to give ribulose-5-phosphate. In this phase two NADPH (NADH equivalent) is formed. The fundamental difference between NADPH and NADH is that NADH is oxidised by the respiratory chain to generate ATP whereas NADPH serves as a hydrogen and electron donor in reductive biosynthesis of molecules such as fatty acids and cholesterol.

In cells that are not using ribose-5-phosphate for biosynthesis, the nonoxidative phase recycles six molecules of the pentose into five molecules of the hexose, glucose-6-phosphate, allowing continued production of NADPH and converting glucose-6-phosphate (in six cycles) to CO_2.



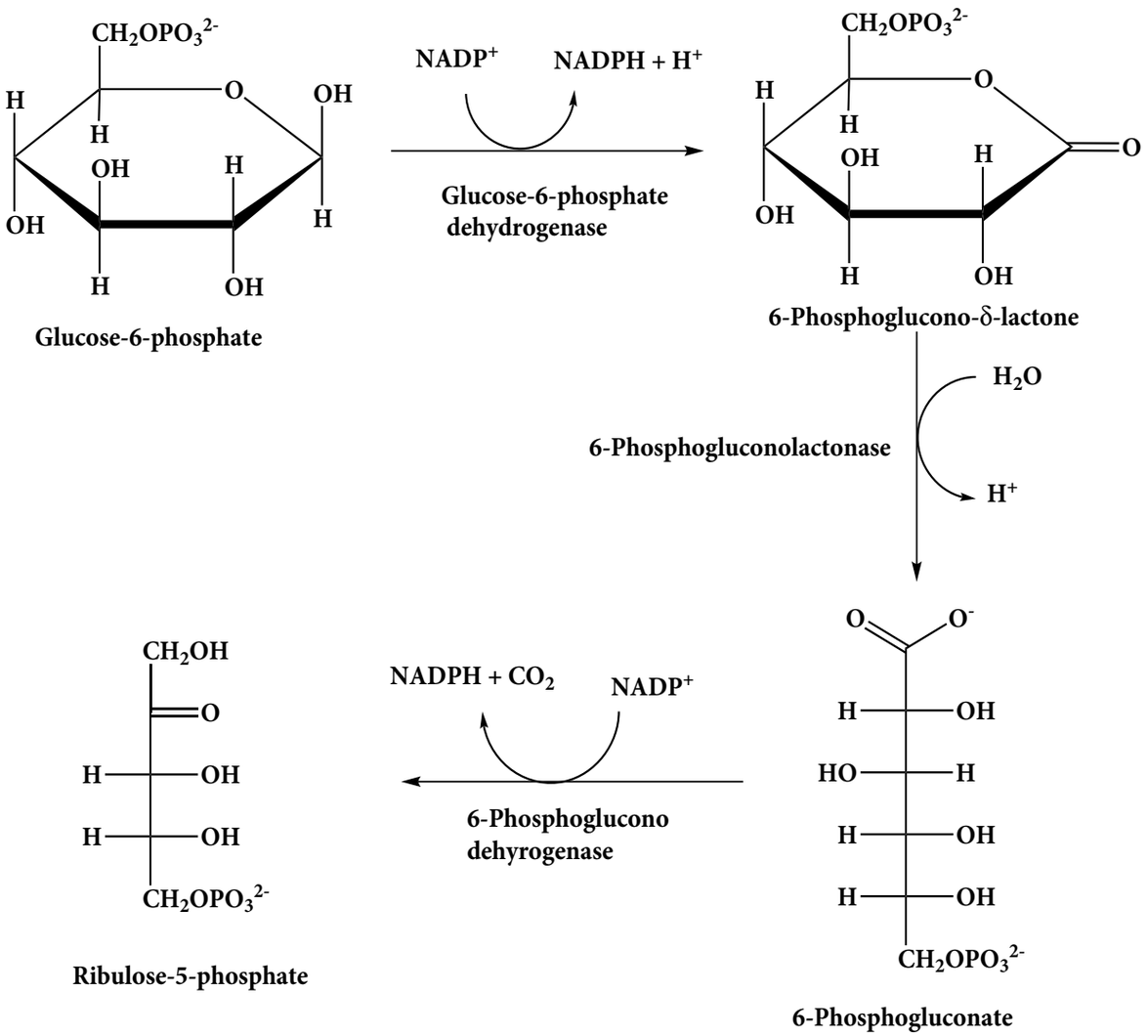
3.5.1. Reactions of oxidative phase

Three enzymes are required in the oxidative phase:

Step 1: In the first step, glucose-6-phosphate dehydrogenase oxidizes glucose-6-phosphate to 6-phosphoglucono-δ-lactone and reduces one molecule of $NADP^+$ to NADPH.

Step 2: In the second step, gluconolactonase cleaves the internal ester bond, which gives 6-phosphogluconate.

Step 3: In the third step, 6-Phosphogluconate dehydrogenase reduces another molecule of $NADP^+$ and decarboxylates 6-phosphogluconate to the pentose, ribulose-5-phosphate.



3.5.2. Non-oxidative phase

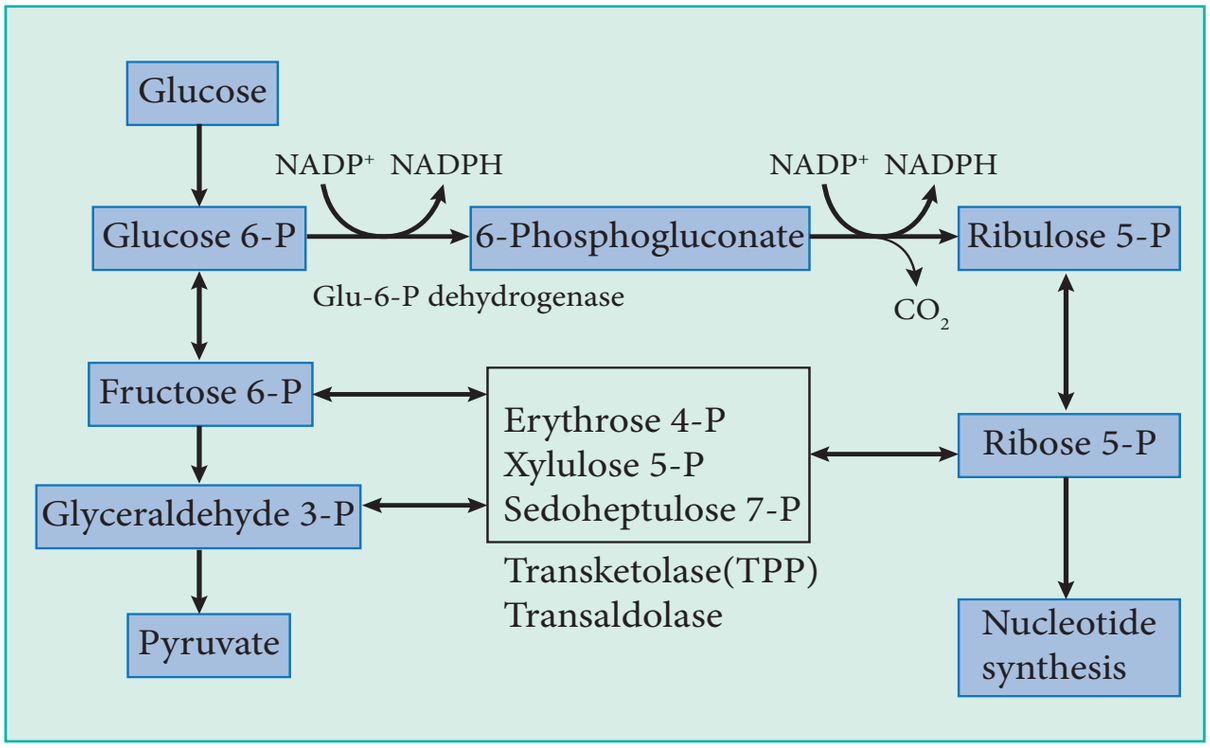
The non-oxidative phase involves the enzymes ribulose-5-phosphate epimerase (RE), ribulose-5-phosphate isomerase (RI), transketolase (TK), and transaldolase (TA). They bring about the following reactions:

Step 1. Two molecules of ribulose-5-phosphate are converted to xylulose-5-phosphate by ribulose-5-phosphate epimerase, and a third one is converted to ribose-5-phosphate by ribulose-5-phosphate isomerase.

Step 2: Transketolase transfers a C2 unit from one xylulose-5-phosphate to the ribose-5-phosphate, yielding glyceraldehyde-3-phosphate and the C7 sugar sedoheptulose-7-phosphate.

Step 3: Transaldolase transfers a C3 unit from sedoheptulose-7-phosphate back to glyceraldehyde-3-phosphate, which yields fructose-6-phosphate and the C4 sugar erythrose-4-phosphate.

Step 4: Transketolase transfers a C2 unit from the second molecule of xylulose-5-phosphate to erythrose-4-phosphate. This yields a second molecule of fructose-6-phosphate and again glyceraldehyde-3-phosphate



3.6. GLYCOGEN METABOLISM

Glycogen is the major storage form of carbohydrates in animals, present in all cells, but found mostly in liver and muscle, where it occurs as cytoplasmic granules. It is a highly branched form of amylopectin, with branch points occurring every 8 to 14 glucose residues. Glycogen's highly branched structure, which has many non-reducing ends, permits the rapid mobilization of glucose in times of metabolic need.

3.6.1. Glycogenesis

The biosynthesis of glycogen from glucose is called Glycogenesis. Glycogenesis takes place when blood glucose levels are sufficiently high to allow excess glucose to be stored.

Glycogenesis is stimulated by the hormone insulin. Insulin facilitates the uptake of glucose into muscle cells, though it is not required for the transport of glucose into liver cells. However,

insulin has profound effects on glucose metabolism in liver cells, stimulating glycogenesis and inhibiting glycogenolysis, the breakdown of glycogen into glucose.

The pathway of glycogenesis includes a series of steps that result in complex glycogen formation in the cytoplasm of the liver and cells of the muscles. The steps of glycogenesis are as follows:

Step 1: Glucose phosphorylation – In the initial phase, glucose is phosphorylated into glucose-6-phosphate, a usual reaction in glycolysis. It is catalyzed by glucokinase (liver) and hexokinase (muscle).

Step 2: Conversion of Glucose-6-Phosphate to Glucose-1-Phosphate – An enzyme Phosphoglucomutase will catalyze the conversion of glucose-6-P is to glucose-1-phosphate.

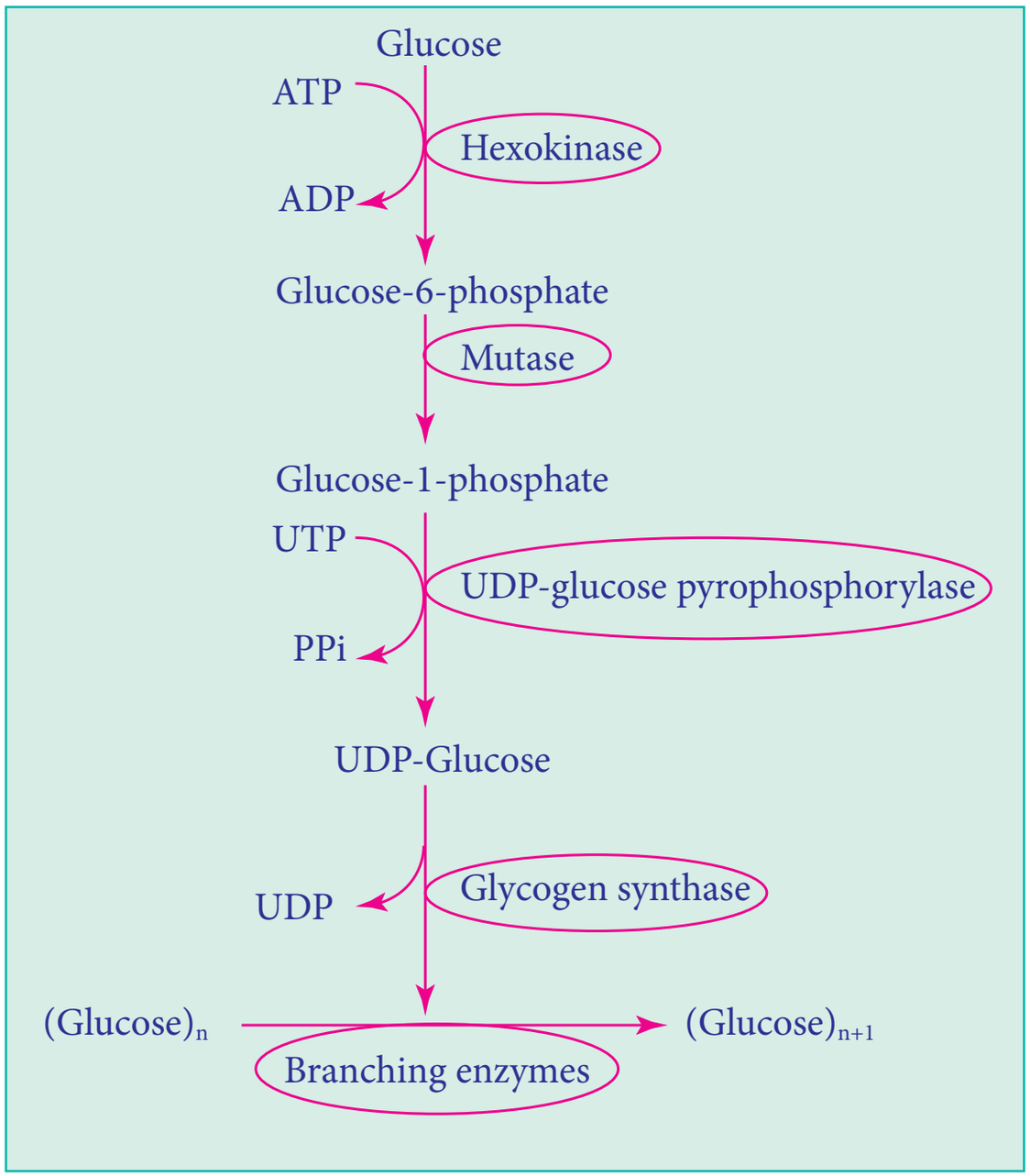
Step 3: UTP (uridine triphosphate) attaches to Glucose-1-phosphate in the third step. This step focuses on the reaction of glucose-1-phosphate to UTP thereby forming active nucleotide UDP-Glucose (Uridine diphosphate glucose). The one responsible for such reaction is the enzyme UDP Glucose pyrophosphorylase.

Step 4: UDP-Glucose attaches to glycogen primer, a small fragment of already existing glycogen that serves as a primer, in order to stimulate the synthesis of glycogen. The glucose from UDP-Glucose will be accepted by glycogenin (a protein that acts as a primer). The initial glucose unit is attached to the hydroxyl group of tyrosine of glycogenin. The first molecule of glucose is transferred to glycogenin, which will then take up for glucose residues forming a fragment of primer.

Step 5: Glycogen synthase transfers glucose from UDP-Glucose to glycogen (non-reducing end) forming alpha 1,4-linkages. The same enzyme catalyzes the synthesis of the unbranched molecule with alpha-1,4-glycosidic linkages.

Step 6: The formation of glycogen branches – The final step is the formation of glycogen branches caused by the effect of branching enzyme, which transfers a small fragment of about five to eight residues of glucose from the non-reducing end of the glycogen chain to another glucose residue linked by alpha-1,6 bond. This action causes the formation of a new non-reducing end. The final result is the elongation and branching out of the glycogen chain.

The process of glycogenesis utilizes two molecules of ATP. One molecule is needed for glucose phosphorylation and another molecule is needed to convert UDP to UTP.

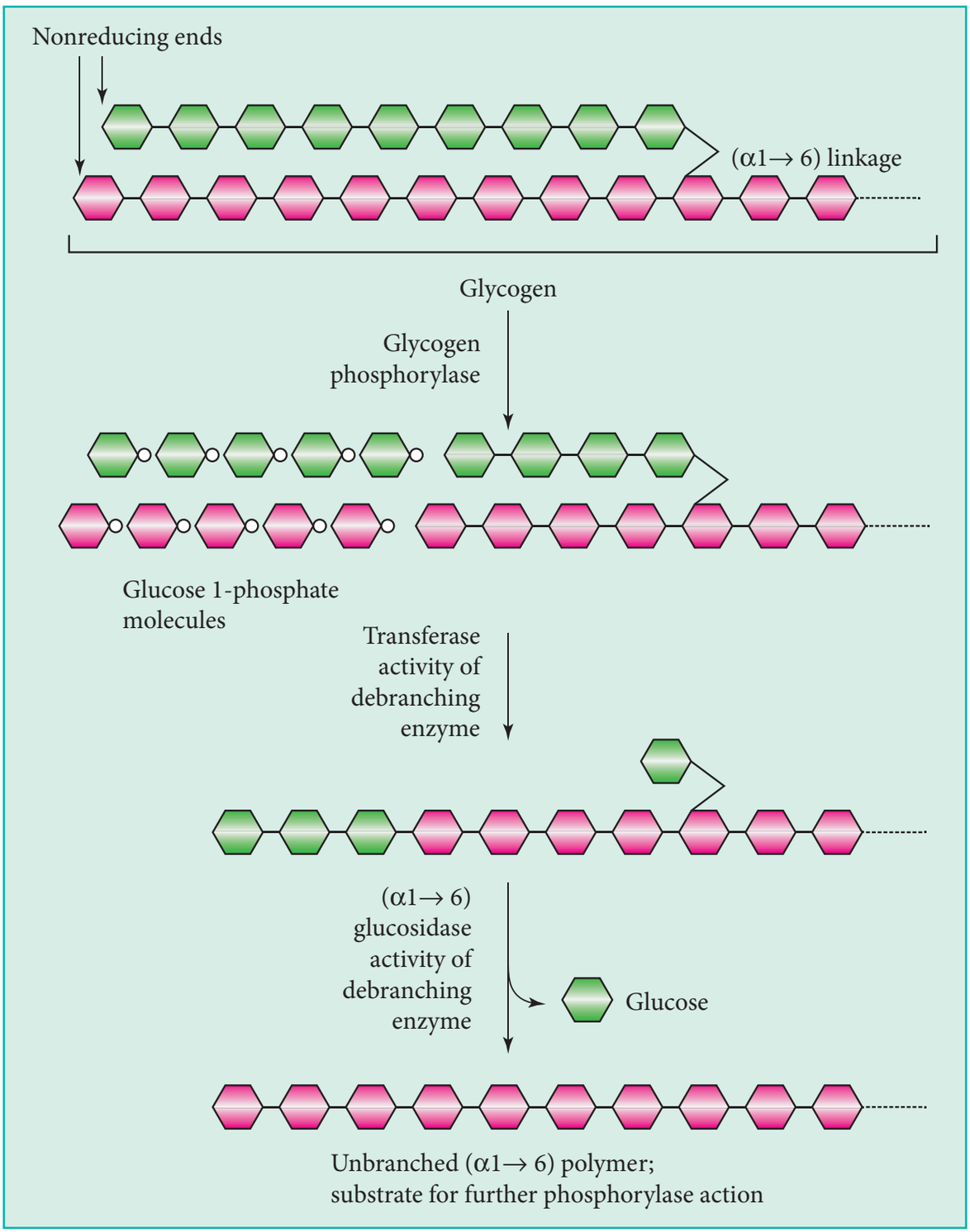


3.6.2. Glycogenolysis

Glycogenolysis is the biochemical breakdown of glycogen to glucose, to provide immediate energy. It takes place in the cells of muscle and liver tissues in response to hormonal and neural signals. In particular, glycogenolysis plays an important role in the adrenaline-induced fight-or-flight response and the regulation of glucose levels in the blood. It is stimulated by the hormones glucagon and epinephrine (adrenaline).

The process of glycogenolysis involves the sequential removal of glucose monomers by phosphorolysis, a reaction catalysed by the phosphorylated 'a' form (active form) of the enzyme glycogen phosphorylase. This enzyme cleaves the glycosidic bond linking a terminal glucose to a glycogen branch by substituting a phosphoryl group for the α [1→4] linkage producing glucose-1-phosphate and glycogen that contains one less glucose molecule.

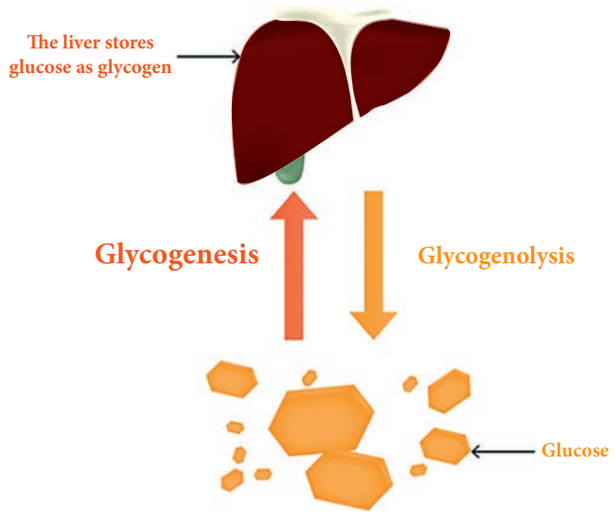
A second enzyme, phosphoglucomutase, converts the glucose-1-phosphate to glucose-6-phosphate. Glycogen involves two types of glycosidic linkage: the linear α [1→4] linkage and the branching α [1→6] linkages. During glycogenolysis glucose units are phosphorolysed from branches of glycogen until four residues before a glucose that is branched with an α [1→6] linkage.



A third enzyme, glycogen debranching enzyme transfers three of the remaining four glucose units to the end of another glycogen branch, exposing the α [1→6] branching point. This glycosidic bond is hydrolysed by a fourth enzyme α [1-6] glucosidase which eliminates the branch by removing the final glucose as a molecule of glucose, rather than glucose-1-phosphate. In muscle, but not liver cells, the glucose is subsequently phosphorylated to glucose-6-phosphate by a fifth enzyme hexokinase and enters the glycolytic pathway.

In liver cells, the main purpose of the breakdown of glycogen is for the release of glucose into the bloodstream for uptake by other cells. The phosphate group of glucose-6-phosphate is removed by the enzyme glucose-6-phosphatase and the free glucose exits the cell via the membrane localized GLUT2 glucose transporter.

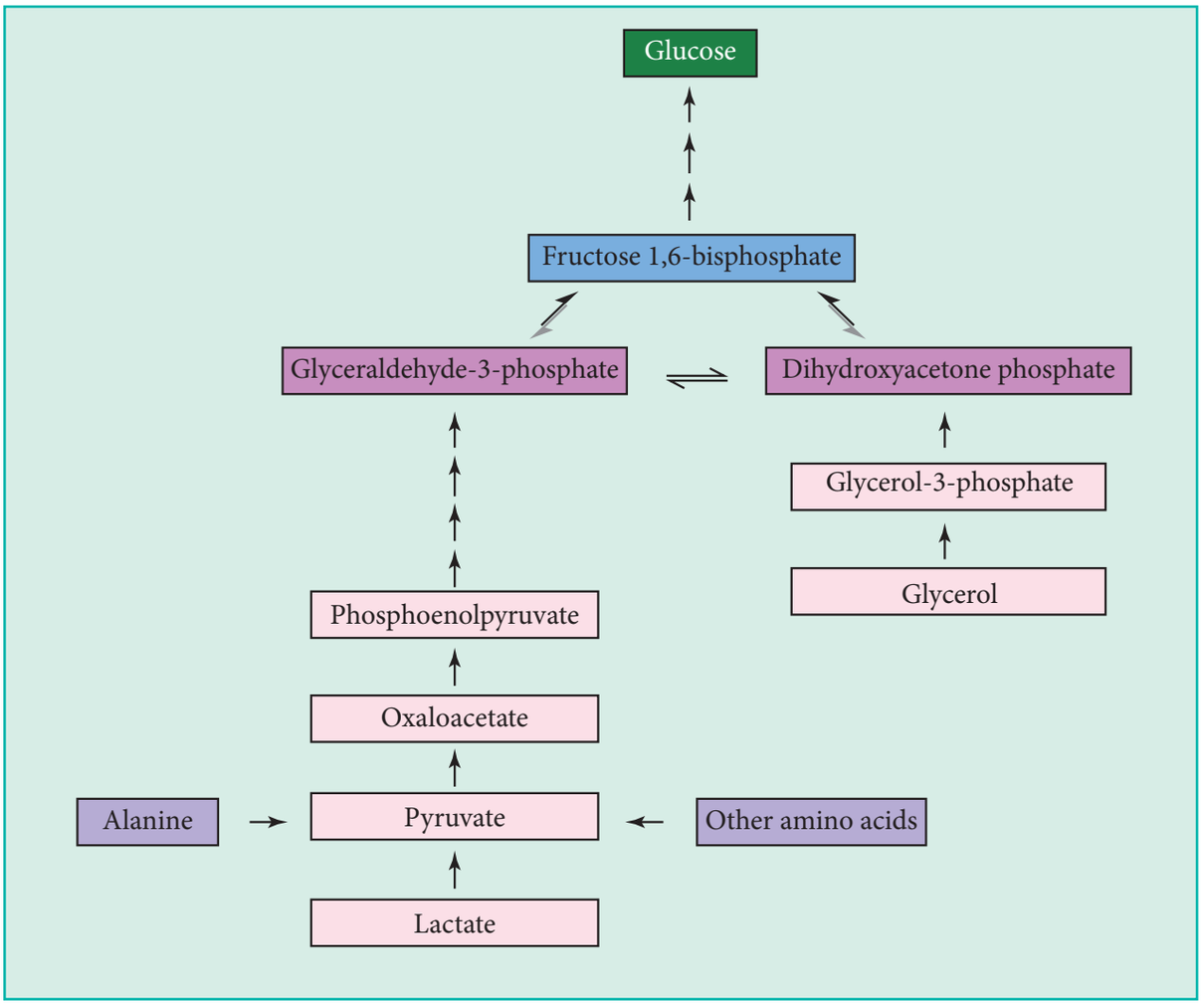
In muscle, glycogenolysis serves to provide an immediate source of glucose-6-phosphate for glycolysis to provide energy for muscle contraction but not for other body tissues. Muscle cells lack the enzyme glucose-6-phosphatase and thus cannot convert glucose-6-phosphate (which cannot be transported across the cell membrane) to glucose.



3.7. GLUCONEOGENESIS

Gluconeogenesis is a process through which noncarbohydrate precursors such as lactate, pyruvate, glycerol, and amino acids are converted to glucose.

Glucose occupies a central role in metabolism, both as a fuel and as a precursor of essential structural carbohydrates and other biomolecules. The brain and red blood cells are almost completely dependent on glucose as an energy source. Yet the liver's capacity to store glycogen is only sufficient to supply the brain with glucose for about half a day under fasting or starvation conditions. Thus, when fasting, most of the body's glucose needs must be met by gluconeogenesis (literally, new glucose synthesis), the biosynthesis of glucose from noncarbohydrate precursors.

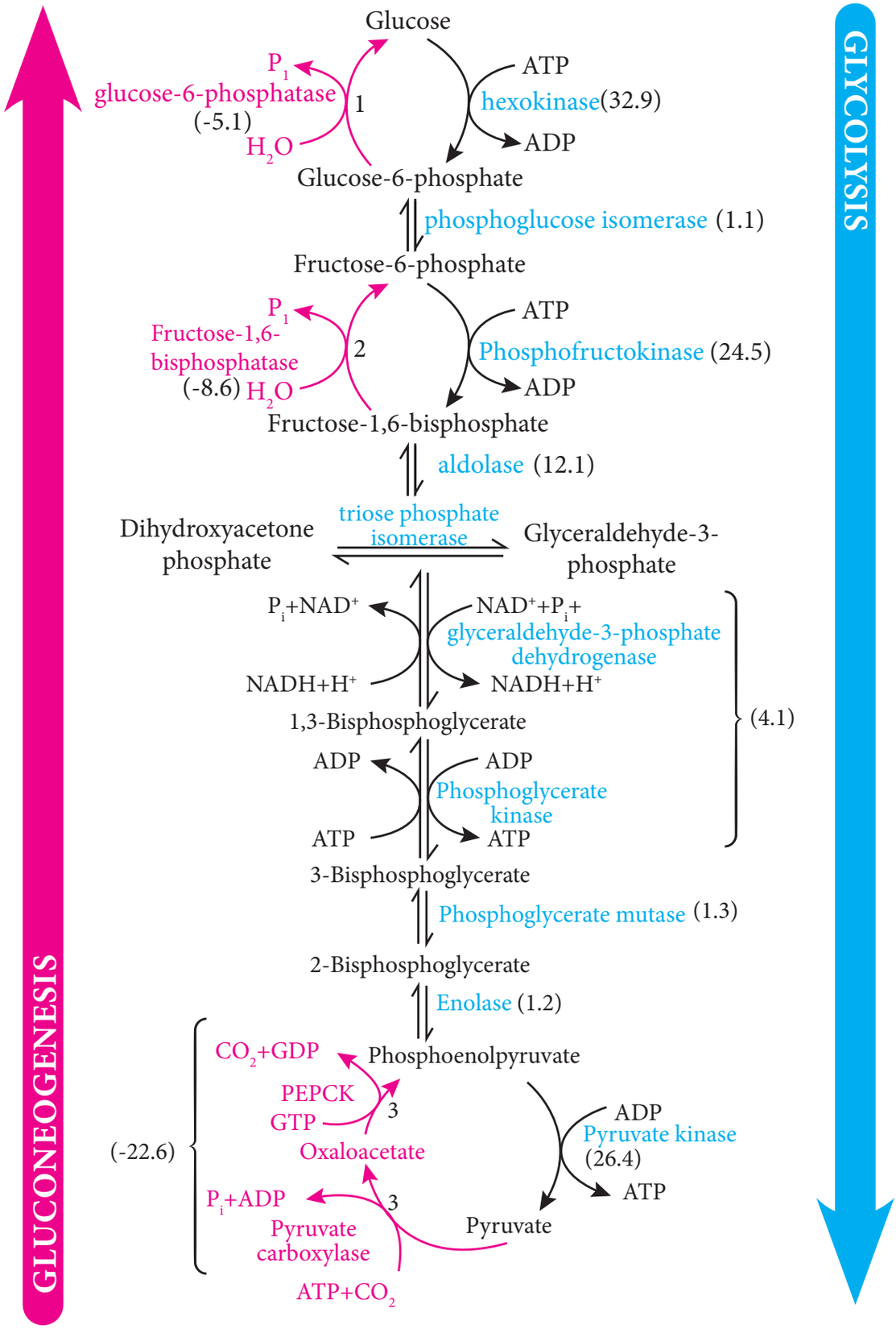


3.7.1 Key reactions in gluconeogenesis

The noncarbohydrate precursors that can be converted to glucose include the glycolysis products lactate and pyruvate, citric acid cycle intermediates, and the carbon skeletons of most amino acids. First, however, all these substances must be converted to oxaloacetate, the starting material for gluconeogenesis.

3.7.2 Reactions of gluconeogenesis

Gluconeogenesis is more or less similar to the reverse reactions of the glycolysis. However, there are some differences between these two pathways. Biological reactions can occur in both the forward and reverse direction. If the reaction occurs in the reverse direction the energetics also reversed. i.e. in the forward reaction is exergonic then the reverse reaction should be endergonic. In glycolysis, there are three irreversible steps (1, 3, 10) catalysed by the enzymes hexokinase, phosphofructokinase, and pyruvate kinase respectively. If gluconeogenesis were to simply occur in reverse direction then the reverse of the above mentioned three reactions which would require three different enzymes. The beauty of the nature is that, the above reactions of gluconeogenesis are catalysed by different enzymes namely glucose-6-phosphatase, fructose-1,6-bisphosphatase and PEP carboxykinase.



The ΔG's for the reactions in the direction of gluconeogenesis under physiological conditions in liver are given in parentheses in kJ mol^{-1}.

Step 1: The first step in gluconeogenesis is the conversion of pyruvate to phosphoenolpyruvic acid (PEP). In order to convert pyruvate to PEP there are several steps and several enzymes required. Pyruvate carboxylase, PEP carboxykinase and malate dehydrogenase are the three enzymes responsible for this conversion. Pyruvate carboxylase is found on the mitochondria and converts pyruvate into oxaloacetate. Because oxaloacetate cannot pass through the mitochondrial membranes it must be first converted into malate by malate dehydrogenase. Malate can then cross the mitochondria membrane into the cytoplasm where it is then converted back into oxaloacetate with another malate dehydrogenase. Lastly, oxaloacetate is converted into PEP via PEP carboxykinase.

The next several steps are exactly the reverse reactions of glycolysis. These reactions are catalysed by the same glycolytic enzymes, except the following two reactions.

The second step that differs from glycolysis is the conversion of fructose-1,6-bisphosophate to fructose-6-phosphate with the use of the enzyme fructose-1,6-phosphatase.

The last step that differs from glycolysis is the conversion of glucose-6-P to glucose with the enzyme glucose-6-phosphatase. This enzyme is located in the endoplasmic reticulum.

Because it is important for organisms to conserve energy, they have derived ways to regulate those metabolic pathways that require and release the most energy. In glycolysis and gluconeogenesis seven of the ten steps occur at or near equilibrium. In gluconeogenesis the conversion of pyruvate to PEP, the conversion of fructose-1,6-bisphosphate, and the conversion of glucose-6-phosphate to glucose all occur spontaneously and these reactions are highly regulated. It is important for the organism to conserve as much energy as possible. When there is an excess of energy available, gluconeogenesis is inhibited. When energy is required, gluconeogenesis is activated.

3.7.3. Precursors for glucose

Gluconeogenesis from amino acids: Amino acids which could be converted to glucose are called glucogenic amino acids. Most of the glucogenic amino acids are converted to the intermediates of citric acid cycle either by transamination or deamination

Gluconeogenesis from Propionate: Propionate is a major source of glucose in ruminants, and enters the main gluconeogenic pathway via the citric acid cycle after conversion to succinyl CoA.

Gluconeogenesis from Glycerol: At the time of starvation, glycerol can also undergo gluconeogenesis. When the triglycerides are hydrolysed in the adipose tissue, glycerol is released. Further metabolism of glycerol does not take place in the adipose tissue because of the lack of glycerol kinase necessary to phosphorylate it. Instead, glycerol passes to the liver where it is phosphorylated to glycerol 3-phosphate by the enzyme glycerol kinase.

$$\text{Glycerol+ ATP} \xrightarrow{\text{Glycerol kinase}} \text{Glycerol-3-phosphate+ADP}$$

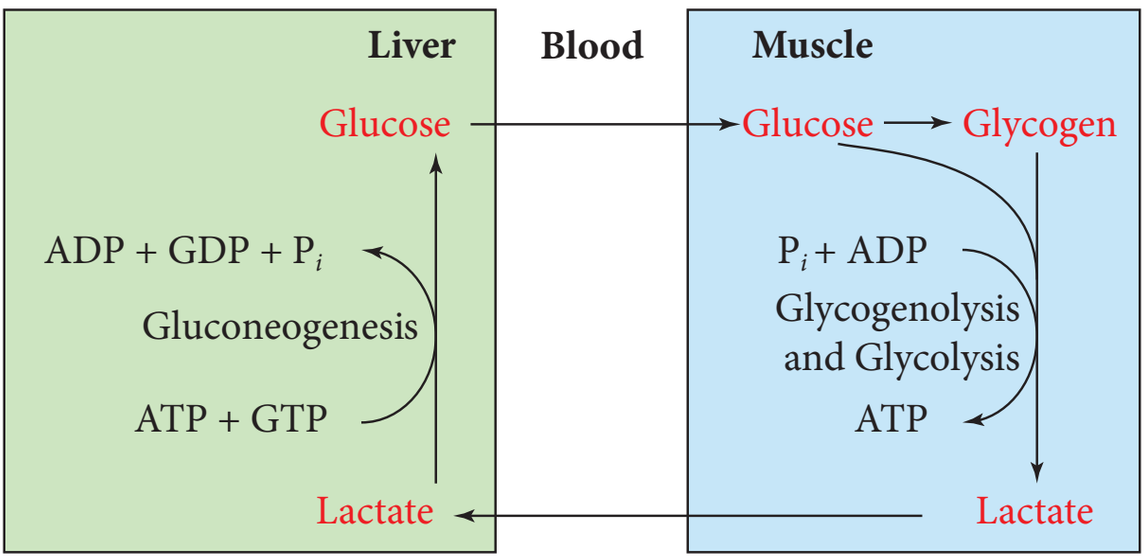
This pathway connects the triose phosphate stage of glycolysis, because glycerol 3-phosphate is oxidized to dihydroxy acetone phosphate in the presence of NAD+ and glycerol 3-phosphate dehydrogenase.

$$\text{Glycerol-3-phosphate} + NAD^+ \xrightarrow{\text{Glycerol-3-phosphate dehydrogenase}} \text{Dihydroxyacetone phosphate} + NADH + H^+$$

This dihydroxy acetone phosphate enters gluconeogenesis pathway and gets converted to glucose. Liver and kidney are able to convert glycerol to blood glucose by making use of the above enzymes.

3.7.4. Cori cycle

Muscle contraction is powered by hydrolysis of ATP, which is then regenerated through oxidative phosphorylation in the mitochondria of slow-twitch (red) muscle fibers and by glycolysis yielding lactate in fast-twitch (white) muscle fibers. Slow-twitch fibers also produce lactate when ATP demand exceeds oxidative flux. The lactate is transferred, via the bloodstream, to the liver, where it is reconverted to pyruvate by lactate dehydrogenase and then to glucose by gluconeogenesis. Thus, through the intermediacy of the bloodstream, liver and muscle participate in a metabolic cycle known as the Cori cycle.



This is similar to ATP-consuming glycolysis/gluconeogenesis, however, instead of occurring in the same cell, the two pathways occur in different organs. Liver ATP is used to resynthesize glucose from lactate produced in muscle. The resynthesized glucose is returned to the muscle, where it is stored as glycogen and used, on demand, to generate ATP for muscle contraction. The ATP utilized by the liver for this process is regenerated by oxidative phosphorylation. After vigorous exertion, it often takes at least 30 min for all

of the lactate so produced to be converted to glycogen and the oxygen consumption rate to return to its resting level, a phenomenon known as oxygen debt.

3.8. DIABETES MELLITUS

Diabetes mellitus is the most common metabolic disease, caused by the lack of insulin activity (not secreted in sufficient amounts or does not efficiently stimulate its target cells) and results in chronic excretion of large volume of urine containing glucose.

The β cells of the pancreas produces a hormone insulin, a 51 amino acid protein in response to high blood glucose levels. Insulin acts mainly on muscle, liver, and adipose tissue cells to stimulate the synthesis of glycogen, fats, and proteins while inhibiting the breakdown of these metabolic fuels.

Insulin functions by binding to the protein receptors on the outer cell surfaces and facilitate the entry of glucose into the cells. Insulin stimulates the uptake and utilization of glucose by most cells and also elevates the rate of glycolysis, glycogen synthesis and fatty acid synthesis. Thus, it lowers the blood glucose level and together with glucagon, insulin acts to maintain the proper level of blood glucose.

The lack of insulin disrupts the regulation and balance of many metabolic pathways. As a consequence, blood glucose levels become so elevated. Yet, despite these high blood glucose levels, cells "starve" since insulin-stimulated glucose entry into cells is impaired.

3.8.1. Types

There are two major forms of diabetes mellitus:

1. **Insulin-dependent, Type 1,** or **juvenile-onset diabetes mellitus**, which most often strikes suddenly in childhood. It is caused by deficiency of pancreatic β cells or inadequate insulin secretion of insulin by β cells.
2. **Noninsulin-dependent, Type 2,** or **maturity-onset diabetes mellitus**, which usually develops rather gradually after the age of 40, mostly in overweight individuals. It is characterized by insulin resistance as well as impaired insulin secretion, where some of the insulin receptors in the cell membrane fail to recognize insulin.

3.8.2. Symptoms

The symptoms of diabetes mellitus include,

- Decreased permeability of the cell membrane for glucose resulting in the accumulation of glucose in the blood. This condition is known as hyperglycemia. Glucose concentration increases as high as 500 mg/100 ml of blood.
- **Polyuria**: This means excretion of increased quantity of urine. This is to excrete the additional quantity of glucose in urine (glucosuria).
- **Polydypsia**: The excessive thirst which leads to increased consumption of water.This condition is known as Polydypsia. This is to replace the volume of water excreted due to polyuria.

- **Polyphagia**: Excessive appetite leads to polyphagia and increased intake of food. This is to replace the lost nourishment. The diabetic has voracious appetite, but inspite of over eating, they lose weight and become lean and emaciated.
- Presence of ketones in the urine (ketones are a byproduct of the breakdown of muscle and fat that happens when there's not enough available insulin)
- Fatigue
- Irritability
- Blurred vision
- Slow-healing sores
- Frequent yeast or urinary tract infections

3.8.3. Diagnosis

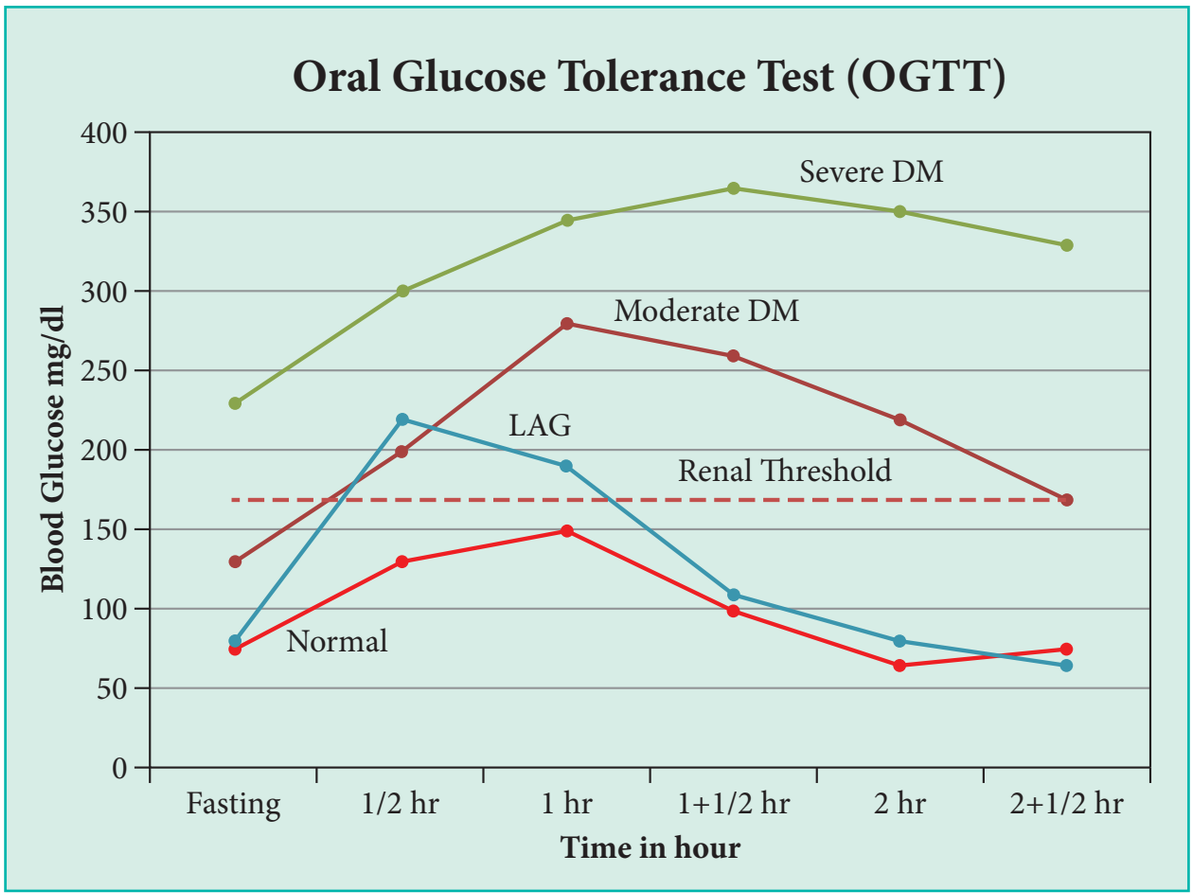
Diabetes is diagnosed with fasting sugar blood tests or with A1C blood tests, also known as **glycated hemoglobin tests**. You do not have to be in fasting for an A1C blood test. A fasting blood sugar test is performed after you have had nothing to eat or drink for at least eight hours. Normal fasting blood sugar is less than **100 mg/dl (5.6 mmol/l)**.

Test	Normal glucose level	Level of glucose in diabetes patient
Fasting Glucose Test	Less than 100 mg/dl	126 mg/dl or higher
Random (anytime) Glucose Test	Less than 140 mg/dl	200 mg/dl or higher
A1C Test	Less than 5.7%	6.5% or higher

3.8.3.1. Glucose tolerance test

The glucose tolerance test, also known as the **oral glucose tolerance test** (OGTT), measures your body's response to sugar (glucose). The glucose tolerance test can be used to screen for type 2 diabetes.

Before the test begins, a sample of blood will be taken.



You will then be asked to drink a liquid containing a certain amount of glucose (usually 75 grams). Your blood will be taken again every 30 to 60 minutes after you drink the solution.

The test may take up to 3 hours.

More commonly, a modified version of the glucose tolerance test is used to diagnose gestational diabetes — a type of diabetes that develops during pregnancy.

EVALUATION

I. Choose the correct answer from the given four alternatives

1. How many NADHs are produced from 7.5 mol of acetyl CoA by the citiric acid cycle?

   a. 15 mol b. 7.5 mol

   c. 75 mol d. none of these

2. Blood sugar is

   a. Sucrose b. Lactose

   c. Glucose d. Fructose

3. How many ATP molecules are generated during anaerobic glycolysis

   a. 2 b. 10

   c. 6 d. 8

4. Which one of the following enzyme is involved in substrate level phosphorylation

   a. Citrate synthase b. Isocitrate dehydrogenase

   c. Succinyl CoA synthetase d. Fumarase

5. The glucose renal threshold is reached when

   a. a condition of hyper glycemia occurs

   b. blood glucose levels are too low

   c. insulin levels are to high

   d. a fasting state occurs

6. The end product of aerobic glycolysis is

   a. Pyruvate b. Citrate

   c. Acetyl CoA d. Lactate

7. The important reducing power produced in HMP shunt pathway is

   a. NADH b. NADPH

   c. FAD d. FADH2

8. Pyruvate is converted to oxaloacetate by

   a. Pyruvate carboxylase b. Pyruvate kinase

   c. PFK d. Phosphoenol pyruvate carboxylase

9. Lactate is converted to glucose in

   a. Skeletal muscle b. liver

   c. Kidney d. lung

10. How many irreversible steps occurs in glycolysis?

    a. 2 b. 4 c.3 d. 5

11. Insulin is secreted by

    a. Liver b. Kidney

    c. Pancreas d. Thyroid

12. Glycolysis converts glucose to two pyruvate, the sum of the oxidation numbers of six carbon atoms is changed from 'x' (glucose) to 'y' (pyruvate) x and y are

    a. 0 and 4 b. 4 and 0

    c. 0 and 2 d. none of these

13. Glycolysis is termed anerobic because

a. molecular oxygen is required

b. only small amounts of ATP are formed

c. molecular oxygen is not required

d. NADH is produced

14. In citric acid cycle the acetyl group of acetyl CoA is

a. reduced to two carbondioxide

b. oxidised to two carbondioxide

c. oxidised to two NADGHs

d. reduced to two NADHs

15. Which of the following enzyme links glycolysis and TCA cycle?

a. Glucokinase b. PFK

c. LDH d. Pyruvate dehydrogenase

16. Which of the following compounds cannot be used to synthesis glucose in gluconeogenisis

a. glycerol d. lactate

c. acetyl CoA d. aminoacid

II. Fill up the blanks

1. Glucokinase acts on glucose to form _____
2. 2 - phosphoglycerate is converted to _____ by the enzyme enolase
3. In the anaerobic phase one molecule of glucose produces _____ molecules of ATP
4. Tricarboylic acid cycle occurs in _____
5. Glycogen biosynthesis is known as _____
6. The major source of glucose in ruminants is

III. Say true of false

1. Phosphoglycerate kinase converts 1,3 bisphosphoglycerate to 3 - phosphoglycerate
2. Pyruvate kinase acts reversibly
3. 24 molecules of ATP are formed in TCA cycle
4. UDP glucose pyrophosphorylase is involved in the synthesis of glycogen
5. Degradation of glucose is also known as glycolysis
6. Pyruvate is the end product of glycolysis

IV. Match the following

1.	Glycolysis	-	Ribose 5 - phosphate
2.	PDH	-	Insulin
3.	HMP shunt pathway	-	Cytosol
4.	Debranchin enzyme	-	Acetyl CoA
5.	Diabetes mellitus	-	Glycogenolysis
6.	TCA	-	Glycerol
7.	Lipid	-	Mitochondira

V. Answer the following

1. Give short note on cori cycle.
2. Briefly discuss several common fats for glucose produced through glucoreogenesis in the liver.
3. Name the enzymes which are involved for NADH formation in TCA cycle?
4. What is the mean by aerobic and anaerobic phases?
5. What is the difference between NADH and NADPH?
6. Describe the steps involved in TCA cycle.
7. Write a short notes on GTT.
8. What are glucogenic amino acids?
9. What are the reaction sequences of glycolysis ?
10. How many moles of ATP can be formed form 0.5 mol of acetyl CoA using the citric acid cycle, electron transport and oxidative phosphorylation?
11. What are the steps involved in glycogen metabolism?
12. What is glycogenesis?
13. Would you define glycogenesis as anabolic or catabolic? Explain briefly.
14. If 2.5 mol of glucose is partially oxidised in glycolysis
    a. how many moles of pyruvate are produced
    b. how many moles of ATP are produced
15. Explain the glycogenolysis.
16. How pyruvate is converted to lactate?
17. Explain the HMP shunt pathway.
18. How pyruvate is converted to glucose?

19. Explain the diabetes mellitus.
20. Whether the ATP productions that is associated with the citric acid cycle is substrate level phosphorylation (or) oxidative phosphorylation?
21. After meal, have a blood glucose concentration of 250 mg/dl of blood. Is this hyper glycemic (or) hypoglycemic?
22. What are the end products of the anerobic catabolism of glucose in muscle tissue.
23. Write the three important irreversible reactions in glycolysis?
24. How does gluconeogenesis get around the three irreversible steps of glycolysis.

CONCEPT MAP

Carbohydrate metabolism