

GLYCOLYSIS |

Structure

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2.1 INTRODUCTION

You have been introduced to general terms, concepts and role of metabolism in unit 1. We also discussed its common features, types of reactions, energy currency and redox carriers. In this unit, we shall begin carbohydrate metabolism with glycolysis, an almost universal pathway of sugar catabolism

Glycolysis is the central and primitive pathway of glucose catabolism. It is the initial route of oxidative catabolism in both anaerobic and aerobic systems. It also acts as a source of energy and metabolites for anabolism.

In this unit you will learn about the elucidation of this multistep pathway. We shall discuss the reactions involved in glycolysis and how other sugars enter into this pathway. You would also study how this pathway yields different products under different conditions as well as in different tissues. Finally we shall also explain how this pathway is regulated.

Expected Learning Outcomes

After studying this unit, you should be able to:

- ❖ explain the glycolytic pathway and its outcome;
- ❖ write the structure and point out the step (s) where oxidation and ATP synthesis occurs;

Unit 2

- ❖ indicate the fates of pyruvate under different conditions;
- ❖ describe the feeder pathways for glycolysis and their relevance;
- ❖ explain the Cori cycle and state its importance under anaerobic conditions; and
- ❖ describe how the key reactions of glycolysis are regulated.

2.2 THE ROAD TO GLYCOLYSIS

Before we go into the details of the glycolytic pathway, let us look at some important leads which were instrumental in the elucidation of the pathway. In 1897 the German brothers, **Hans Buchner and Eduard Buchner** accidentally found that addition of sucrose to yeast extract led to evolution of bubbles from the solution. The addition of sucrose was meant to preserve yeast extract. Eduard Buchner concluded that fermentation, a process described by **Pasteur** was occurring. He isolated the enzyme from yeast extract and called it 'zymase'. It was demonstrated for the first time that fermentation could take place outside the cell and discounted the existing idea of a vital force to carry out life processes. This work allowed chemists to identify individual steps and characterise them under controlled conditions. Above all it opened the era of enzymatic theory of metabolism. Eduard Buchner was rewarded with the Nobel Prize in 1907.

In 1906, **Arthur Harden and William John Young** made two very important observations. They found that inorganic phosphate was required for fermentation and is incorporated into fructose 1,6 biphosphate (Harden and Young ester). They also elaborated Buchner's work and showed that a cell free extract can be separated by dialysis into two fractions. One of them was non dialysable heat labile fraction or zymase and the other is heat stable and dialysable or **cozymase**. Both of them are necessary for fermentation. They also discovered NAD^+ . Today we know that each of these fractions includes a mix of enzymes and coenzymes / other low molecular weight substances.

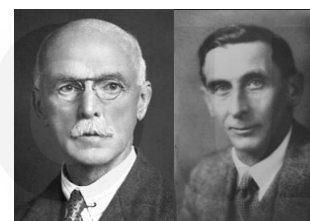
Later studies on muscle extracts showed that many reactions of lactic acid fermentation were same as those of alcoholic fermentation. The complete glycolytic pathway was elucidated in 1940 by pioneers in the field including Gustav Embden, Otto Meyerhof, Carl Neuberg, Robert Robison, Jacob Parnas, Otto Warburg, Gerty Cori and Carl Cori.

The discovery by Otto Meyerhof and his students that some phosphorylated compounds are rich in energy revolutionised our concepts and significance of cellular metabolism. One of his associates, K. Lohmann was the first to discover ATP.

Meyerhof and his colleagues not only discovered the intermediates of the cycle but played a key role in piecing together the complex puzzle of glycolysis. He had the gift of integrating a variety of phenomenon. Glycolysis is also known as Embden- Meyerhof –Parnas pathway. Meyerhof was awarded, together with the English physiologist A.V. Hill, the Nobel Prize for Physiology or Medicine in 1922.



Hans and Eduard Buchner



Harden and Young



**Otto Fritz Meyerhof
(1884-1951)**

2.3 GLYCOLYSIS OR EMBDEN-MEYERHOF-PARNAS (EMP) PATHWAY

Glycolysis is the initial stage of glucose metabolism. It occurs in the cytosol. It does not involve oxygen. It produces 2 ATP for each glucose oxidised. Its end product is pyruvate.

It is important to note that the division into phases is for the ease of understanding. In fact, the product of one reaction serves as the substrate for the next reaction in the pathway.

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

Let us see what makes glycolysis an almost universal pathway. Since all these reactions can take place in the absence of oxygen therefore, it is an important pathway for extraction of energy from nutrients in anaerobic organisms. Even aerobes begin glucose metabolism with glycolysis and then enter the citric acid cycle for complete breakdown. In addition, it becomes the major source of energy in cells lacking mitochondria such as red blood cells and cornea of the eye or in rapidly contracting skeletal muscles experiencing transient anaerobic conditions.

The overall pathway of glycolysis is energetically favourable and unidirectional. It can be represented by the following equation:



Let us proceed to learn about the reactions of glycolysis. You would notice that all the intermediates of the pathway are **phosphorylated**. The purpose of phosphorylation is two- fold. It activates the intermediate and polarises it, thereby preventing it from leaving the cell. Generally, the plasma membrane lacks transporters for phosphorylated sugars.

The glycolytic pathway is divided conventionally into two phases. They are called preparatory or energy investment phase and energy yielding / pay off phase (Fig. 2.1). We shall discuss these phases one by one.

A. Preparatory or energy investment phase

This phase has two reactions which require input of energy in the form of ATP. The situation is similar to day to day life situations where we invest small amounts of money to get better returns later. The phase ends with the splitting of activated fructose 1,6- bisphosphate to two sugars.

Step 1: Phosphorylation of glucose

The first step of glycolysis is catalysed by a ubiquitous enzyme, **hexokinase**. It is relatively a non specific enzyme as it also phosphorylates mannose, fructose, glucosamine and 2-deoxyglucose in addition to glucose. It catalyses the phosphoryl group transfer from ATP to the hydroxyl group at C-6 of glucose in presence of Mg^{2+} . The reaction is highly exergonic as the phosphorylated product, glucose-6- phosphate is a low energy ester and the reaction is essentially irreversible under *in vivo* conditions. In some tissues,

specific kinases also exist like glucokinase that is specific only for glucose.

Glucokinase has restricted distribution and low affinity for glucose. This first step of glycolysis is not subject to stringent regulation because the product formed has multiple fates.

Step 2: Isomerisation of glucose-6 phosphate

The next reaction is the isomerisation of glucose-6-phosphate to fructose-6-phosphate by **phosphohexose isomerase** (phospho-glucose isomerase). The reaction proceeds readily in both directions and the aldoses-ketose isomerisation involves the formation of an enzyme bound enediol intermediate.

Enediol is an organic compound in which two hydroxyl groups are attached; one each to carbon atoms of a double bond
 $(>C(OH)=C(OH)<$

Step 3: Phosphorylation of fructose-6 phosphate

You learnt that first irreversible reaction is not unique to glycolysis as glucose-6-phosphate is an intermediate for other metabolic pathways also.

Phosphorylation of fructose-6 phosphate is the first committed reaction to glycolysis, which means once this reaction occurs; glycolysis will proceed till the last reaction. This first unique step of glycolysis is catalysed by Mg^{2+} dependent **phosphofructokinase-I (PFK-I)**. It catalyses the irreversible phosphorylation of fructose-6-phosphate to fructose-1,6-bisphosphate. In this reaction, one molecule of ATP is consumed. In many organisms including plants, some bacteria and protists pyrophosphate (PPi) is the phosphoryl donor in place of ATP. Due to its uniqueness, this reaction is important in regulation of glycolysis.

When two phosphate groups are present on two different carbons of the same molecule, it is named bisphosphate as in fructose-1,6-bisphosphate. When both phosphates are present on the same carbon, it is notated as diphosphate as in ADP.

The next two reactions first split the six carbon bisphosphate intermediate to 2 three carbon sugars and then triose phosphate isomerase interconvert the two split sugars so that both products can be utilised for oxidation and generation of ATP in the pay off phase.

Step 4: Cleavage of fructose 1, 6-bisphosphate

The enzyme **aldolase** splits fructose 1,6-bisphosphate to 3-phosphoglyceraldehyde (an aldose) and dihydroxyacetone phosphate (a ketose). This reaction has a positive standard free energy change but at lower concentration of reactant, the reaction is reversible. The enzyme is capable of splitting a number of ketose mono- and bis-phosphates. This reaction also operates in gluconeogenesis and Calvin cycle in the reverse direction.

Step 5: Inter conversion of triose phosphates (aldose-ketose)

The production of two sugars by aldolase completes the preparatory phase of glycolysis. The energy generation phase begins with the oxidation of glyceraldehyde 3-phosphate. Therefore, dihydroxyacetone phosphate (DHAP) is isomerised to glyceraldehyde 3-phosphate by triose phosphate isomerase so that both sugars are degraded by this pathway. The enzyme is extremely active and is generally dubbed as a perfect enzyme which means that the product is formed as soon as the enzyme and substrate collide.

So far, we learnt that The cleavage of fructose 1,6 bisphosphate results in two sugar derivatives; DHAP comes from C-1 to C-3 and glyceraldehyde is derived from C-4 to C-6. But following the isomerase reaction C-1, C-2 and C-3 are

indistinguishable from C-6, C-5 and C-4, respectively. This is because in glyceraldehyde 3-phosphate, the carbonyl group is C-1. **You must always keep this in mind while tracing the fate of C-14 labelled glucose.**

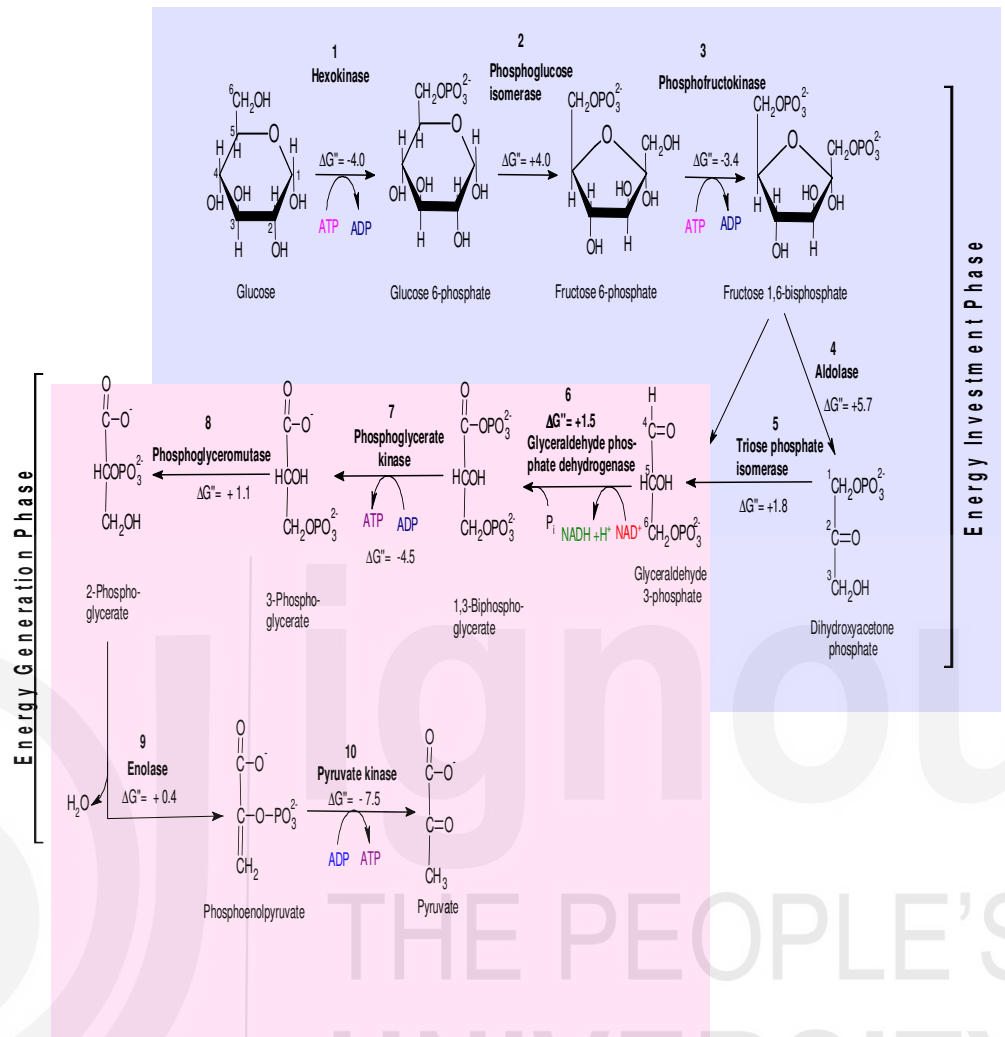


Fig. 2.1: The Glycolysis Pathway.

B. Energy generation phase

The last five reactions of glycolysis include a single step of oxidation and two reactions in which ATP is synthesised by substrate level phosphorylation. Since two molecules of glyceraldehyde 3-phosphate produced in the preparatory phase enter the energy generation phase, therefore you must double the final outcome of each reaction in the pay off phase.

Step 6: Oxidation of glyceraldehyde 3-phosphate

The oxidation of glyceraldehyde-3-phosphate is catalysed by a NAD^+ dependent **glyceraldehyde-3-phosphate dehydrogenase**. It has tightly bound NAD^+ which is an exception to the generalisation discussed in unit 1. The substrate is oxidised to 1,3 bisphosphoglycerate (1,3 BPG) with concomitant production of NADH and H^+ . Formation of 1,3 BPG, a mixed anhydride (acyl phosphate) conserves the energy released during oxidation of the carbonyl group to an acid. During the reaction, the substrate is covalently bound to the -SH group of cysteine in enzyme. Therefore, heavy metals like Hg^{2+} which react with cysteine can irreversibly inhibit it. Even arsenate, an

analog of phosphate can replace it and forms an unstable intermediate that can be non- enzymatically hydrolysed back to 3- phosphoglyceric acid (3 PGA).

You will see that in the next reaction that phosphate group of 1,3 BPG is essential for ATP synthesis, therefore, in presence of heavy metals, glycolysis proceeds without generation of ATP.

Step 7: Substrate level phosphorylation by 1, 3 BPG

In this step, ATP is synthesised by phosphoryl group transfer from 1,3BPG to ADP. The reaction is catalysed by phosphoglycerate kinase generating 3PGA. The two steps (6 and 7) represent an example of how energy from oxidation of a substrate is coupled to ATP synthesis.

The next two reactions of glycolysis help to generate another energy rich intermediate (PEP) starting from an energy poor 3-PGA.

Step 8: Isomerisation of 3-phosphoglyceric acid (3-PGA)

The inter conversion between 3-PGA and 2-PGA is catalysed by phosphoglycerate mutase. The reaction goes through 2,3 bisphosphoglycerate (2,3 BPG). The product 2-PGA is obtained by transferring the phosphate at C-3 to the active site. A small amount of 2, 3 BPG is needed as a cofactor to initiate the cyclic process.



Step 9: Dehydration of 2-PGA

The dehydration of 2 PGA by the enzyme **enolase** yields an energy rich enolic phosphate, phosphoenol pyruvate (PEP). This reaction requires Mg^{2+} or Mn^{2+} and it is inhibited by fluoride.

Step 10: Substrate level phosphorylation by PEP

The enzyme **pyruvate kinase** catalyses irreversible phosphoryl group transfer from PEP to ADP. The enol form of pyruvate non- enzymatically tautomerises to the more stable keto form. The reaction is irreversible under physiological conditions.

Thus each molecule of 3-phosphoglyceraldehyde is processed in the second phase to produce $1\text{NADH} + \text{H}^+$ and 2 ATP. We will double this to account for both molecules of 3-phosphoglyceraldehyde. In the preparatory phase two ATP were consumed and so the net yield of ATP is only two ($4 - 2$).

Many of the intermediates of this pathway also provide anabolic precursors; DHAP is the precursor for the glycerol backbone of glycerolipids, 3-PGA can be converted to serine and other amino acid and PEP is one of the precursors for the synthesis of aromatic amino acids.

We learnt that 2 NAD^+ are consumed in one cycle of glycolysis. This NAD^+ must be regenerated in all organisms to allow oxidative catabolism. There is more than one way to do it depending on the presence or absence of oxygen. In the following section, we will cover fermentation and demonstrate its role in regeneration of NAD^+ by taking suitable examples.

SAQ 1

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

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

2.4 FERMENTATION

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

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

It is our general understanding that microorganisms are employed for fermentation of sugar to produce alcohol in brewing industry. In the microbial world we encounter enormous variation in the end product of fermentation. In some microbes more than one end product is produced. The type of fermentation is named on the end products such as homo lactate fermentation, mixed acid fermentation, etc. Some of these organic end

products for instance, citric acid, propionic acid, butanol, acetone and ethanol are produced commercially

Fermentation is an inefficient mode of energy generation as the end products are organic compounds rather than CO₂ that still conserve lot of energy. Let us study homo lactate and alcohol fermentation reactions. In both examples you will note that there is no net change in the oxidation state of carbon which means that H: C ratio is same for glucose and the end product.

Lactic acid fermentation

Lactic acid fermentation is carried out by bacteria (*Lactobacillus* species), some fungi and rapidly contracting skeletal muscle. A single reaction catalysed by **lactate dehydrogenase (LDH)** reduces pyruvate to lactate (Fig. 2.2). It is not specific for pyruvate and reduces a number of other keto acids, including phenylpyruvic acid. If the end product is only lactate it is homo lactate fermentation. The NADH utilized in this step is obtained from the reaction catalyzed by glyceraldehyde-3-phosphate dehydrogenase in glycolysis. The formation of lactate allows the regeneration of NAD⁺ which can be reused in glycolysis. In this case, glycolysis results in net formation of 2 ATP for each molecule of glucose and no net production of NAD⁺/NADH.

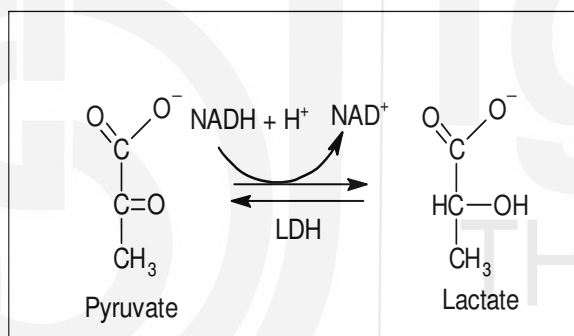


Fig. 2.2: Reduction of pyruvate to lactate: Pyruvate formed in glycolysis is reduced to lactate under anaerobic conditions to regenerate NAD⁺.

In vertebrates multiple **isozymes of LDH** exist that are encoded by two genes. The active form of this enzyme is a homo tetramer and the isozymes differ in ratio of the two polypeptides (M or H chain). The skeletal muscles have predominantly LDH₅ that four M (muscle) chains while the major variant in the heart is LDH₁ with four H (heart) chains. The other tissues have a mix of H and M polypeptides. Isozymes have different amino acid composition, kinetic and immunological properties. The heart enzyme has a low K_m (high affinity for the substrate) while the muscle enzyme works best at higher concentration of pyruvate (high K_m). These properties are consistent with their roles.

K_m is defined as the substrate concentration at which half of the maximum velocity of a reaction is attained. Its value for an enzyme is inversely proportional to the affinity of the enzyme for the substrate.

CO₂ released during alcohol fermentation gives the characteristic carbonation of champagne in brewing and allows dough to rise in baking.

Alcohol fermentation

In some bacteria and fungi (e.g. yeast), pyruvate is fermented to ethyl alcohol in two steps. In the first step, pyruvate is decarboxylated irreversibly to acetaldehyde by a thiamine pyrophosphate (TPP) dependent **pyruvate decarboxylase**. It also requires Mg²⁺. This enzyme is absent in animal tissues.

In the second step, acetaldehyde is reduced to ethyl alcohol by **alcohol dehydrogenase** (Fig. 2.3). Ethyl alcohol is excreted by microorganisms as

accumulation of alcohol beyond a certain limit can kill them. In many organisms including humans, the enzyme metabolises ethyl alcohol.

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

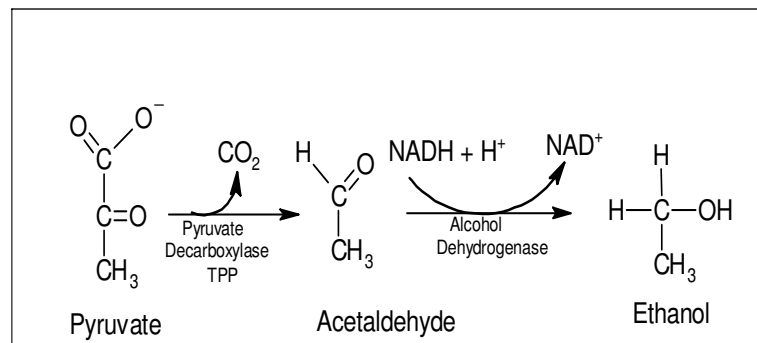


Fig. 2.3: The conversion of pyruvate to ethanol reoxidizes NADH.

SAQ 2

Fill in the blanks:

- The general term used for the anaerobic degradation of glucose to obtain energy is _____.
- The net yield of ATP during lactate fermentation is _____.
- _____ is the example of human tissue that can ferment glucose to _____.
- The end products of alcohol fermentation are _____ and _____.
- In both lactate and alcohol fermentation the purpose of going beyond glycolysis is to _____.

2.5 CORI CYCLE

In section 2.3, we discussed that lactic acid is formed in skeletal muscles during strenuous exercise. It is a temporary measure taken by the muscles to cope up with the energy requirements under hypoxic (low oxygen) conditions. As far as the muscle is concerned it is a dead end. Lactate must be converted back to pyruvate. The muscles use the inefficient mode to buy time and then shift their burden to the liver for gluconeogenesis (synthesis of carbohydrates from non carbohydrate sources). Liver, in turn, makes glucose again available to the muscles to generate energy or storage. Some tissues like liver, kidney and intestine can hydrolytically cleave glucose 6-phosphate to glucose that leaves the cells with ease. We shall discuss about gluconeogenesis in unit 5, however, you may note in Fig. 2.4 that gluconeogenesis is an expensive process and uses 6 ATP to get back glucose while lactate fermentation in muscles results in net synthesis of 2 ATP molecules.

The synthesis of lactate in the skeletal muscles and its conversion back to glucose by the liver for use largely by the muscles constitutes the **Cori cycle**

Carl and Gerty Cori



(Fig. 2.4). This cyclic route was worked out by the husband and wife team of Carl Ferdinand Cori and Gerty Theresa Cori. Their contributions in glycogen metabolism were recognised with the 1947 Nobel Prize in Physiology or Medicine along with Bernardo Houssay.

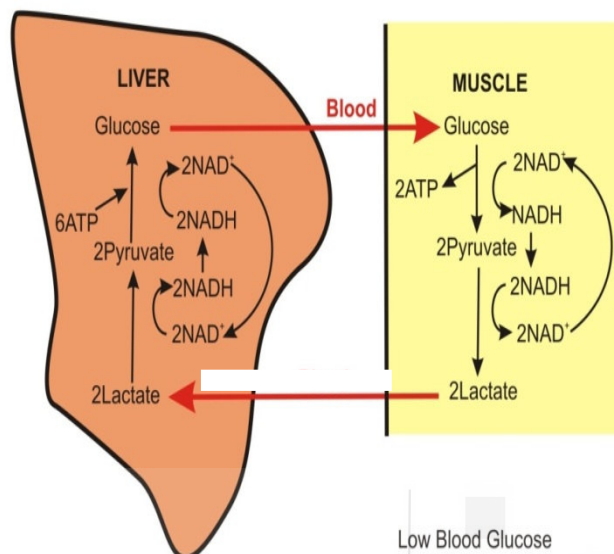


Fig. 2.4: The Cori cycle illustrates metabolic interdependence between the muscle and liver.

In situations, where strenuous activity continues for a long time, the formation of lactate exceeds the capacity of the liver to regenerate glucose, resulting in rise of lactic acid concentration in blood. The mildly acidic lactate will lower blood pH (lactic acidosis) leading to tissue damage and symptoms associated with panic, such as hyperventilation, abdominal cramps, vomiting, etc. All these symptoms are a part of the body's natural defence mechanisms designed to slow down rigorous activity, so that permanent damage can be avoided.

So far we have discussed metabolism of glucose through glycolysis and the fate of lactate in the skeletal muscle. In the next section, we shall learn how carbohydrates other than glucose are processed into intermediates of the glycolysis. They may be either obtained from diet or synthesised endogenously. The additional reactions that allow their entry into glycolysis constitute the feeder pathways.

2.6 FEEDER PATHWAYS FOR GLYCOLYSIS

Glycolysis is the central metabolic pathway for generation of energy. We also know that whenever our body needs energy, glycogen stores are mobilized. In addition, polysaccharides, oligosaccharides and disaccharides are also present in our diet. The complex dietary carbohydrates are hydrolyzed into their constituent monosaccharides prior to absorption and assimilation. When there is a need to degrade carbohydrates; the first step is to break them into monosaccharides and process sugars other than glucose to an intermediate in glycolysis. Fig. 2.5 gives an overview of the feeder pathways of glycolysis.

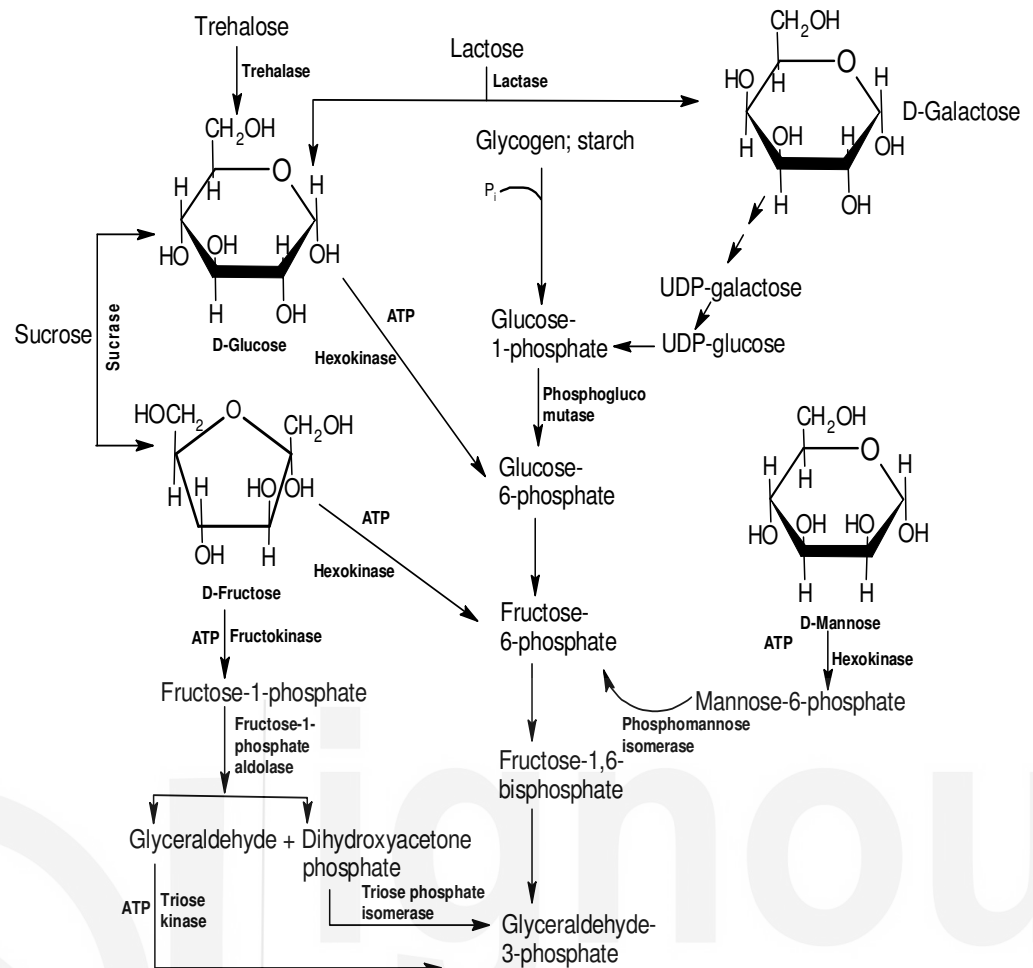


Fig. 2.5: Feeder pathways for glycolysis.

Fig. 2.5 shows the breakdown of storage polysaccharides (glycogen and starch), disaccharides (lactose, maltose, trehalose and sucrose) and monosaccharides (fructose, galactose and mannose) to glycolytic intermediates.

Phosphorolysis of glycogen releases glucose-1-phosphate which enters glycolysis at fructose-6-phosphate, thereby, bypassing the first step in which ATP is consumed. This is advantageous as it increases the yield of ATP to three per glucose from glycolysis instead of two.

The dietary polysaccharides, oligosaccharides and disaccharides are hydrolysed to monosaccharides by a combination of digestive enzymes that act step by step, as the food moves down the digestive tract. On the other hand, the endogenous glycogen undergo phosphorolytic cleavage from the non reducing end, releasing glucose 1-phosphate one by one until they reach a branch point. The debranching enzyme takes over to remove branches. The details of this process will be dealt in glycogen metabolism. Finally, glucose-1 phosphate is isomerised to the glycolytic intermediate glucose-6 phosphate by a phosphoglucomutase (PGM).

The disaccharides like sucrose, lactose, maltose and trehalose are hydrolysed to monosaccharides (Fig.2.5). The entry of fructose, mannose and galactose to the glycolytic pathway is explained in detail below.

Metabolism of Fructose

Fructose is a ketohexose that is released from the hydrolysis of sucrose (table sugar) and is also present in free form in many fruits and honey. It is either directly phosphorylated by hexokinase (muscles and kidney) or goes through

the **fructose 1-phosphate pathway** (liver). The latter pathway is a three step conversion of fructose to glyceraldehyde 3-phosphate, initiated by fructokinase (Fig.2.5):

1. The liver fructokinase transfers the phosphoryl group from ATP to C-1 of fructose.
2. Fructose-1-phosphate is split by **fructose-1-phosphate aldolase** into glyceraldehyde and DHAP. You already know how DHAP enters glycolysis.
3. Glyceraldehyde is converted into glyceraldehyde-3-phosphate by **triose kinase**.

Many people do not produce enough lactase; therefore, cannot digest milk or milk products. As a result, lactose is fermented by gut bacteria leading to symptoms like gastric discomfort, diarrhoea, bloating, nausea and gas.

Metabolism of Galactose

D-Galactose is an aldohexose that is obtained by the hydrolysis of milk sugar, lactose. Lactose is a disaccharide that is hydrolysed by lactase to glucose and galactose by β -galactosidase / lactase. The conversion of galactose to glucose 1-phosphate goes through UDP-linked sugar derivative and uses NAD^+ for both oxidation and reduction (Fig. 2.6).

1. Galactose is phosphorylated to galactose-1-phosphate by **galactokinase**.



2. Galactose-1-phosphate is converted into UDP-Galactose by UDP- glucose: galactose-1-phosphate uridylyl transferase. In this reaction UDP-glucose is the donor of uridine monophosphate (UMP) and itself is converted to glucose-1-phosphate. Glucose-1-phosphate is isomerised to glucose-6-phosphate by PGM.

3. UDP-Galactose is converted to UDP-glucose by UDP galactose-4-epimerase. In this reaction C-4 of galactose is first oxidised and then reduced by NAD^+ that also results in the inversion of its configuration. UDP-glucose can participate in another round.

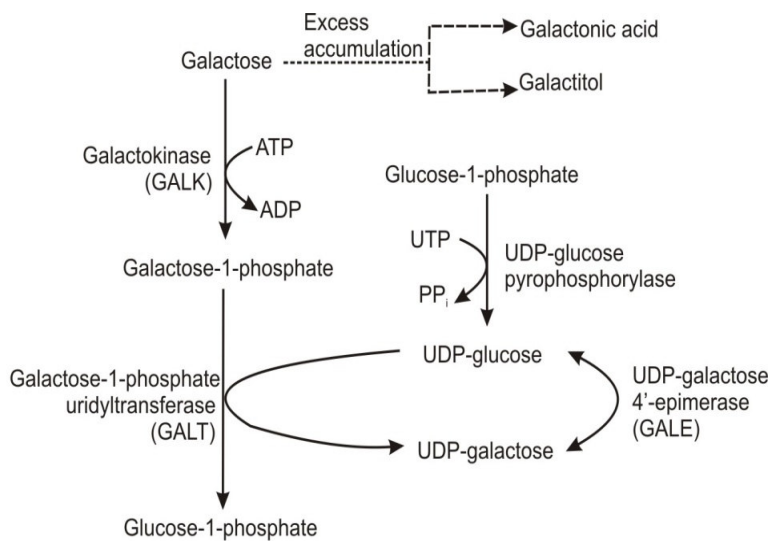


Fig. 2.6: Feeder pathway of galactose.

Individuals with a defect in any of three enzymes of galactose catabolism have **galactosemia**. The most severe condition results from the deficiency of uridylyl transferase in which children have mental retardation, poor growth and liver damage that may lead to death even when they are fed on galactose free diet.

The survivors also suffer from cataract due to deposition of galactitol in lens of the eye. A deficiency of the other two enzymes is relatively less severe especially when dietary control is rigidly followed.

Finally, D-mannose is converted to mannose-6- phosphate by hexokinase and then isomerised to fructose-6- phosphate by phosphomannose isomerase (Fig. 2.5).

SAQ 3

Complete the feeder pathway of galactose with names of missing intermediates and the enzymes involved.

E1 E2 E3

Galactose → _____ → Glucose-1- phosphate → _____ glycolysis.

2.7 FATES OF PYRUVATE

We studied in the previous sections that fate of pyruvate depends on whether it is catabolised under aerobic or anaerobic conditions. We learnt that under anaerobic conditions it is fermented to a variety of end products like lactic acid or ethanol.

In this section we shall discuss what happens to pyruvate under aerobic condition.

Under aerobic conditions pyruvate is completely degraded to CO₂ and water by the combined action of pyruvate dehydrogenase complex (PDH), citric acid cycle and electron transport chain. In eukaryotes, all this takes place in the mitochondria.

Most of the ATP is generated by oxidative phosphorylation. In this respect Louis Pasteur made a very significant observation while studying fermentation in yeast. He discovered that glucose consumption dramatically falls if yeast (facultative anaerobe) is shifted from anaerobic to aerobic conditions.

The basis of '**Pasteur effect**' can now be explained by the stringent regulation of glycolysis at PKF-I catalysed step. The details of catabolism beyond pyruvate under aerobic conditions are discussed in the next unit.

In addition to the catabolic fate, pyruvate also serves as starting material in anabolism in all organisms. Fig. 2.7 shows diverse fates of pyruvate under different conditions.

Pyruvate, the end product of glycolysis can form lactate, ethanol or acetyl CoA under different conditions.

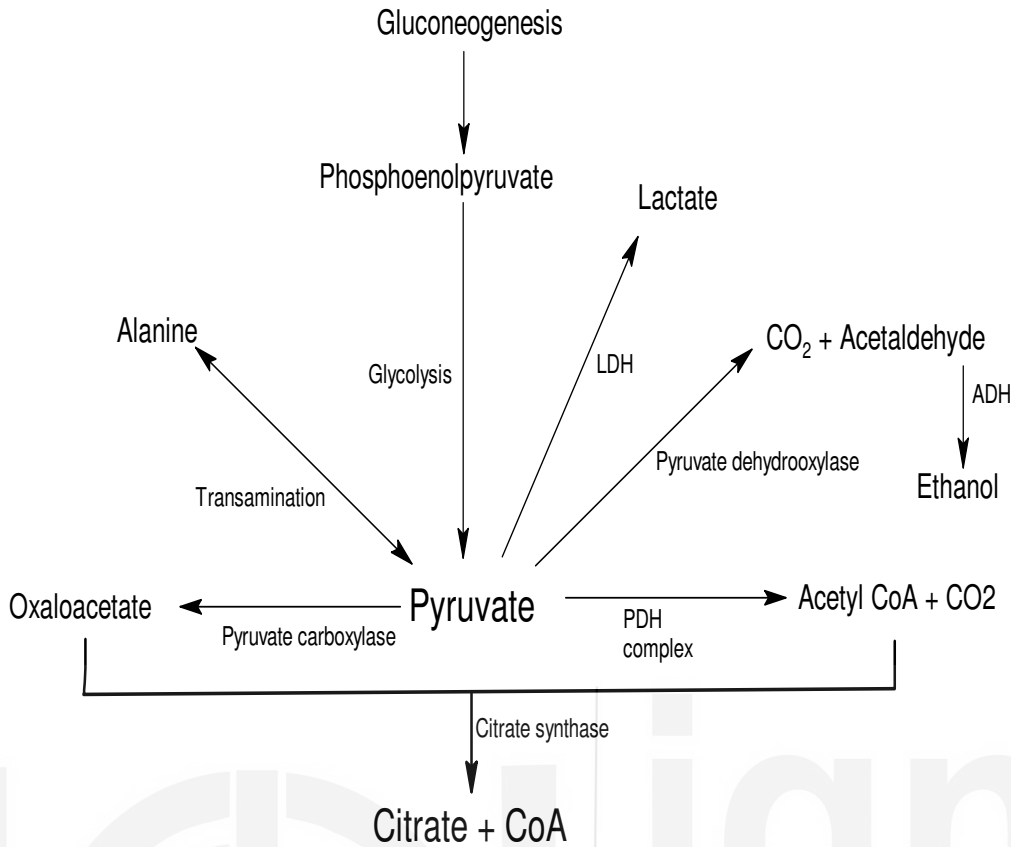


Fig. 2.7: Fates of pyruvate.

2.8 REGULATION OF GLYCOLYSIS

Glycolysis is the first step towards generation of energy from glucose. In addition, it also serves as primary pathway for metabolism of other monosaccharides. In general, the regulation of metabolic pathways is best achieved at steps that are far from equilibrium and are essentially irreversible. The glycolysis pathway is therefore regulated at steps catalysed by hexokinase, phosphofructokinase-I and pyruvate kinase. In this section, allosteric regulation of the three enzymes will be explained. We will come back to this again after gluconeogenesis to understand how the two pathways are reciprocally regulated.

The regulation of carbohydrate breakdown occurs at the level of glycolysis, citric acid cycle and glycogenolysis.

➤ Hexokinase

The enzyme hexokinase is inhibited by its product, glucose-6-phosphate. In many organisms including humans, isozymes of hexokinase exist and they may or may not be inhibited by glucose-6-phosphate; for instance the human liver enzyme is not subject to product inhibition. This reaction is not subject to stringent control so that glucose-6-phosphate can be fed to other pathways such as glycogen synthesis and pentose phosphate pathway.

➤ Phosphofructokinase-I (PFK-I)

The reaction catalysed by PFK-I is the first unique step of glycolysis and the product is only fed to glycolysis. The enzyme is regulated by multiple allosteric activators and inhibitors, although the regulation varies between organisms.

Allosteric inhibitors include ATP, citrate and H^+ ion concentration (low pH). ATP inhibits the enzyme by decreasing its affinity (high K_m) for fructose 6-phosphate. A high concentration of citrate intensifies the inhibitory effect of ATP and it favours the dissociation of PFK-I from an active tetramer to an inactive dimer.

Allosteric activators are fructose 2,6-bisphosphate, ADP, AMP and P_i . Among them fructose 2,6-bisphosphate is the most important regulator of PFK-I. It activates PFK-I at very low concentration as compared to other effectors by increasing the affinity of the enzyme for the substrate and simultaneously decreasing the affinity of ATP and citrate.

Fructose 2,6-bisphosphate (F26BP) is formed from fructose-6-phosphate by a bifunctional enzyme that has both kinase (PFK-2) and phosphatase activity (Fig. 2.8). The activities of PFK-2 and fructose 2,6-bisphosphatase are controlled by reversible covalent modification (phosphorylation/dephosphorylation) that is dependent on hormonal (insulin/glucagon) response. Glucagon released in response to low blood glucose levels results in phosphorylation of enzyme which converts fructose-2,6-bisphosphate back to fructose-6-phosphate which slows down the glycolysis. Insulin has opposite effect.

Insulin is released when blood glucose levels are high and glucagon is released when blood glucose levels drop.

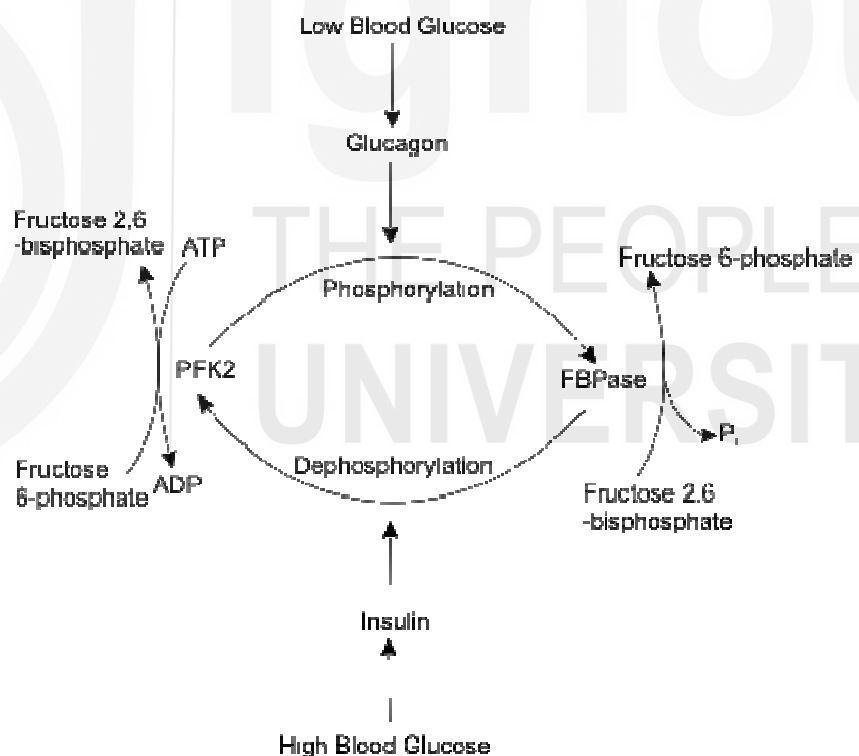


Fig. 2.8: Regulation of PFK –I by fructose-2,6- bisphosphate.

Pyruvate kinase is regulated by allosteric effectors. Some of its isozymes are also regulated by reversible covalent modification. The enzyme is allosterically inhibited by high concentrations of ATP, acetyl CoA and long chain fatty acids. The liver isozyme is regulated by phosphorylation and is active as dephosphoenzyme. The hormone glucagon released in response to low glucose slows down hepatic glycolysis by activating cAMP dependent protein kinase that phosphorylates pyruvate kinase. As conditions change a phosphatase removes the phosphate and makes it active. Fig. 2.9 summarizes

the regulation of glycolysis. In muscles an increase in cAMP stimulates glycolysis.

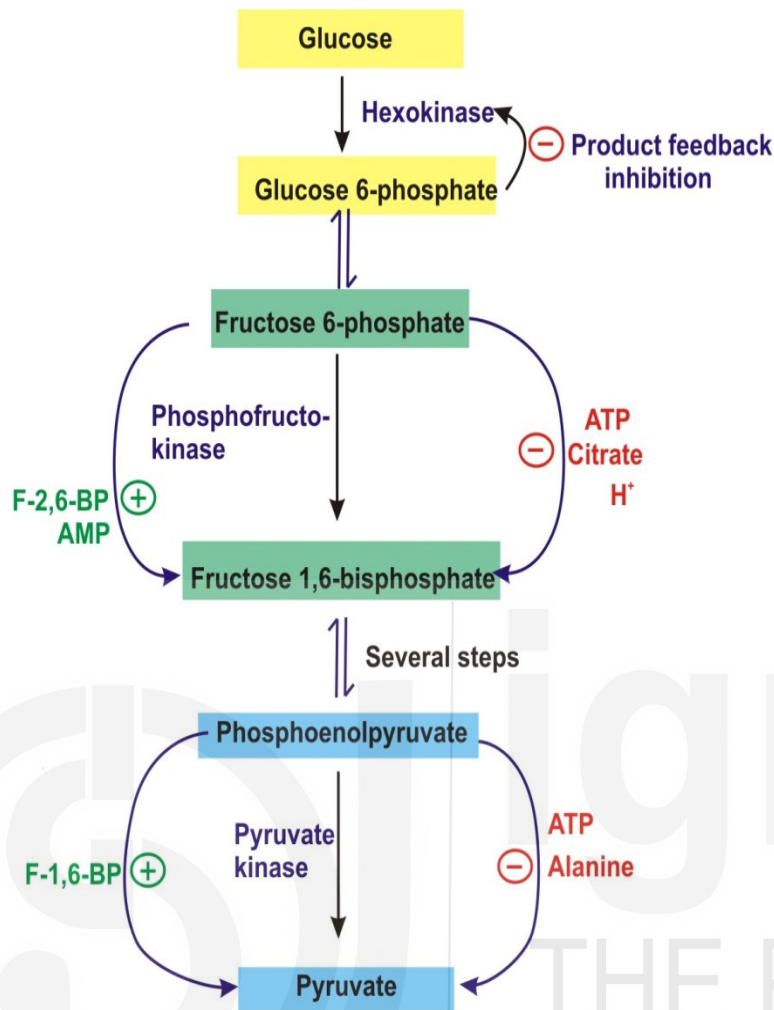


Fig. 2.9: Regulation of glycolysis- An overview.

SAQ 4

Choose the most appropriate glycolytic enzyme:

- Depletion of cell's ATP supply activates ----- (Hexokinase / Pyruvate kinase / Glucokinase / Phosphofructokinase-1).
- High concentration of glucose-6-phosphate inhibits ----- (Hexokinase / Pyruvate kinase / Glucokinase / PFK -1)
- The committed step of glycolysis is catalyzed by ----- (Hexokinase/Pyruvate kinase/ Glucokinase / Phosphofructokinase-1).

2.9 SUMMARY

- Glycolysis is a near universal pathway of ten reactions in which glucose (or related hexoses) is converted to two molecules of pyruvate with net production of two ATP.
- It is divided into two phases: Energy investment / preparatory phase and energy generation / pay off phase.

- The preparatory phase has two reactions which require input of energy in the form of ATP to synthesise activated phosphorylated intermediates. It ends with the splitting of fructose 1,6- biphosphate to two 3 carbon sugars.
- The pay off phase includes one oxidation and two steps of substrate level phosphorylation from each three carbon sugar. It ends with the generation of two molecules of pyruvate, 2 NADH + H⁺ and 4 ATP. The net ATP yield of glycolysis is therefore only 2.
- Under anaerobic conditions, pyruvate is converted to either lactate or ethanol. This process is known as fermentation and its purpose is to regenerate NAD⁺. It is an inefficient process.
- The glycolytic pathway is regulated at steps catalysed by hexokinase, phosphofructokinase-I and pyruvate kinase. These steps are essentially irreversible under physiological conditions.
- The synthesis of lactate in the skeletal muscles and its conversion back to glucose by the liver for use largely by the muscles constitutes the **Cori cycle**. It is a temporary measure taken by the muscles to cope up with the energy requirements under hypoxic (low oxygen) conditions.
- Monosaccharides other than glucose such as fructose, mannose and galactose can be transformed into glycolytic intermediates and catabolised.
- Under aerobic conditions pyruvate is completely degraded to CO₂ and water by the combined action of pyruvate dehydrogenase complex (PDH), citric acid cycle and electron transport chain.

2.10 TERMINAL QUESTIONS

1. What are the two phases of glycolysis? What is the net outcome of each phase?
2. What is substrate level phosphorylation? Give an example of glycolytic reaction that synthesises ATP by substrate level phosphorylation.
3. Indicate the significance of the Cori cycle.
4. Briefly explain the regulation of glycolysis. Why is step 3 and not step 1 of glycolysis the major control point?
5. What is the role of feeder pathways in carbohydrate metabolism? Support your answer with suitable examples.
6. If C-1 of glucose is ¹⁴C labelled, which carbon(s) of ethanol will be labelled?
7. Differentiate between PFK-1 and PFK-2
8. What would be the net yield of ATP if glucose is fermented to lactate in the presence of arsenate?

2.11 ANSWERS

Self-Assessment Questions

1. i) f) ii) d) iii) a) iv) e) v) b) vi) c)
2. i) Fermentation
ii) Two
iii) Skeletal muscle/ cornea of eye;
iv) Ethanol and CO₂
v) Regenerate NAD⁺
3. E1- galactokinase; E2- galactose-1-phosphate uridylyl transferase; E3- phosphoglucomutase

Intermediates: galactose-1-phosphate; glucose-6- phosphate
4. i) Pyruvate kinase and Phosphofructokinase-1
ii) Hexokinase
iii) Phosphofructokinase-1

Terminal Questions

1. Investment (preparatory) and pay off phase. The investment phase consumes two molecules of ATP where as the pay off phase results in formation of 4 ATP molecules by substrate level phosphorylation and 2 molecules of NADH. Refer to section 2.3 for more details.
2. When ATP is synthesised by phosphoryl group transfer from activated phosphorylated substrate to ADP is known as substrate level phosphorylation. For example the conversion of 1,3 BPG to 3- PGA..
3. The synthesis of lactate in the skeletal muscles and its conversion back to glucose by the liver for use largely by the muscles constitutes the **Cori cycle**. Lactic acid formation is temporary measure taken by the muscles to cope up with the energy requirements under hypoxic (low oxygen) conditions.
4. The glycolytic pathway is regulated at the steps catalysed by hexokinase, phosphofructokinase-I and pyruvate kinase. Step 1 of glycolysis is not subject to stringent control because glucose-6-phosphate also fed to pathways such as glycogen synthesis and pentose phosphate pathway. Reaction 3 catalysed by PFK-I is the first unique step of glycolysis and the product is only directed to glycolysis. Therefore, it is more important for regulation of glycolysis. Refer to Fig.2.11 for an overview of regulation of glycolysis.
5. Feeder pathways allow use of sugars other than glucose, either obtained from diet or synthesised endogenously to be processed by glycolysis. Refer to section 2.5 for more details.

6. None; labelled C-1 will be removed as CO_2 because following the isomerase reaction C-1, C-2 and C-3 are indistinguishable from C-6, C-5 and C-4.
7. PFK-1 catalyzes conversion of fructose-6- phosphate to fructose-1,6- phosphate and is the stringently regulated step of glycolysis. PFK-2 is part of a bifunctional enzyme and converts fructose-6- phosphate to fructose-2,6- phosphate that functions as a positive allosteric regulator of PFK-1 activity. PFK-1 is affected by ATP concentration but PFK-2 is not.
8. Arsenate resembles phosphate and may replace phosphate in glyceraldehyde-3-phosphate dehydrogenase reaction. As a result, ATP is not generated by substrate level phosphorylation, although glycolysis will proceed. Therefore, there would be no net yield of ATP.



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