Al-Mustaqbal University

جامعة المستقبل
AL MUSTAQBAL UNIVERSITY

College of Medical and Health Techniques

Medical Laboratories Techniques Departments

Biochemistry Lectures for 2nd Year Students

First Semester

(2 Credit Hrs. Theory + 4 Credit Hrs. Practice / Week = 4 Credit Unit

Academic Year: 2024 - 2025

Course Organizers:

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Lecture No. 8

Date: Dec., 1st , 2024

Carbohydrate Metabolism - Glycolysis

Objectives:

The reader will be able to know and answer questions on the following topics:

1. Digestion of carbohydrates
2. Absorption of glucose and glucose transporters
3. Glycolysis pathway and its regulation
4. Energy yield from glycolysis
5. Roles of pyruvate dehydrogenase complex with its regulations.

The major pathways of glucose metabolism include glycolysis (aerobic and anaerobic), gluconeogenesis, and pentose phosphate pathway. Glucose occupies a central position in the metabolism of plants, animals, and many microorganisms. It is relatively rich in potential energy, and thus a good fuel; the complete oxidation of glucose to CO_2 and H_2O proceeds with a standard free-energy change of −2,840 kJ/mol. In animals and vascular plants, glucose has four major fates:

1. Used in the biosynthesis of complex polysaccharides.
2. Stored in cells (as a polysaccharide as starch or glycogen or as sucrose).
3. Oxidized to a three-carbon compound (pyruvate) via glycolysis to provide ATP and metabolic intermediates.
4. Oxidized via the pentose phosphate pathway to yield ribose-5-phosphate for nucleic acid synthesis and NADPH for reductive biosynthesis, (Fig. 1).

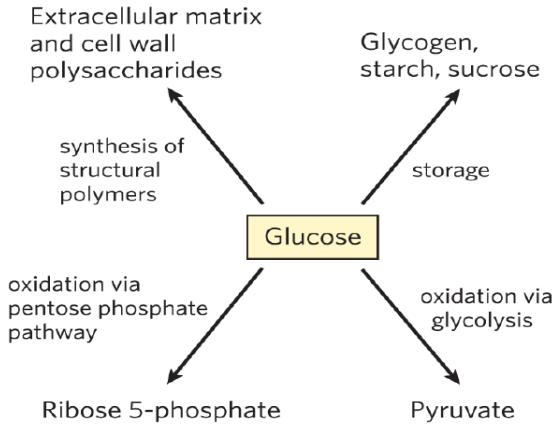


Fig. 1: Major pathways of glucose utilization.

These principles are central to understanding glucose metabolism, but many apply to all metabolic pathways:

P1 Metabolites like glucose are often activated with a high-energy group before their catabolism. Glycolysis is a nearly universal 10-step metabolic pathway for producing ATP by the oxidation of glucose. In this process, two molecules of ATP are invested to activate glucose, but the products of the pathway include four ATP, as well as NADH + H^+ (a form of reducing power) and the triose pyruvate, which can be metabolized further in other pathways.

P2 Glucose and other hexoses and hexose phosphates obtained from stored polysaccharides or dietary carbohydrates feed into the glycolytic pathway.

P3 Pyruvate formed under anaerobic conditions is reduced to lactate with electrons from NADH + H^+, recycling NADH to NAD^+ and allowing continued glycolysis in the processes of lactate or alcohol fermentation. Manipulation of the fermentable material and the microorganisms present allows the synthesis of a variety of industrial products and foods.

P4 Gluconeogenesis is the synthesis of glucose from simpler precursors like pyruvate and lactate.

P5 Glycolysis and gluconeogenesis are reciprocally regulated so that both processes don't occur simultaneously. Most regulatory mechanisms act on reactions that are unique to each pathway.

P6 The pentose phosphate pathway is an alternative pathway for glucose oxidation. It yields pentoses for nucleotide synthesis and reduced cofactors for biosynthesis of fatty acids, sterols, and many other compounds.

A Metabolic Strategy

Central to energy metabolism is the maintenance of a blood glucose concentration of about 5 mM, which is essential for normal cerebral function. Confusion and coma can result if blood glucose falls below 3 mM, while serious vascular damage may occur if its levels exceed 8 mM for significant periods.

After a meal, glucose concentrations in the portal venous blood can easily reach 20 mM. Much of this excess will be removed by the liver. Stimulation of insulin release results in the uptake of glucose by the peripheral tissues (muscle and adipose tissue). Excess glucose is stored locally in skeletal muscles and liver as glycogen, but mostly it is converted into fats.

Blood glucose of 5 mM is sufficient for just a few minutes of normal activity. This level of glucose is actively defended by the liver, which removes glucose when too high, and replenishes it when too low. Both the supply and the demand for glucose may vary more than 20-fold over a 24-hour period; both can change suddenly and sometimes without warning. The liver can both uptake and secrete glucose; it is one of the few tissues in the body to permit bi-directional glucose transport (enterocytes and kidney are others). Although glucose is the essential cerebral energy supply, most tissues preferentially use fat as an energy source.

Biomedical Importance

Most tissues have at least some requirements for glucose. In the brain, the requirement is substantial, and even in prolonged fasting the brain can meet no more than about 20% of its energy needs from ketone bodies. Glycolysis, the

major pathway for glucose metabolism, occurs in the cytosol of all cells. It can function either aerobically or anaerobically, depending on the availability of oxygen and the electron transport chain. Erythrocytes, which lack mitochondria, are completely reliant on glucose as their metabolic fuel, and metabolize it by anaerobic glycolysis. However, to oxidize glucose beyond pyruvate (the end product of glycolysis) requires both oxygen and mitochondrial enzyme systems: the pyruvate dehydrogenase complex, the citric acid cycle, and the respiratory chain.

Figure-2 indicates the major pathways of glucose utilization; these three pathways are the most significant in terms of the amount of glucose that flows through them in most cells.

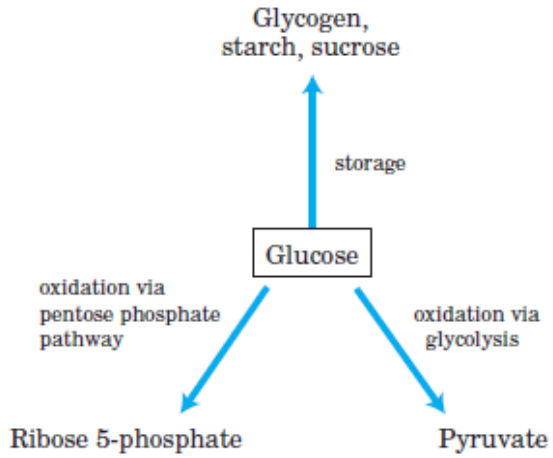


Fig. 2. Major pathways of glucose utilization

Digestion of Carbohydrates:

1. In the diet, carbohydrates are present as complex polysaccharides (starch, glycogen), and to a minor extent, as disaccharides (sucrose and lactose). They are hydrolyzed to monosaccharide units in the gastrointestinal tract.
2. The process of digestion starts in mouth by the salivary alpha-amylase. However, the time available for digestion in the mouth is limited, because the gastric hydrochloric acid will inhibit the action of salivary α-amylase.
3. In the pancreatic juice another α-amylase is available, which will hydrolyze the α-1,4-glycosidic linkages randomly, so as to produce smaller subunits like maltose, isomaltose and branched or unbranched oligosaccharides.
4. The cells of brush border of intestine contain the enzymes, sucrase, maltase, isomaltase and lactase. They hydrolyze the corresponding disaccharides into component monosaccharides, which are then absorbed.

Absorption of Carbohydrates:

Only monosaccharides are absorbed by the intestine. Absorption rate is maximum for galactose; moderate for glucose; and minimum for fructose. Glucose has specific transporters, which are transmembrane proteins. Table-1 shows a summary of some glucose transporters.

Table-1. Some glucose transporters

Transporter	Present in	Properties
GluT1	RBC, brain, kidney, colon, retina, placenta	Glucose uptake in most of cells
GluT2	Serosal surface of intestinal cells, liver, beta cells of pancreas	Low affinity, glucose uptake in liver, glucose sensor in beta cells
GluT3	Neurons, brain	High affinity, glucose into brain cells
GluT4	Skeletal, heart muscle, adipose tissue	Insulin-mediated glucose uptake
GluT5	Small intestine, testis, sperms, kidney	Fructose transporter, poor ability to transport glucose

Glucose Transporter 4

1. GluT4 is the major glucose transporter in skeletal muscle and adipose tissue.
2. GluT4 is under the control of insulin. But other glucose transporters are not under the control of insulin.
3. Clinical application: Insulin promotes the translocation of intracellular GluT4 molecules to the cell surface and thus increases glucose uptake. In type 2 diabetes mellitus, membrane GluT4 is reduced, leading to insulin resistance in muscle and fat cells. In diabetes, entry of glucose into muscle is only half of normal cells.

The glucose transporters are facilitative transporters that carry hexose sugars across the membrane without requiring energy. They comprise a family of at least 14 members. The well-characterized members of the family are GluT1, GluT2, GluT3, GluT4 and GluT5. The transporters belong to a family of proteins called the solute carriers, see Figure 3.

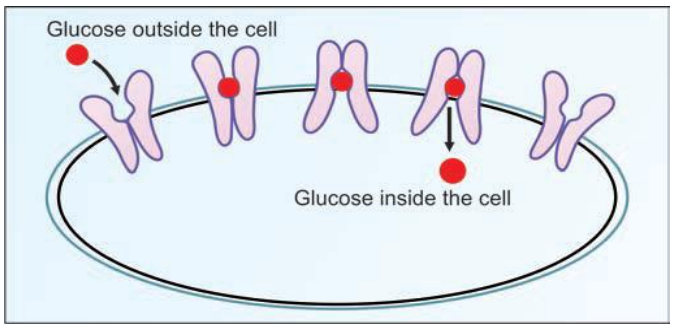


Fig. 3. GluT- Glucose transport in cells.

Glucose Entry into Cells

Glucose transporter-4 (GluT4) transports glucose from the extracellular fluid to muscle cells and adipocytes (Table 1). This translocase is under the influence of insulin. In diabetes mellitus, insulin deficiency hinders the entry of glucose into the peripheral cells. But GluT2 is the transporter in liver cells; it is not under the control of insulin.

Glucose Metabolism:

Clinical importance of glucose

1. Glucose is the preferred source of energy for most of the body tissues. Brain cells derive the energy mainly from glucose.
2. When the glucose metabolism is deranged, life threatening conditions may occur. A minimum amount of glucose is always required for normal functioning.
3. Normal fasting plasma glucose level is 70 to 110 mg/dL. After a heavy carbohydrate meal, in a normal person, this level is below 150 mg/dL. The catabolic oxidation of glucose, to provide cellular energy, occurs principally through three 'linked' catabolic pathways (glycolysis, tricarboxylic acid cycle (TCA cycle) and mitochondrial electron transfer/oxidative phosphorylation).

Glycolysis or Embden-Meyerhof-Parnas Pathway:

Definition: In glycolytic pathway glucose is converted to pyruvate (aerobic condition) or lactate (anaerobic condition), along with production of a small quantity of energy.

Site of reactions: All the reaction steps take place in the cytoplasm. Sugars such as glucose and fructose are catabolized to pyruvate. This is a central pathway for the catabolism of monosaccharides in which the six-carbon sugars are split to three-carbon compounds, with subsequent net production of cellular energy in the form of ATP. This pathway is almost universal to living organisms. Pyruvate is an intermediate in several metabolic pathways; mostly it is converted to acetyl-CoA to feed into the TCA cycle. Under anaerobic conditions, pyruvate is converted to lactate by the enzyme lactate dehydrogenase, thereby re-oxidizing NADH + H^+ to NAD^+ for re-use in glycolysis.

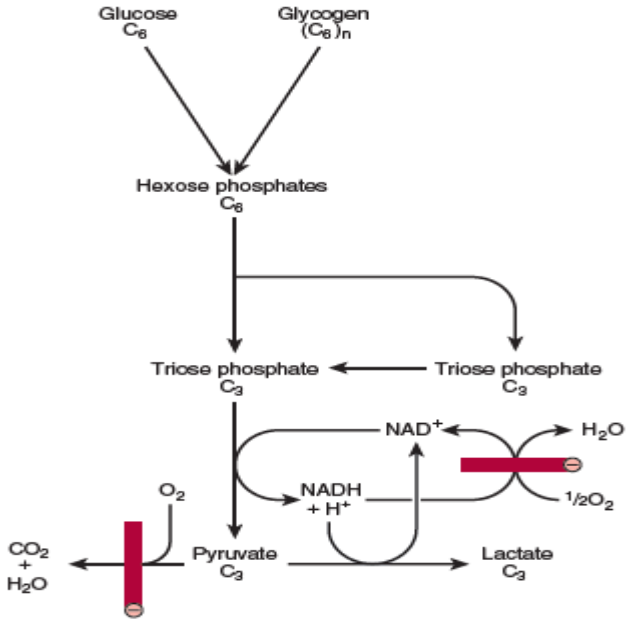


Fig. 4. Summary of glycolysis blocked under anaerobic conditions or by absence of mitochondria containing key respiratory enzymes, as in erythrocytes.

In glycolysis, a molecule of glucose is degraded in a series of enzyme-catalyzed reactions to yield two molecules of pyruvate. During the sequential reactions of glycolysis, some of the free energy released from glucose is conserved in the form of ATP and NADH + H^+. The glycolytic breakdown of glucose is the sole source of metabolic energy in some mammalian tissues and cell types (erythrocytes, renal medulla, brain, and sperm, for example).

Significance of Glycolysis Pathway

1. It is the only pathway that is taking place in all the cells of the body.
2. Glycolysis is the only source of energy in erythrocytes.
3. In strenuous exercise, when muscle tissue lacks enough oxygen, anaerobic glycolysis forms the major source of energy for muscles.
4. The glycolytic pathway may be considered as the initial steps before complete oxidation.
5. The glycolytic pathway provides carbon skeletons for synthesis of non-essential amino acids as well as glycerol part of fat.
6. Most of the reactions of the glycolytic pathway are reversible, which are also used for gluconeogenesis. A summary is shown in Figure-4 and 5.

Steps 1, 3 and 10 are key enzymes; these reactions are irreversible. Steps 6 and 9 produce energy. Steps 5 and 10 are coupled for regeneration of NAD^+. These figures indicate the two phases of glycolysis. For each molecule of glucose that passes through the preparatory phase (a), two molecules of glyceraldehyde-3-phosphate are formed; both pass through the payoff phase (b).

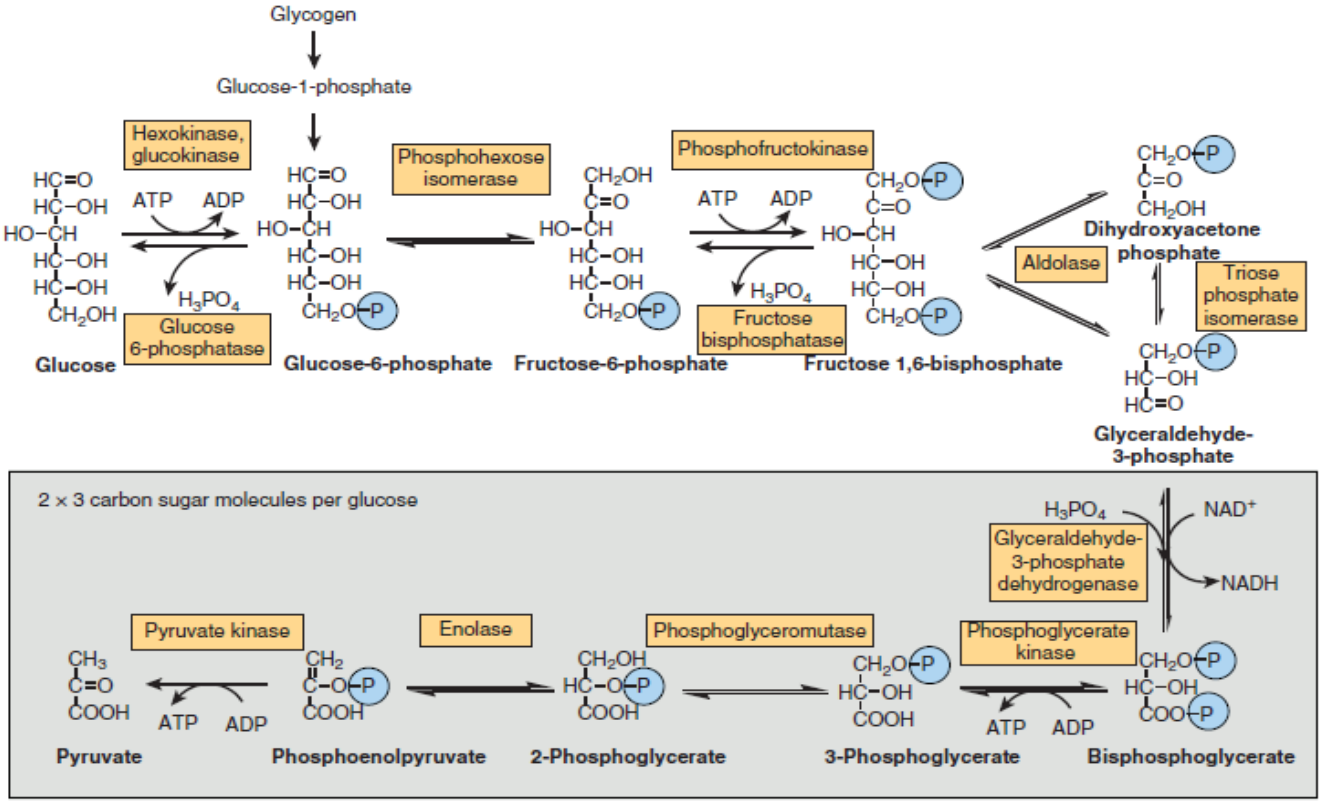


Fig. 5. Summary of glycolysis.

Pyruvate is the end product of the second phase of glycolysis. For each glucose molecule, two ATP are consumed in the preparatory phase and four ATP are produced in the payoff phase, giving a net yield of two ATP per molecule of glucose converted to pyruvate. Keep in mind that each phosphoryl group, represented here as P, has two negative charges (OPO_3^{-2}).

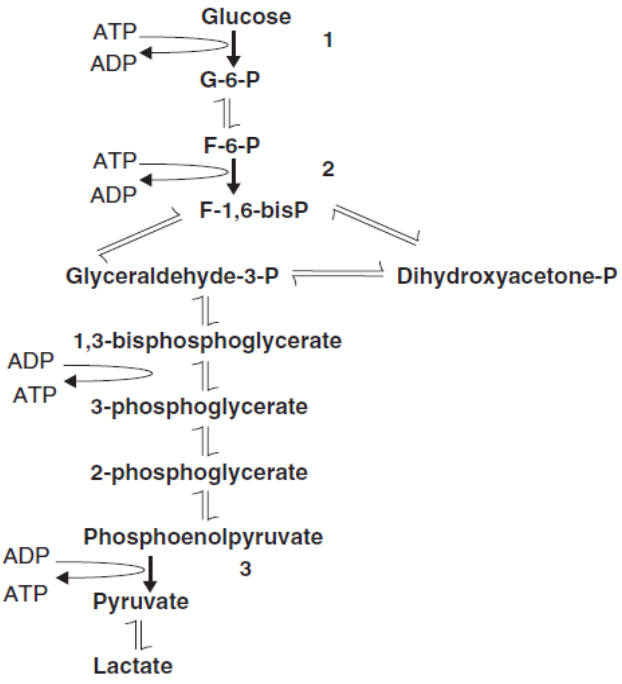


Fig. 6. The glycolytic pathway. The first and third reactions of glycolysis require the input of energy (ATP); the energy investment stage. The 6-carbon sugar is then cleaved to two3-carbon sugars. In the energy generation stage, ATP is formed. As a consequence, for each molecule of glucose (6-carbon) oxidized, two molecules of ATP are invested and four molecules of ATP are generated, giving a net production of two molecules of ATP. Reactions 1–3 are exergonic and essentially irreversible.

Steps of Glycolytic Pathway

Step 1 of Glycolysis

1. Glucose is phosphorylated irreversibly to glucose-6-phosphate (Figure 6).
2. The enzyme is hexokinase (HK), which splits the ATP into ADP, and the Pi is added on to the glucose. The energy released by the hydrolysis of ATP is utilized for the forward reaction.
3. Hexokinase is a key glycolytic enzyme. The kinase reaction is irreversible.
4. Hexokinase and glucokinase may be considered as iso-enzymes; their properties are compared in Table 2. Glucokinase is under the influence of insulin; but hexokinase is not. Hexokinase is present in most tissues. Glucokinase with a high k_m for glucose is present in liver and beta cells. Glucokinase is induced by insulin.
5. The metabolic fates of glucose-6-phosphate are shown in Figure 7. The phosphorylation of glucose traps it within the cells. Once phosphorylated, glucose-6- phosphate is trapped within the cell and has to be metabolized.

Table 2. Comparison of hexokinase and glucokinase

	Hexokinase	Glucokinase
Occurrence	In all tissues	Only in liver
k_m value	10^{-2} mmol/L	20 mmol/L
Affinity to substrate	High	Low
Specificity	Acts on glucose, fructose and mannose	Acts only on glucose
Induction	Not induced	Induced by insulin, and glucose
Function	Even when blood sugar is low, glucose is utilized by body cells	Acts only when blood glucose level is more than 100 mg/dL, then glucose is taken up by liver cells for glycogenesis

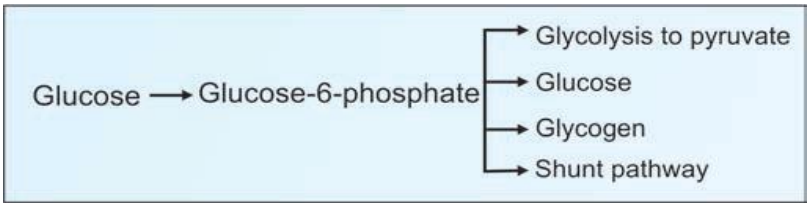


Fig. 7. Fate of glucose-6-phosphate

Step 2 of Glycolysis

Glucose-6-phosphate is readily reversibly isomerized to fructose-6-phosphate by phosphohexose isomerase, Figure 6 which catalyzes the reversible isomerization of glucose-6-phosphate, an aldose, to fructose-6-phosphate

Step 3 of Glycolysis

Fructose-6-phosphate is further phosphorylated to fructose1,6-bisphosphate. The enzyme is phosphofructokinase (PFK) which is the rate-limiting enzyme of glycolysis. It is an allosterically regulated enzyme. The enzyme catalyzes the second phosphorylation step of glycolysis using a second molecule of ATP. It is an important key enzyme of this pathway. This is again an activation process, and the energy is derived from ATP. This reaction is an irreversible step in glycolysis. The steps 1,2 and 3 together are called as the preparatory phase.

Note// Diphosphate and bisphosphate are different (When two phosphate groups are linked together and then attached to a parent compound, it is called diphosphate, e.g. adenosine-di-phosphate. But when phosphoric acid groups are present at two different sites of the compound, it is named as bisphosphate, e.g. fructose-1,6-bisphosphate).

Step 4 of Glycolysis

The fructose-1,6-bisphosphate is cleaved reversibly into two 3 carbon units; one glyceraldehyde-3-phosphate and another molecule of dihydroxy acetone phosphate (DHAP) by aldolase.

Only one of the two triose phosphates formed by aldolase, glyceraldehyde-3-phosphate, can be directly degraded in the subsequent steps of glycolysis. The other product, dihydroxyacetone phosphate, is immediately and reversibly

isomerized and converted to glyceraldehyde-3-phosphate by the fifth enzyme of the glycolytic sequence, triose phosphate isomerase.

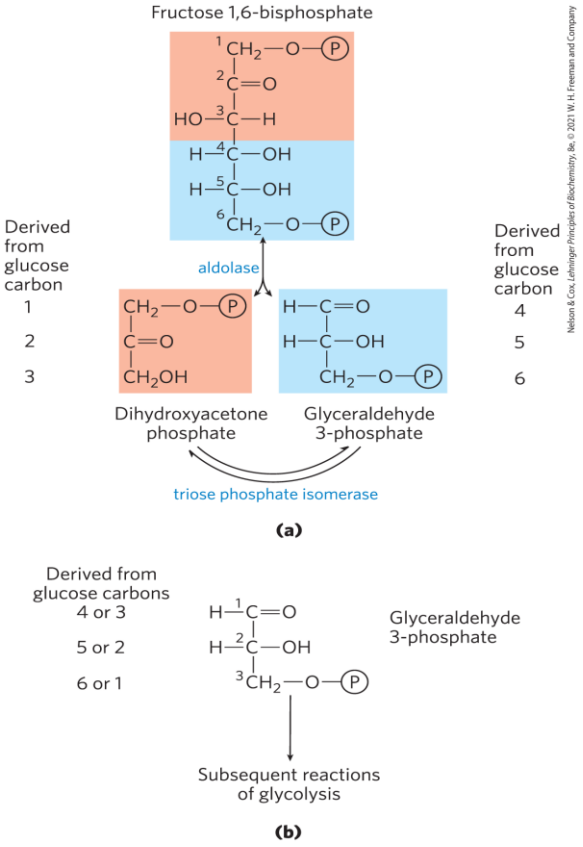


Fig.8. Isomerization reaction and fate of the glucose carbons in the formation of glyceraldehyde-3-phosphate.

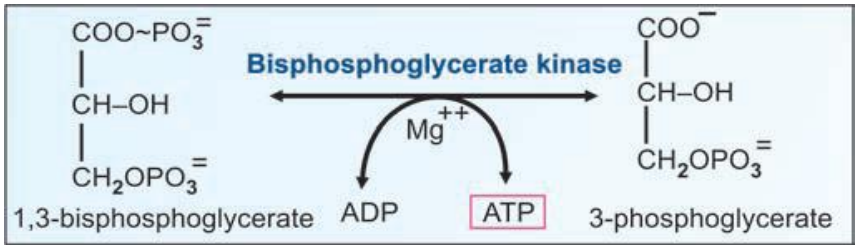
The steps 4 and 4-A are together called the Splitting Phase. Glycerol portion of the neutral fat can enter into glycolytic or gluconeogenic pathways at this point. Similarly for neutral fat synthesis, glycerol is required which can be derived from glucose through DHAP.

Step 5 of Glycolysis

Glyceraldehyde-3-phosphate is dehydrogenated and simultaneously phosphorylated to 1,3-bisphosphoglycerate (1,3-BPG) with the help of NAD^+. The enzyme is glyceraldehyde-3-phosphate dehydrogenase. The product contains a high energy bond. This is a reversible reaction. The enzyme has a cysteinyl SH group at the active center and is inhibited by iodoacetate. During this reaction, NAD^+ is reduced to NADH.

Step 6 of glycolysis.

The energy of 1,3-BPG is trapped to synthesize one ATP molecule with the help of bisphosphoglycerate kinase, see below.



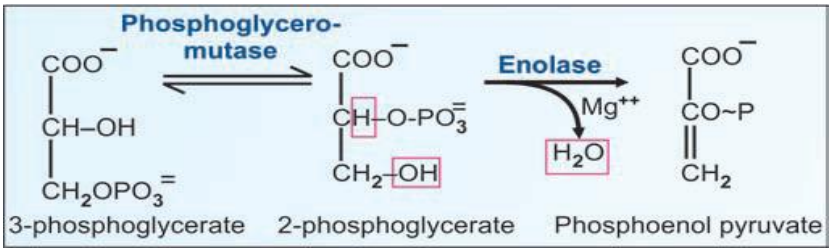
1. This is an example of substrate level phosphorylation, where energy is trapped directly from the substrate, without the help of the complicated electron transport chain reactions.
2. When energy is trapped by oxidation of reducing equivalents such as NADH, it is called oxidative phosphorylation.
3. Arsenate (As) inhibits this reaction since it interferes with the incorporation of the inorganic phosphate into 1,3-BPG. But the oxidation occurs and 3-phosphoglycerate is formed which can undergo further reactions of glycolysis, but ATP is not formed in this step. The two steps of glycolysis together constitute an energy coupling process in which 1,3-bisphosphoglycerate is the common intermediate; it is formed in the first reaction (which would be endergonic in isolation), and its acyl phosphate group is transferred to ADP in the second reaction (which is strongly exergonic). The sum of these two reactions is:

Glyceraldehyde 3-phosphate + ADP + Pi + NAD^+ ⇌ 3-phosphoglycerate + ATP + NADH + H^+

$\Delta G'^\circ = -12.2$ kJ/mol, thus the overall reaction is exergonic.

Step 7 of Glycolysis

3-phosphoglycerate is isomerized to 2-phosphoglycerate by shifting the phosphate group from 3rd to 2nd carbon atom as shown below. The enzyme is phospho-glyceromutase. This is a readily reversible reaction.



Step 8 of Glycolysis

1. 2-phosphoglycerate is converted to phosphoenol pyruvate by the enzyme enolase. One water molecule is removed.
2. A high energy phosphate bond is produced. The reaction is reversible.
3. Enolase requires Mg^{++}, and by removing magnesium ions, fluoride will irreversibly inhibit this enzyme. Thus, fluoride will stop the whole glycolysis. So when collecting blood for sugar estimation, fluoride is added to blood. If not, glucose is metabolized by the blood cells, so that lower blood sugar values are obtained.

Step 9 of Glycolysis

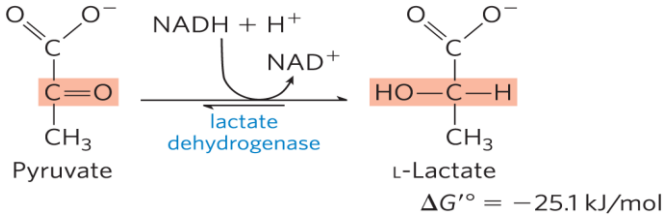
During the reaction, PEP is first converted to pyruvate, the stable form.

1. Phosphoenol pyruvate (PEP) is dephosphorylated to pyruvate, by pyruvate kinase. First PEP is made into a transient intermediary of enol pyruvate; which is spontaneously isomerized into a stable form keto pyruvate.

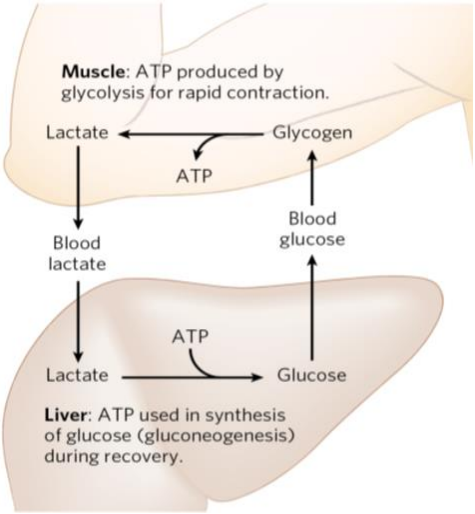
2. One mole of ATP is generated during this reaction. This is again an example of substrate level phosphorylation.
3. The pyruvate kinase is a key glycolytic enzyme. This step is irreversible.

Step 10 of Glycolysis

When animal tissues cannot be supplied with sufficient oxygen to support aerobic oxidation of the pyruvate and NADH produced in glycolysis, NAD^+ is regenerated from NADH by the reduction of pyruvate to lactate. Some tissues and cell types (such as erythrocytes, which have no mitochondria and thus cannot oxidize pyruvate to CO_2) produce lactate from glucose even under aerobic conditions. The reduction of pyruvate is catalyzed reversibly by lactate dehydrogenase (LDH) as shown in Figure below which has 5 iso-enzymes, LDH1, LDH2, LDH3, LDH4 and LDH5. Their presence in normal sera as follows: LDH2>LDH1>LDH3>LDH4>LDH5.

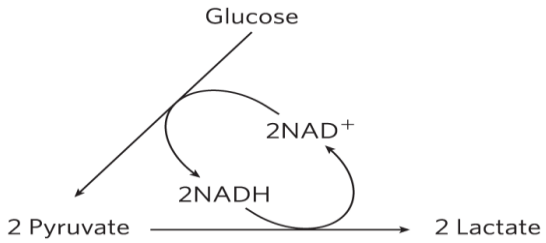


In aerobic conditions, the pyruvate enters the citric acid cycle for complete oxidation. The end product of anaerobic glycolysis is lactate which enters the Cori's cycle in which glucose is converted to lactate in the muscle; and in the liver this lactate is re-converted into glucose as shown in Figure below.



The overall equilibrium of the reaction strongly favors lactate formation, as shown by the large negative standard free-energy change.

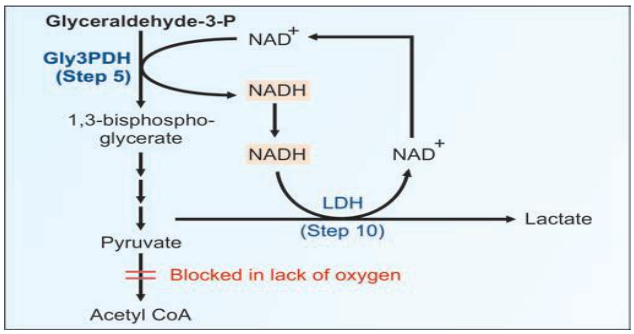
In glycolysis, dehydrogenation of the two molecules of glyceraldehyde-3-phosphate derived from each molecule of glucose converts two molecules of NAD^+ to two of NADH. Because the reduction of two molecules of pyruvate to two of lactate regenerates two molecules of NAD^+, there is no net change in NAD^+ or NADH + H^+ as shown below which indicate the regeneration of NAD+ in anaerobic glycolysis.



The lactate formed by active skeletal muscles (or by erythrocytes or retinal cells) can be recycled; it is carried in the blood to the liver, where it is converted to glucose during the recovery from strenuous muscular activity. When lactate is produced in large quantities during vigorous muscle contraction, the acidification that results from ionization of lactic acid in muscle and blood limits the period of vigorous activity. The best-conditioned athletes can sprint at top speed for no more than a minute.

Significance of Lactate Production

In the 5th step, for each molecule of glucose entering in the pathway, two molecules of NAD^+ are reduced to NADH. The availability of co-enzymes inside a cell is limited. For smooth operation of the pathway, the NADH is to be reconverted to NAD^+. This can be done by oxidative phosphorylation in respiratory chain. However, during exercise, there is lack of oxygen. So, this reconversion is not possible. Therefore, the cell has to couple some other reaction in which NAD^+ is regenerated in the cytoplasm itself. Hence pyruvate is reduced to lactate; the NAD^+ thus generated is reutilized for uninterrupted operation of the 5th step. In RBCs, there are no mitochondria and the energy derive only through glycolysis, where the end product is lactic acid, see figure below in which lactate formation is necessary for reconversion of NADH to NAD^+ during anaerobic condition.



Energy Yield from Glycolysis

1. During anaerobic (oxygen deficient) condition, when one molecule of glucose is converted to 2 molecules of lactate, there is a net yield of 2 molecules of ATP.
2. Four molecules of ATP are synthesized by the 2 substrate level phosphorylations (steps 6 and 9). But 2 molecules of ATP are used in the steps 1 and 3, hence the net yield is only 2 ATP (Table 3).
3. The whole reaction is summarized as

Glucose + 2 Pi + 2 ADP -------> 2 Lactate + 2 ATP

Table- 3: Energy yield (number of ATP generated) per molecule of glucose in the glycolytic pathway, under anaerobic conditions (oxygen deficiency)

Step	Enzyme	Source	No. od ATPs gained per glucose mol
1	Hexokinase	------	Minus 1
3	Phosphofructokinase	-----	Minus 1
6	1,3-bisphosphgoglycerate kinase	ATP	1 x 2 = 2
9	Pyruvate kinase	ATP	1 x 2 = 2
Total : 4 minus 2 = 2			

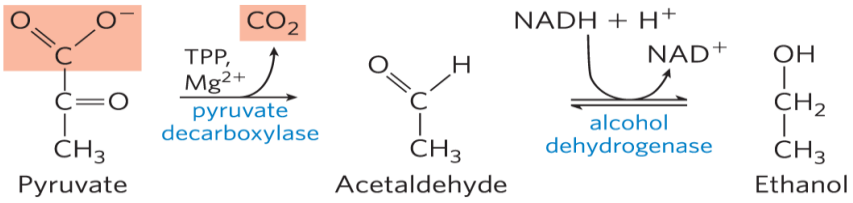
4. But when oxygen is sufficient, the two NADH molecules, generated in the glyceraldehyde-3-phosphate dehydrogenase reaction (step 5), can enter the mitochondrial electron transport chain for complete oxidation. As each NADH provides 2.5 ATPs, this reaction generates 2.5 × 2 = 5 ATPs. Thus when oxygen is available, the net gain of energy from the glycolysis pathway is 7 ATPs (Table 4).
5. Hence the ATP yield from glycolysis is different in anaerobic and aerobic conditions.
6. In aerobic conditions, pyruvate is converted to acetyl-CoA which enters the TCA cycle for complete oxidation. Complete oxidation of glucose through glycolysis plus citric acid cycle will yield a net 32 ATPs.

Table 4. Energy yield (number of ATP generated) per molecule of glucose in the glycolytic pathway, under aerobic conditions (oxygen is available)

Step	Enzyme	Source	No. of ATPs gained per glucose molecule
1	Hexokinase	------	Minus 1
3	Phosphofructokinase	-----	Minus 1
5	Glyceraldehyde-3-phosphate dehydrogenase	$NADH+H^+$	2.5 x 1 = 2.5
6	1,3-Bisphosphoglycerate kinase	ATP	1 x 2 = 2
9	Pyruvate kinase	ATP	1 x 2 = 2
Total : 9 minus 2 = 7			

Ethanol Fermentation

Yeast and other microorganisms ferment glucose to ethanol and CO_2, rather than to lactate. Glucose is metabolized to pyruvate by glycolysis, and the pyruvate is converted to ethanol and CO_2 in a two-step process, see Figure below:



In the first step, pyruvate is decarboxylated to form acetaldehyde in an irreversible reaction catalyzed by pyruvate decarboxylase. This reaction is a simple decarboxylation and does not involve the net oxidation of pyruvate. Pyruvate decarboxylase requires Mg^{2+} and has a tightly bound coenzyme, thiamine pyrophosphate. In the second step, acetaldehyde is reduced to ethanol through the action of alcohol dehydrogenase, with the reducing power furnished by NADH derived from the dehydrogenation of glyceraldehyde-3-phosphate. Ethanol and CO_2 are thus the end products of ethanol fermentation, and the overall equation is

$$\text{Glucose} + 2ADP + 2Pi \rightarrow 2\text{Ethanol} + 2CO_2 + 2ATP + 2H_2O$$

Regulation of Glycolysis:

The regulatory enzymes or key enzymes of glycolysis are:

1. Hexokinase, step 1 (glucokinase, in liver)
2. Phosphofructokinase, step 3 (Table 6)
3. Pyruvate kinase, step 9.

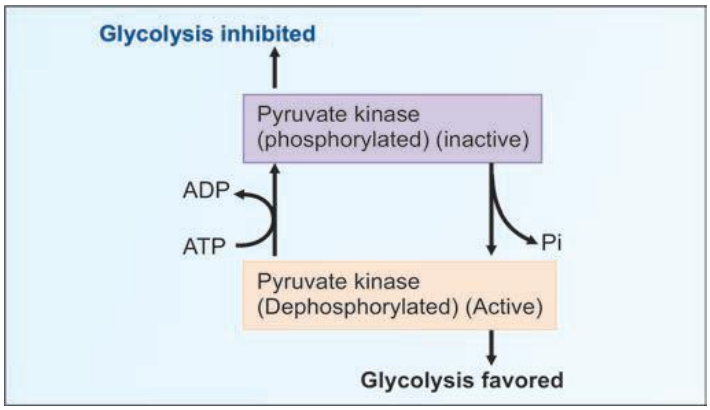
Table 5. Regulatory enzymes of glycolysis

Enzyme	Activation	Inhibition
HK	-----	G-6-P
GK	Insulin	Glucagon
PK	Insulin, F-1,6-BisP	Glucagon, ATP, cyclic AMP
PFK	Insulin, AMP, F-6-P	Glucagon, ATP, citrate, Low pH, cyclic AMP

Factors Regulating Glycolysis

1. Hexokinase having a high affinity for glucose will phosphorylate glucose even at low glucose concentrations to provide energy to tissues that depend on glycolysis for energy needs, e.g. brain and RBCs. However, glucose-6-phosphate has a feedback inhibitory effect on the enzyme.
2. Glucokinase with a low affinity and high k_m for glucose is present only in tissues where the phosphorylation has to take place when glucose is available in plenty. In the liver glucokinase phosphorylates glucose which can be used for glycogen synthesis. Insulin also induces glucokinase.
3. Phosphofructokinase (PFK) (step 3) is the most important rate-limiting enzyme for glycolysis pathway. ATP and citrate are the most important allosteric inhibitors. AMP acts as an allosteric activator.
4. Pyruvate kinase catalyzes an irreversible step and is a regulatory enzyme of glycolysis. When energy is in plenty in the cell, glycolysis is inhibited. Insulin increases its activity whereas glucagon inhibits. Pyruvate kinase is inactive

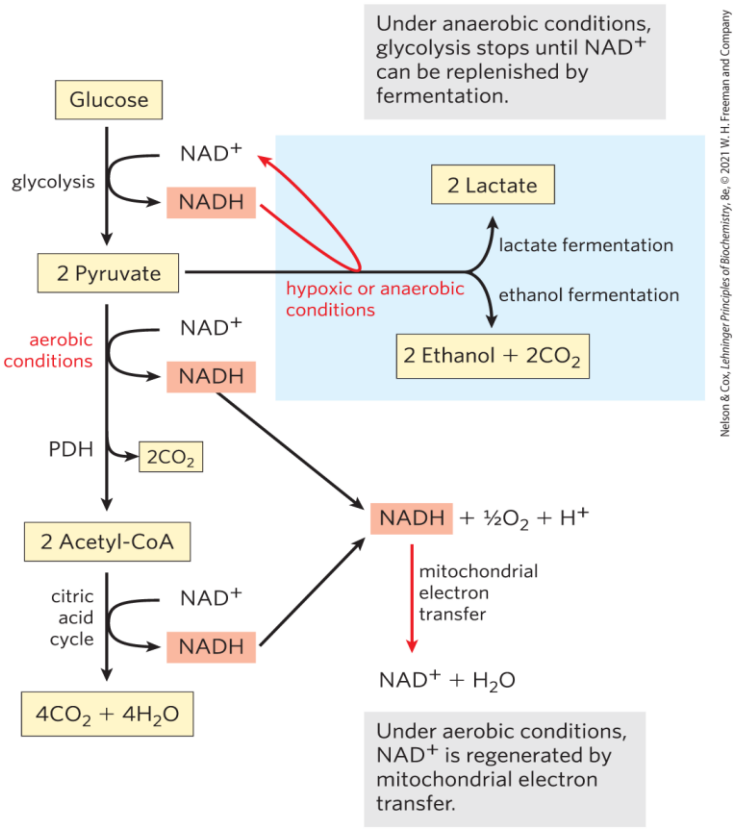
in the phosphorylated state as shown below which indicate the covalent modification of pyruvate kinase reaction.



5. Insulin favors glycolysis by activating the key glycolytic enzymes.
6. Glucocorticoids inhibit glycolysis and favors gluconeogenesis.

Metabolic Fate of Pyruvate:

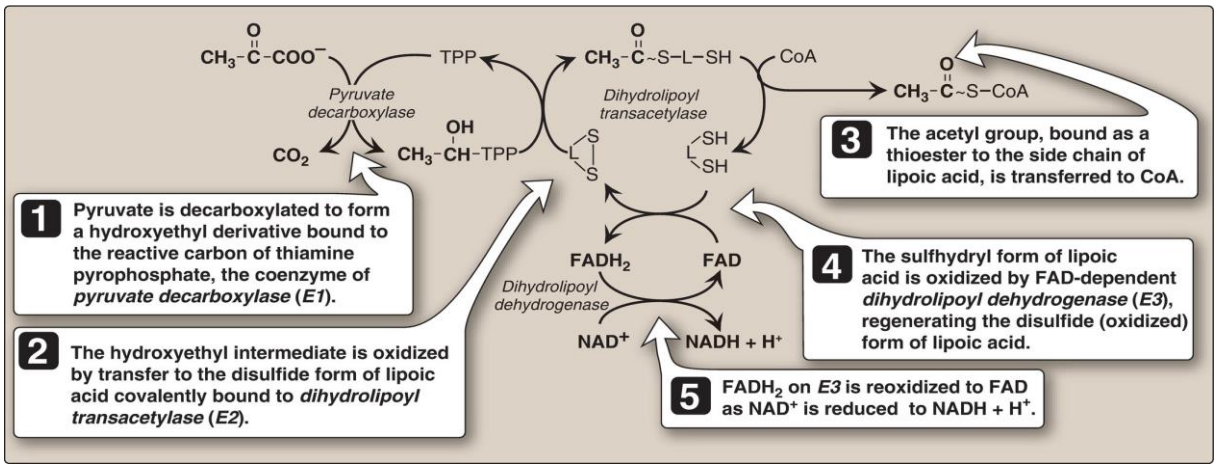
Under aerobic conditions, pyruvate is converted to acetyl-CoA which enters the TCA cycle to be oxidized to CO_2. ATP is generated. Glycolysis is taking place in cytoplasm. So, pyruvate is generated in cytoplasm. This is transported into mitochondria by a pyruvate transporter as shown in the following Figure.



Pyruvate Dehydrogenase Complex

Inside the mitochondria, pyruvate is oxidatively decarboxylated to acetyl-CoA by pyruvate dehydrogenase (PDH). It is a multi-enzyme complex with 5 co-enzymes and 3 apo-enzymes. The co-enzymes needed are (Thiamine pyrophosphate (TPP), Co-enzyme A (CoA), FAD, NAD^+, and Lipoamide). The lipoic acid has two Sulphur atoms and 8 carbon atoms. It can accept or donate

hydrogen atoms. The enzyme part of the PDH complex is made up of three component enzymes as shown below.



Regulation of Pyruvate Dehydrogenase Complex:

PDH is subject to regulation by allosteric mechanisms and covalent modification. Allosteric inhibitors are the products acetyl-CoA and NADH.

Importance of Pyruvate Dehydrogenase

1. Completely irreversible process. Glucose through this step is converted to acetyl-CoA from which fatty acids can be synthesized.
2. But the backward reaction is not possible, and so there is no net synthesis of glucose from fat.
3. Pyruvate may be channeled back to glucose through gluconeogenesis. But oxidative decarboxylation of pyruvate to acetyl-CoA is irreversible. Hence, PDH reaction is the committed step towards complete oxidation of glucose.
4. **Energetics:** The NADH generated in this reaction, enters the electron transport chain to produce 2.5 ATP molecules.
5. Pyruvate dehydrogenase is regulated by end product inhibition as well as by covalent modification. Phosphorylation of the enzyme by a kinase decreases the activity of the enzyme. Dephosphorylation activates the enzyme.
6. Arsenite and mercuric ions react with the -SH groups of lipoic acid and inhibit pyruvate dehydrogenase.
7. Many alcoholics are thiamin deficient (both because of a poor diet and also because alcohol inhibits thiamin absorption), and may develop potentially fatal pyruvic and lactic acidosis.
8. Patients with inherited pyruvate dehydrogenase deficiency, which can be the result of defects in one or more of the components of the enzyme complex, also present with lactic acidosis, particularly after a glucose load. Because of the dependence of the brain on glucose as a fuel, these metabolic defects commonly cause neurological disturbances.