**AL-RASHEED UNIVERSITY COLLEGE**

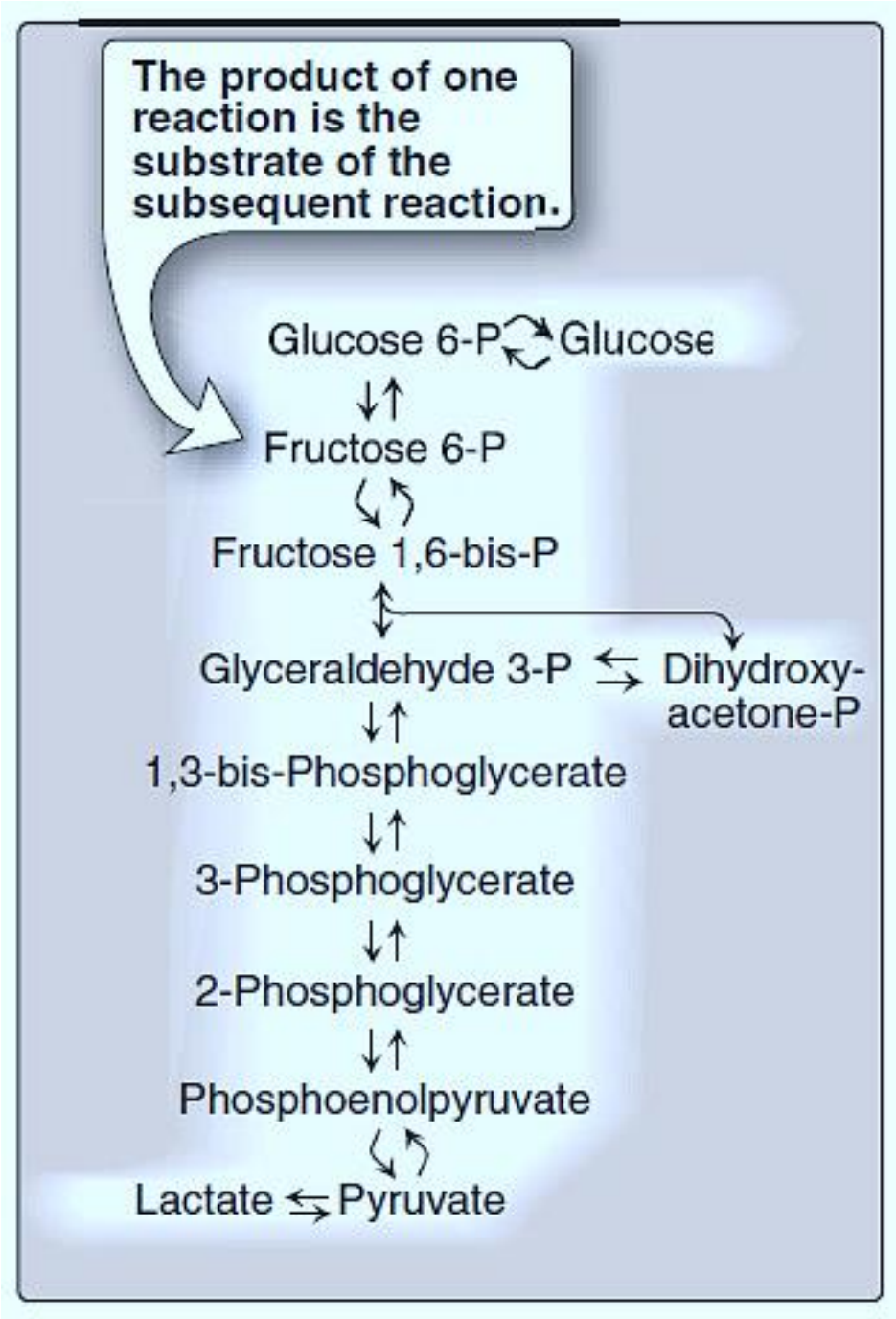
**DEPARTMENT OF MEDICAL LABORATORY TECHNIQUES**

# Introduction to Metabolism and Glycolysis

## Lecture 7

***Prepared By***

***Dr. Kutaiba I. Alzand & Dr. Rusul H. Hamza***



## الوحدة الثانية - المحاضرة الثالثة - الزمن: 90 دقيقة

## أهداف المحاضرة الثالثة:

**يتوقع في نهاية المحاضرة أن يكون الطالب قادراً على:**

By the end of the lecture, the student should be able to:

1. Define the term glycolysis.
2. Know glycolysis shown as one of the essential pathways of energy metabolism.
3. Explain reactions of aerobic glycolysis.
4. Discus reactions of anaerobic glycolysis.
5. Describe the transport of glucose into cells.

# موضوعات المحاضرة الثالثة:

- **OVERVIEW OF GLYCOLYSIS**
- **TRANSPORT OF GLUCOSE INTO CELLS**
  - Sodium-independent facilitated diffusion transport system
    - Tissue specificity of glucose transporter gene express ion
    - Specialized functions of glucose transporter isoforms
  - Sodium–monosaccharide cotransport system

# III. OVERVIEW OF GLYCOLYSIS

- The glycolytic pathway is employed by all tissues for the oxidation of glucose to provide energy (in the form of ATP) and intermediates for other metabolic pathways.
- Glycolysis is at the hub of carbohydrate metabolism because virtually all sugars, whether arising from the diet or from catabolic reactions in the body, can ultimately be converted to glucose (Figure 2.9A).
- Pyruvate is the end product of glycolysis in cells with mitochondria and an adequate supply of oxygen.
- This series of ten reactions is called aerobic glycolysis because oxygen is required to reoxidize the NADH formed during the oxidation of glyceraldehyde 3-phosphate (Figure 2.9B).

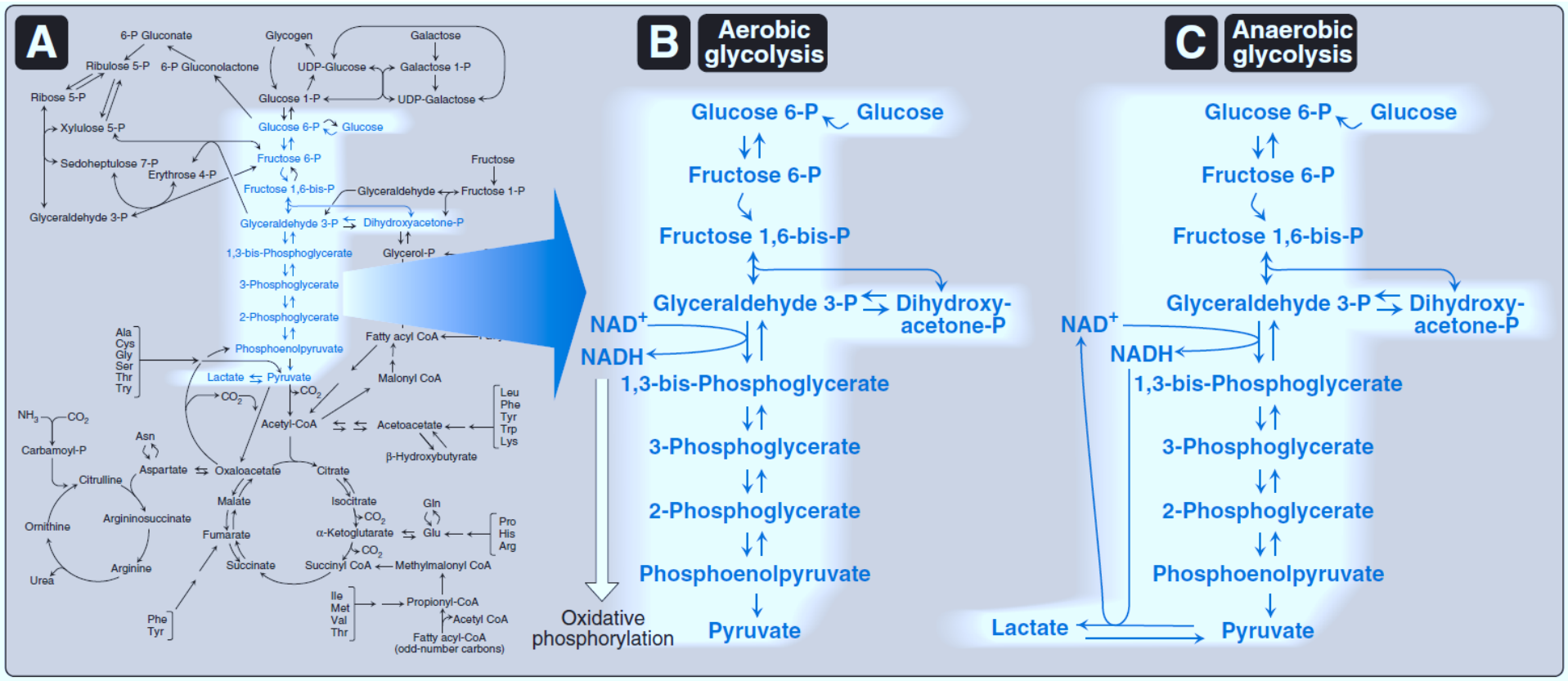



Figure 2.9 A. Glycolysis shown as one of the essential pathways of energy metabolism. B. Reactions of aerobic glycolysis. C. Reactions of anaerobic glycolysis. NAD(H) = nicotinamide adenine dinucleotide; P = phosphate.

- Aerobic glycolysis sets the stage for the oxidative decarboxylation of pyruvate to acetyl CoA, a major fuel of the TCA cycle.
- Alternatively, pyruvate is reduced to lactate as NADH is oxidized to $NAD^+$ (Figure 2.9C).
- This convers ion of glucose to lactate is called anaerobic glycolysis because it can occur without the participation of oxygen.
- Anaerobic glycolysis allows the production of ATP in tissues that lack mitochondria (for example, red blood cells and parts of the eye) or in cells deprived of sufficient oxygen.

# IV. TRANSPORT OF GLUCOSE INTO CELLS

Glucose cannot diffuse directly into cells but enters by one of two transport mechanisms:

- a $Na^+$-independent, facilitated diffusion transport system or
- an ATP-dependent $Na^+$-monosaccharide cotransport system.

## A. Sodium-independent facilitated diffusion transport system

- This system is mediated by a family of 14 glucose transporters found in cell membranes.
- They are designated GLUT-1 to GLUT-14 (glucose transporter isoforms 1–14).

- These monomeric protein transporters exist in the membrane in two conformational states (Figure 2.10).
- Extracellular glucose binds to the transporter, which then alters its conformation, transporting glucose across the cell membrane.

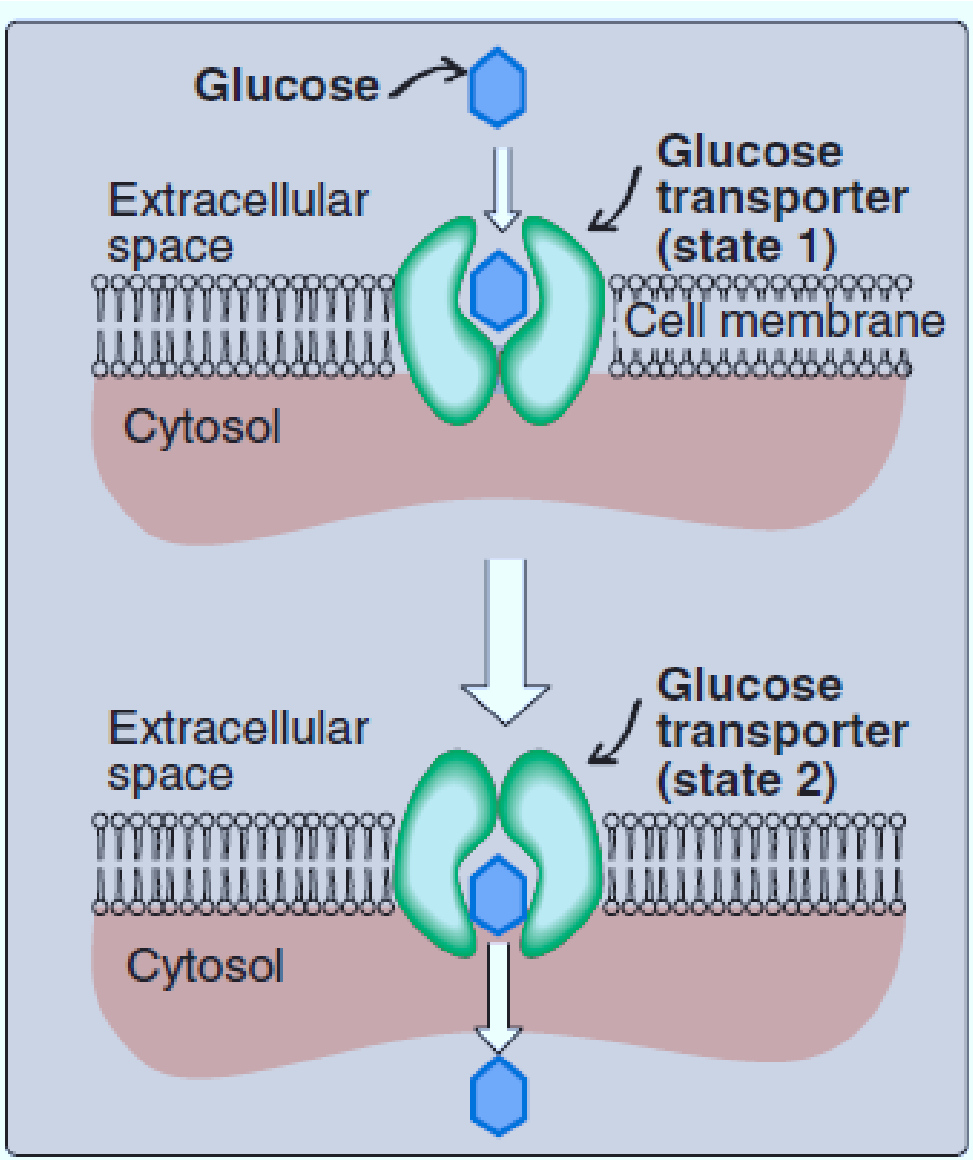



**Figure 2.10** Schematic representation of the facilitate d transport of glucos e through a cell membrane. [Note: Glucose transporter proteins are monomeric and contain 12 transmembrane α helices.]

## 1. Tissue specificity of glucose transporter gene express ion:

The GLUTs display a tissue-specific pattern of expression. For example,

- GLUT-3 is the primary glucose transporter in neurons.
- GLUT-1 is abundant in erythrocytes and the blood–brain barrier but is low in adult muscle, whereas
- GLUT-4 is abundant in muscle and adipose tissue. [Note: The number of GLUT-4 transporters active in these tissues is increased by insulin.]
- GLUT-2 is abundant in liver, kidney, and β cells of the pancreas.
- The other GLUT isoforms also have tissue-specific distributions.

## 2. Specialized functions of glucose transporter isoforms:

- In facilitated diffusion, transporter-mediated glucose movement is down a concentration gradient (that is, from a high glucose concentration to a lower one and, therefore, does not require energy).
- For example, GLUT-1, GLUT-3, and GLUT-4 are primarily involved in glucose uptake from the blood.
- In contrast, GLUT-2, in the liver and kidney, can either transport glucose into these cells when blood glucose levels are high or transport glucose from these cells when blood glucose levels are low (for example, during fasting).
- GLUT-5 is unusual in that it is the primary transporter for fructose (not glucose) in the small intestine and the testes.

# B. Sodium–monosaccharide cotransport system

- This is an energy-requiring process that transports glucose "against" a concentration gradient (that is, from low glucose concentrations outside the cell to higher concentrations within the cell).
- This system is a transporter-mediated process in which the movement of glucose is coupled to the concentration gradient of $Na^+$, which is transported into the cell at the same time.
- The transporter is a sodium-dependent glucose transporter (SGLT).
- This type of transport occurs in the epithelial cells of the intestine, renal tubules, and choroid plexus.
- [Note: The choroid plexus, part of the blood–brain barrier, also contains GLUT-1.]

## نشاط (1/3/2) نشاط فردي

**What are the 2 forms of glycolysis?**

## نشاط (2/3/2) نشاط فردي

Glucose cannot diffuse directly into cells but enters by one of two transport mechanisms, which?

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# Introduction to Metabolism and Glycolysis

## Lecture 8

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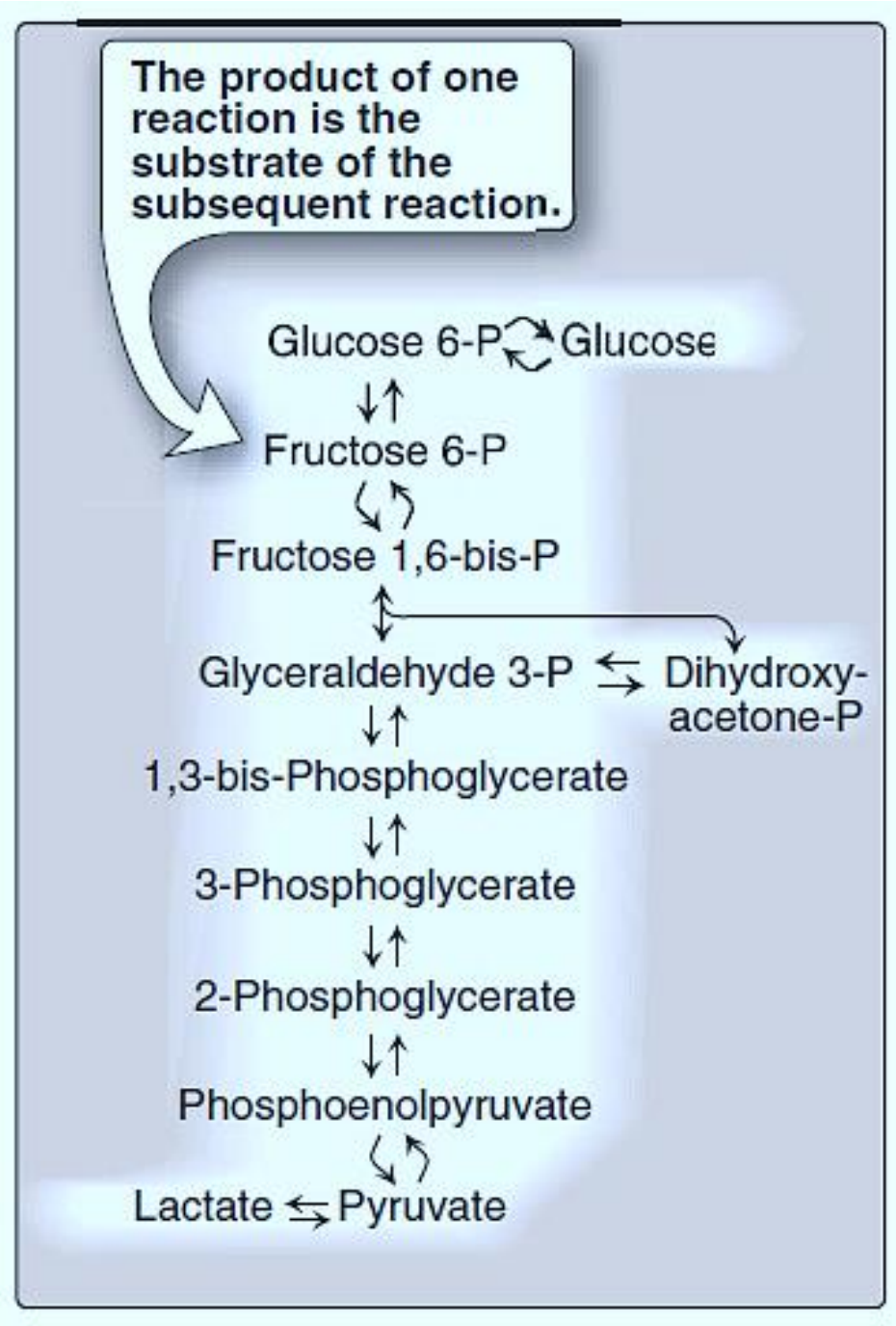

# الوحدة الثانية - المحاضرة الرابعة - الزمن: 90 دقيقة

## أهداف المحاضرة الرابعة:

**يتوقع في نهاية المحاضرة أن يكون الطالب قادراً على:**

By the end of the lecture, the student should be able to:

1. Know the conversion of glucose to pyruvate occurs in two stages
2. List the first five reactions of glycolysis correspond to an energy–investment phase.
3. Phosphorylation of glucose
4. Isomerization of glucose 6-phosphate
5. Phosphorylation of fructose 6-phosphate
6. Cleavage of fructose 1,6-bisphosphate
7. Isomerization of dihydroxyacetone phosphate

# موضوعات المحاضرة الرابعة:

## REACTIONS OF GLYCOLYSIS

- Phosphorylation of glucose
  - Hexokinases I–III
  - Hexokinase IV (or, glucokinase)
    - Kinetics
    - Regulation by fructose 6-phosphate and glucose
- Isomerization of glucose 6-phosphate
- Phosphorylation of fructose 6-phosphate
  - Regulation by energy levels within the cell
  - Regulation by fructose 2,6-bisphosphate
    - During the well-fed state
    - During fasting
- Cleavage of fructose 1,6-bisphosphate
- Isomerization of dihydroxyacetone phosphate

# V. REACTIONS OF GLYCOLYSIS

- The conversion of glucose to pyruvate occurs in two stages (Figure 2.11).
- The first five reactions of glycolysis correspond to an energy–investment phase in which the phosphorylated forms of intermediates are synthesized at the expense of ATP.
- The subsequent reactions of glycolysis constitute an energy–generation phase in which a net of two molecules of ATP are formed by substrate-level phosphorylation per glucose molecule metabolized.

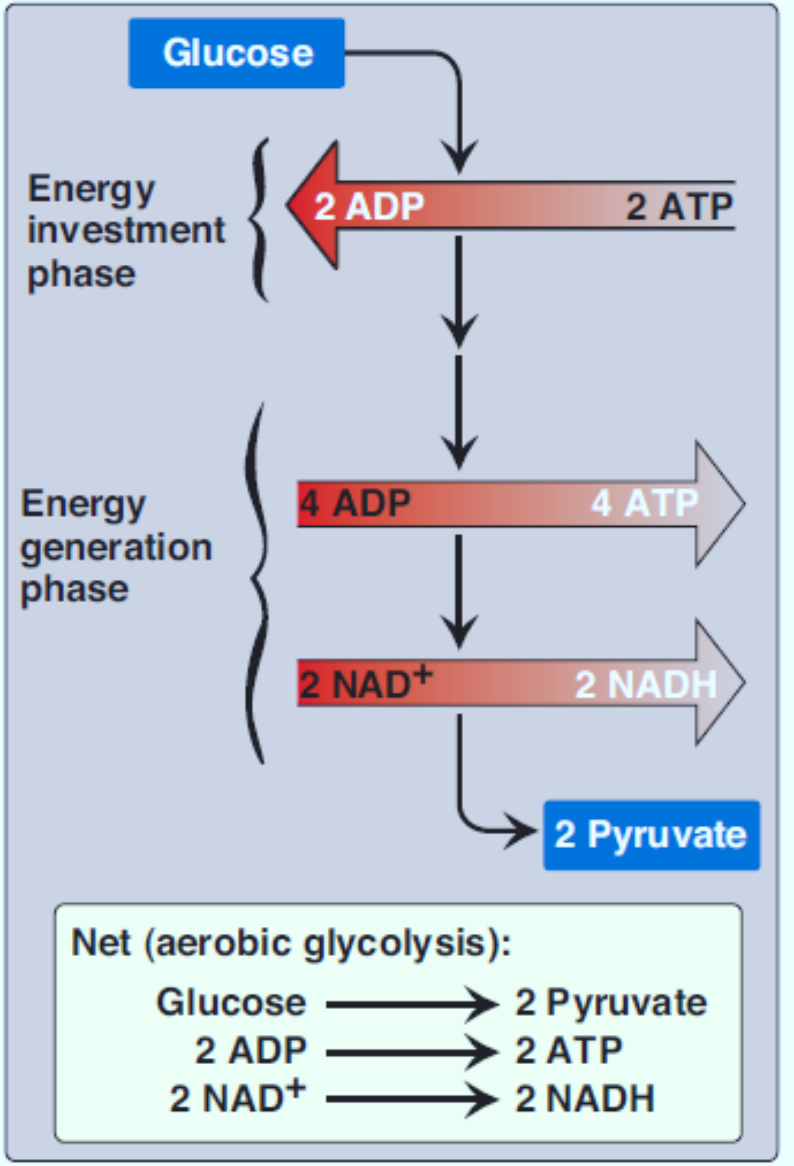


**Figure 2.11** Two phases of aerobic glycolysis. NAD(H) = nicotinamide adenine dinucleotide.

# A. Phosphorylation of glucose

- Phosphorylated sugar molecules do not readily penetrate cell membranes because there are no specific transmembrane carriers for these compounds and because they are too polar to diffuse through the lipid core of membranes.
- The irreversible phosphorylation of glucose (Figure 2.12), therefore, effectively traps the sugar as cytosolic glucose 6-phosphate, thereby committing it to further metabolism in the cell.
- Mammals have four (I–IV) isozymes of the enzyme *hexokinase* that catalyze the phosphorylation of glucose to glucose 6-phosphate.

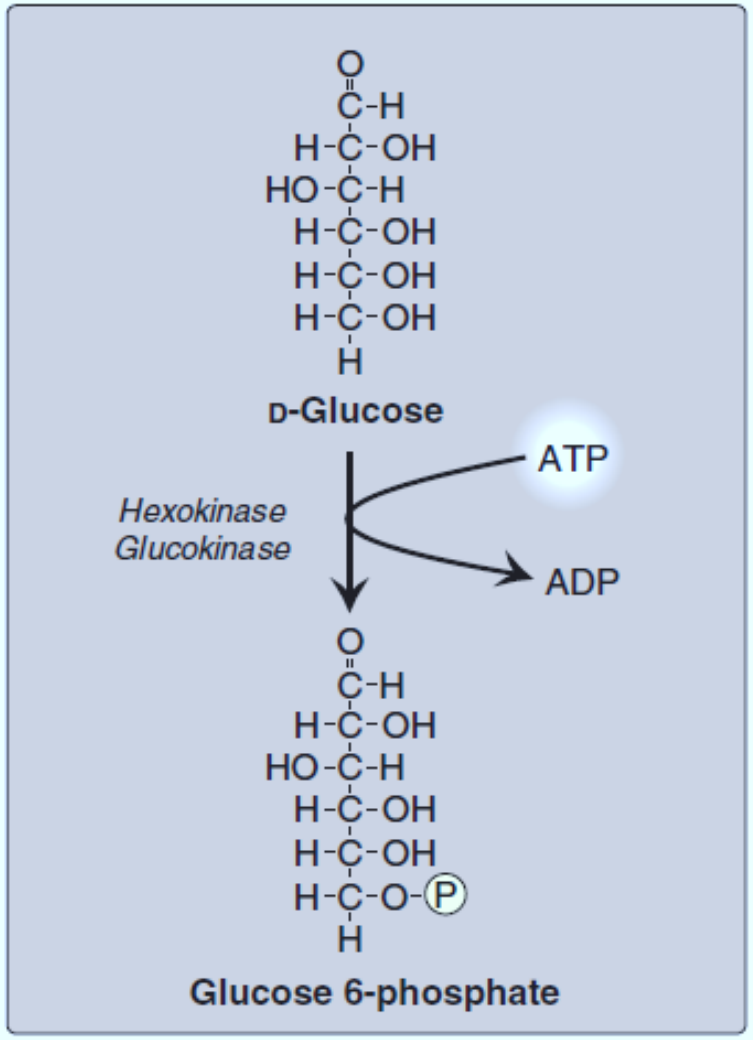


Figure 2.12 Energy-investment phase: phosphorylation of glucose. [Note: Kinases utilize ATP complexed with a divalent metal ion, most typically $Mg^{2+}$.]

# 1. Hexokinases I–III:

- In most tissues, phosphorylation of glucose is catalyzed by one of these isozymes of *hexokinase*, which is one of three regulatory enzymes of glycolysis (see also *phosphofructokinase* and *pyruvate kinase*).
- These isozymes have broad substrate specificity and are able to phosphorylate several hexoses in addition to glucose.
- They are inhibited by the reaction product, glucose 6-phosphate, which accumulates when further metabolism of this hexose phosphate is reduced.

- *Hexokinases I-III* have a low Michaelis constant (Km) (and, therefore, a high affinity) for glucose.
- This permits the efficient phosphorylation and subsequent metabolism of glucose even when tissue concentrations of glucose are low (Figure 2.13).
- These isozymes, however, have a low maximal velocity ([$V_{max}$]) for glucose and, therefore, do not sequester (trap) cellular phosphate in the form of phosphorylated hexoses, or phosphorylate more sugars than the cell can use.

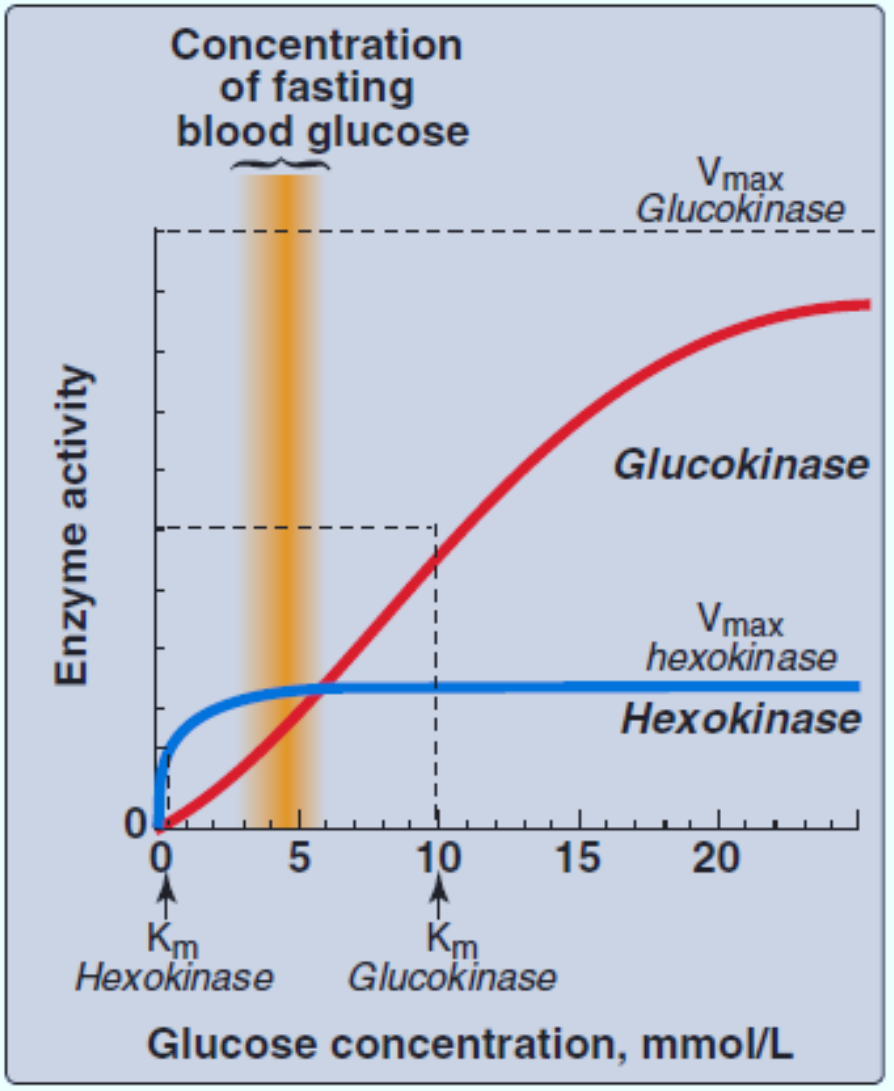


Figure 2.13 Effect of glucose concentration on the rate of phosphorylation catalyzed by *hexokinase* and *glucokinase*. $K_m$ = Michaelis constant; $V_{max}$ = maximal velocity.

## 2. Hexokinase IV (or, glucokinase):

- In liver parenchymal cells and β cells of the pancreas, *glucokinase* (the *hexokinase IV* isozyme ) is the predominant enzyme responsible for the phosphorylation of glucose.
- In β cells, *glucokinase* functions as a glucose sensor, determining the threshold for insulin secretion.
- [Note: *Hexokinase IV* also serves as a glucose sensor in neurons of the hypothalamus, playing a key role in the adrenergic response to hypoglycemia.]
- In the liver, the enzyme facilitates glucose phosphorylation during hyperglycemia.
- Despite the popular but misleading name *glucokinase*, the sugar specificity of the enzyme is similar to that of other *hexokinase* isozymes.

# a. Kinetics:

- *Glucokinase* differs from *hexokinases I–III* in several important properties. For example, it has a much higher $K_m$, requiring a higher glucose concentration for half-saturation (see Figure 2.13).
- Thus, *glucokinase* functions only when the intracellular concentration of glucose in the hepatocyte is elevated such as during the brief period following consumption of a carbohydrate-rich meal, when high levels of glucose are delivered to the liver via the portal vein.
- *Glucokinase* has a high $V_{max}$, allowing the liver to effectively remove the flood of glucose delivered by the portal blood. This prevents large amounts of glucose from entering the systemic circulation following such a meal thereby minimizing hyperglycemia during the absorptive period.
- [Note: GLUT-2 insures that blood glucose equilibrates rapidly across the membrane of the hepatocyte.]

## b. Regulation by fructose 6-phosphate and glucose:

- *Glucokinase* activity is not directly inhibited by glucose 6-phosphate as are the other *hexokinases* but, rather, is indirectly inhibited by fructose 6-phosphate (which is in equilibrium with glucose 6-phosphate, a product of *glucokinase*) and is indirectly stimulated by glucose (a substrate of *glucokinase*) via the following mechanism.

- Glucokinase regulatory protein (GKRP) in the liver regulates the activity of *glucokinase* through reversible binding.

- In the presence of fructose 6-phosphate, *glucokinase* is translocated into the nucleus and binds tightly to the regulatory protein, thereby rendering the enzyme inactive (Figure 2.14).

- When glucose levels in the blood (and also in the hepatocyte, as a result of GLUT-2) increase, *glucokinase* is released from the regulatory protein, and the enzyme reenters the cytosol where it phosphorylates glucose to glucose 6-phosphate.

- [Note: Fructose 1 phosphate inhibits formation of the *glucokinase*–GKRP complex.]

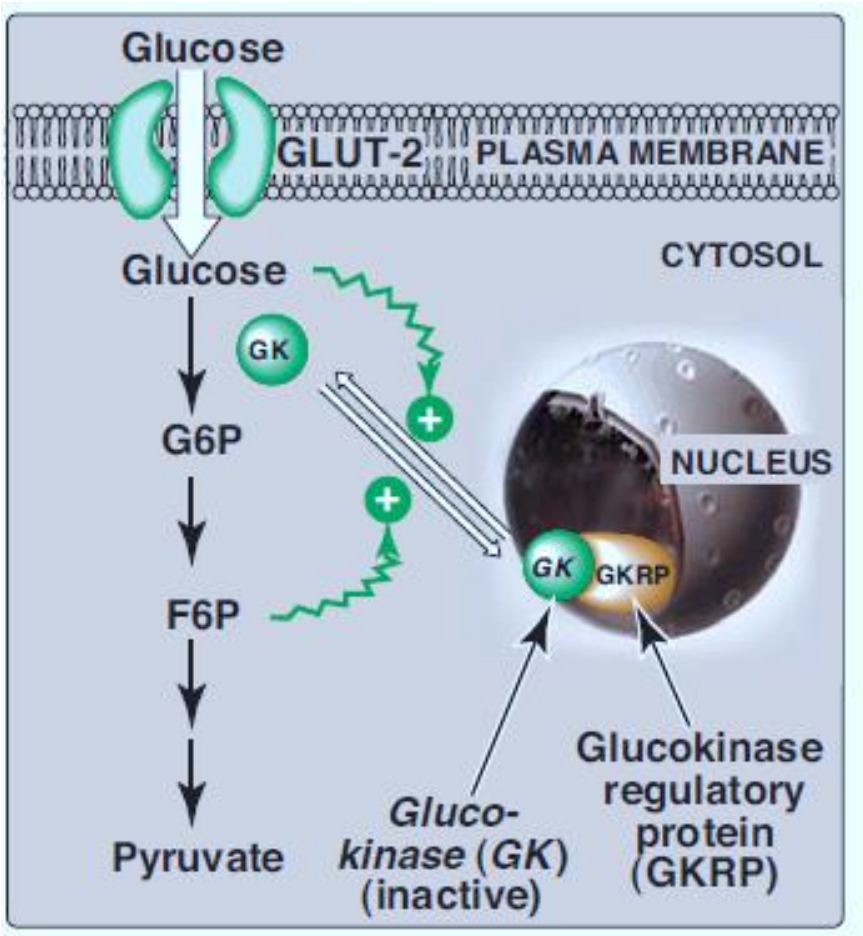


**Figure 2.14** Regulation of glucokinase activity by *glucokinase* regulatory protein. GLUT = glucose transporter.

Glucokinase functions as a glucose sensor in the maintenance of blood glucose homeostasis. Inactivating mutations of glucokinase are the cause of a rare form of diabetes, maturity onset diabetes of the young type 2 (MODY 2) that is characterized by impaired insulin secretion.

# B. Isomerization of glucose 6-phosphate

- The isomerization of glucose 6-phosphate to fructose 6-phosphate is catalyzed by *phosphoglucose isomerase* (Figure 2.15).
- The reaction is readily reversible and is not a rate-limiting or regulated step.

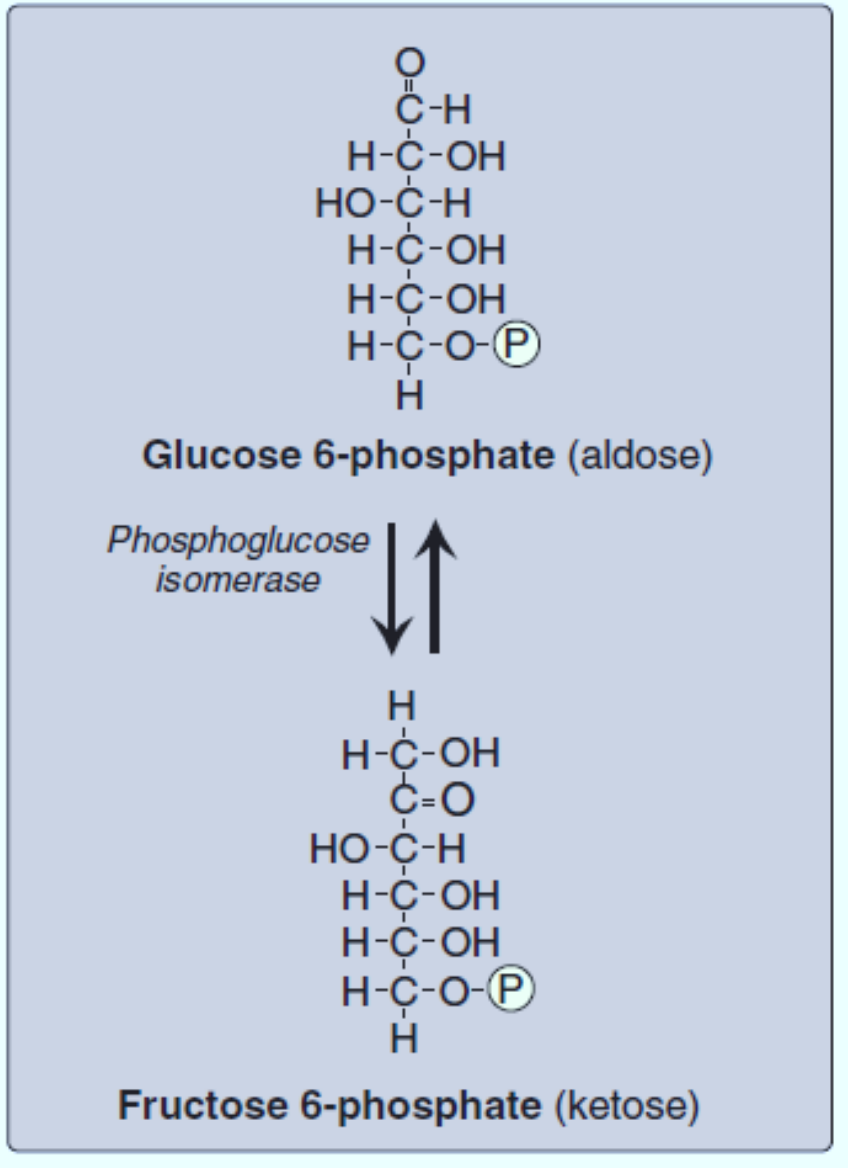


**Figure 2.15** Aldose-ketose isomerization of glucose 6-phosphate to fructose 6-phosphate. P = phosphate.

# C. Phosphorylation of fructose 6-phosphate

- The irreversible phosphorylation reaction catalyzed by *phosphofructokinase-1* (*PFK-1*) is the most important control point and the rate-limiting and committed step of glycolysis (Figure 2.16).
- *PFK-1* is controlled by the available concentrations of the substrates ATP and fructose 6-phosphate as well as by regulatory substances described below.

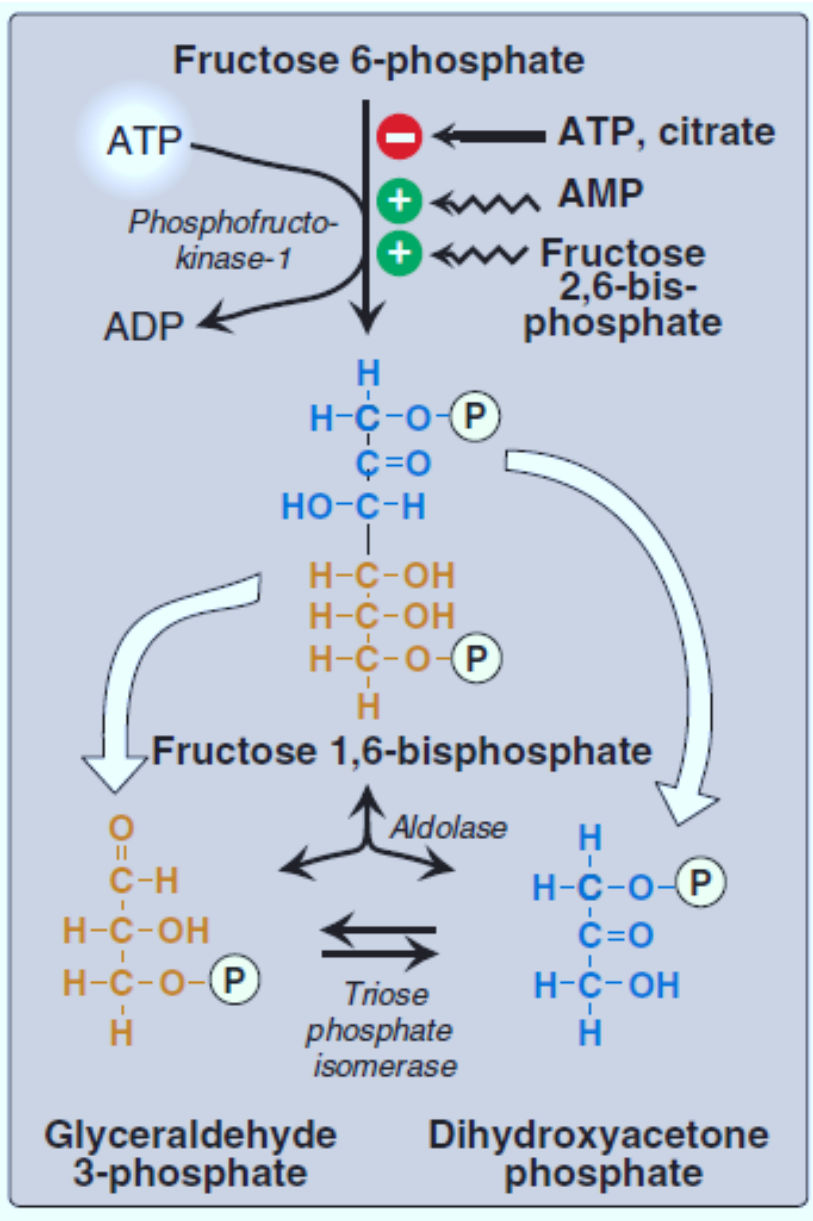


**Figure 8.16** Energy–investment phase (continued): Convers ion of fructose 6-phosphate to triose phosphates. P = phosphate; AMP = adenosine monophosphate .

# 1. Regulation by energy levels within the cell:

- *PFK-1* is inhibited allosterically by elevated levels of ATP, which act as an “energy-rich” signal indicating an abundance of high-energy compounds .
- Elevated levels of citrate, an intermediate in the TCA cycle, also inhibit *PFK-1*.
- [Note: Inhibition by citrate favors the use of glucose for glycogen synthesis.]
- Conversely, *PFK-1* is activated allosterically by high concentrations of AMP, which signal that the cell’s energy stores are depleted.

## 2. Regulation by fructose 2,6-bisphosphate:

- Fructose 2,6-bisphosphate is the most potent activator of *PFK-1* (see Figure 2.16) and is able to activate the enzyme even when ATP levels are high.
- Fructose 2,6-bisphosphate is formed from fructose 6-phosphate by *phosphofructokinase-2* (*PFK-2*), an enzyme different than *PFK-1*.
- *PFK-2* is a bifunctional protein that has both the *kinase* activity that produces fructose 2,6-bisphosphate and the *phosphatase* activity that dephosphorylates fructose 2,6-bisphosphate back to fructose 6-phosphate.
- In the liver, the kinase domain is active if dephosphorylated and is inactive if phosphorylated (Figure 2.17).

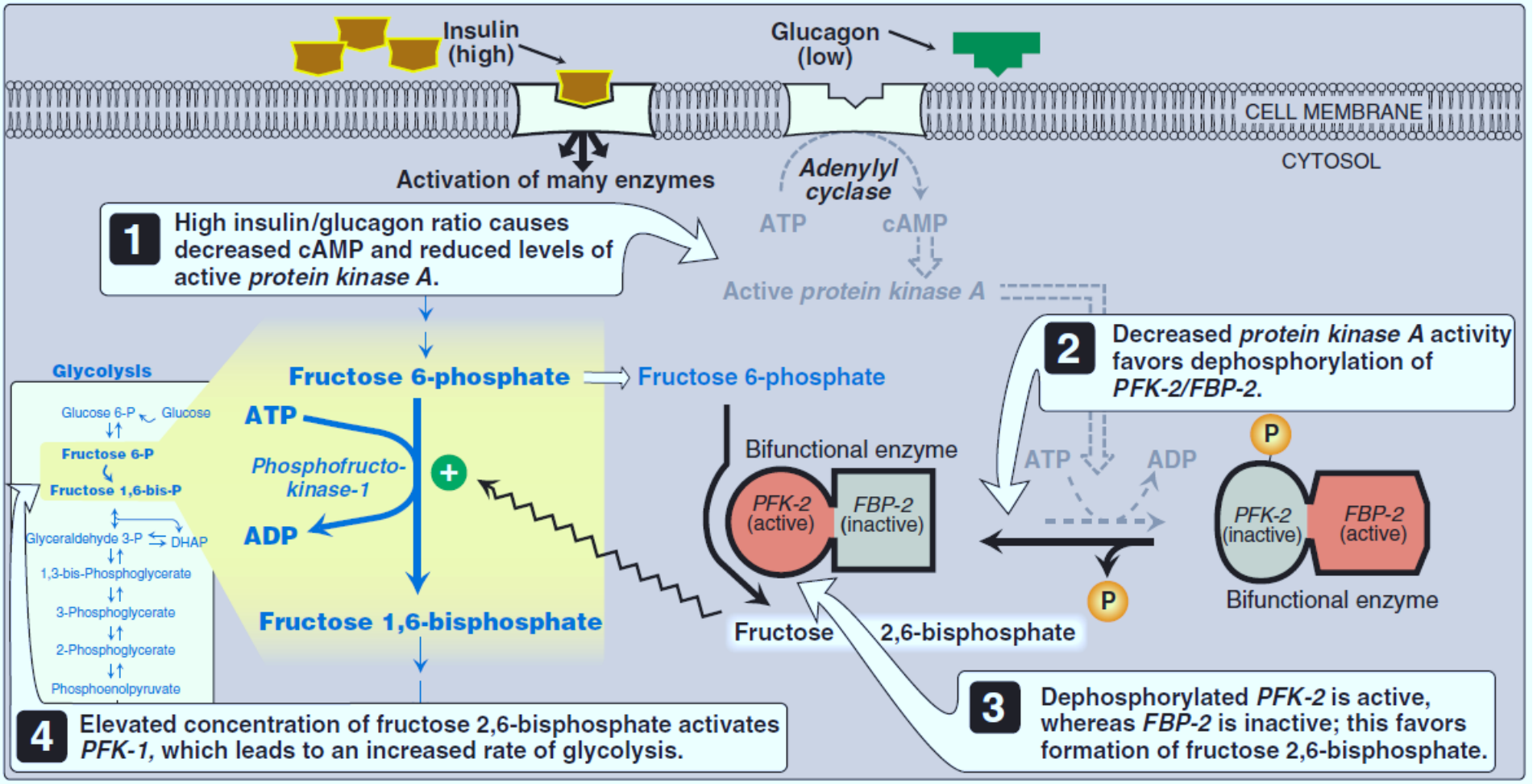


**Figure 2.17** Effect of elevated insulin concentration on the intracellular concentration of fructose 2,6-bisphosphate in liver. *PFK-2 = phosphofructokinase-2*; *FBP-2 = fructose 2,6-bisphosphatase* ; cAMP = cyclic AMP; P = phosphate.

- [Note: Fructose 2,6-bisphosphate is an inhibitor of *fructose 1,6-bisphosphatase*, an enzyme of gluconeogenesis. The reciprocal actions of fructose 2,6-bisphosphate on glycolysis (activation) and gluconeogenesis (inhibition) ensure that both pathways are not fully active at the same time, preventing a futile cycle in which glucose would be converted to pyruvate followed by resynthesis of glucose from pyruvate.]

a. **During the well-fed state:** Decreased levels of glucagon and elevated levels of insulin, such as occur following a carbohydrate-rich meal, cause an increase in fructose 2,6-bisphosphate and, thus, in the rate of glycolysis in the liver (see Figure 2.17). Fructose 2,6-bisphosphate, therefore, acts as an intracellular signal, indicating that glucose is abundant.

b. **During fasting:** Elevated levels of glucagon and low levels of insulin, such as occur during fasting, decrease the intracellular concentration of hepatic fructose 2,6-bisphosphate. This results in inhibition of glycolysis and activation of gluconeogenesis.

# D. Cleavage of fructose 1,6-bisphosphate

- *Aldolase* cleaves fructose 1,6-bisphosphate to dihydroxyacetone phosphate and glyceraldehyde 3-phosphate (see Figure 2.16). The reaction is reversible and not regulated.
- [Note: *Aldolase B*, the isoform found primarily in the liver, also cleaves fructose 1-phosphate and functions in the metabolism of dietary fructose.]

# E. Isomerization of dihydroxyacetone phosphate

- *Triose phosphate isomerase* interconverts dihydroxyacetone phosphate (DHAP) and glyceraldehyde 3-phosphate (see Figure 2.16).
- DHAP must be isomerized to glyceraldehyde 3-phosphate for further metabolism by the glycolytic pathway.
- This isomerization results in the net production of two molecules of glyceraldehyde 3-phosphate from the cleavage products of fructose 1,6-bisphosphate.
- [Note: DHAP is utilized in triacylglycerol synthesis.]

## نشاط فردي (1/4/2) نشاط

**The conversion of glucose to pyruvate occurs in two stages, explain**

## نشاط فردي (2/4/2) نشاط

**Answer the following Questions**

1. Hexokinases I-III has a high or low Km?
2. Hexokinases I-III has a high or low Vmax?

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# Introduction to Metabolism and Glycolysis

## Lecture 9

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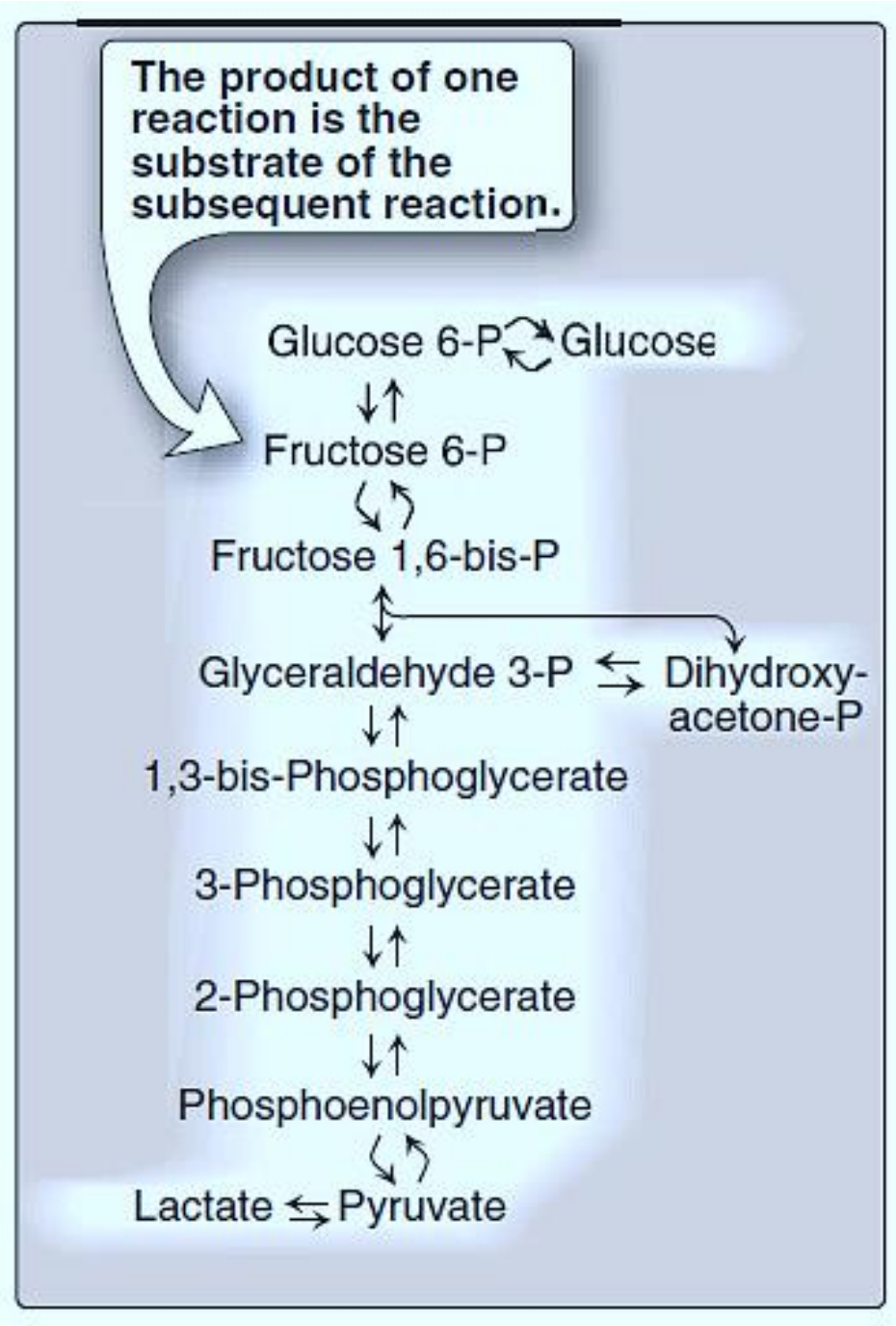
***Dr. Kutaiba I. Alzand & Dr. Rusul H. Hamza***



# الوحدة  الثانية - المحاضرة الخامسة - الزمن: 90 دقيقة

## أهداف المحاضرة الخامسة:

**يتوقع في نهاية المحاضرة أن يكون الطالب قادراً على:**

By the end of the lecture, the student should be able to:

- Explain the oxidation of glyceraldehyde 3-phosphate
- Describe the synthesis of 3-phosphoglycerate producing ATP
- Know the shift of the phosphate group
- Explain the dehydration of 2-phosphoglycerate
- Describe the formation of pyruvate producing ATP
- Describe the reduction of pyruvate to lactate
- Explain the energy yield from glycolysis

# موضوعات المحاضرة الخامسة:

## REACTIONS OF GLYCOLYSIS

- Oxidation of glyceraldehyde 3-phosphate
  - Synthesis of 1,3-bisphosphoglycerate:
  - Mechanism of arsenic poisoning
  - Synthesis of 2,3-bisphosphoglycerate in red blood cells
- Synthesis of 3-phosphoglycerate producing ATP
- Shift of the phosphate group
- Dehydration of 2-phosphoglycerate
- Formation of pyruvate producing ATP
  - Feedforward regulation
  - Covalent modulation of pyruvate kinase:
  - Pyruvate kinase deficiency:
- Reduction of pyruvate to lactate
  - Lactate formation in muscle
  - Lactate utilization
  - Lactic acidosis
- Energy yield from glycolysis
  - Anaerobic glycolysis
  - Aerobic glycolysis

# F. Oxidation of glyceraldehyde 3-phosphate

- The conversion of glyceraldehyde 3-phosphate to 1,3-bisphosphoglycerate (1,3-BPG) by *glyceraldehyde 3-phosphate dehydrogenase* is the first oxidation-reduction reaction of glycolysis (Figure 2.18).

- [Note: Because there is only a limited amount of $NAD^+$ in the cell, the NADH formed by this reaction must be reoxidized to $NAD^+$ for glycolysis to continue. Two major mechanisms for oxidizing NADH are 1) the NADH-linked conversion of pyruvate to lactate (anaerobic; and 2) oxidation of NADH via the respiratory chain (aerobic). The latter requires the malate-aspartate and glycerol 3-phosphate substrate shuttles.]

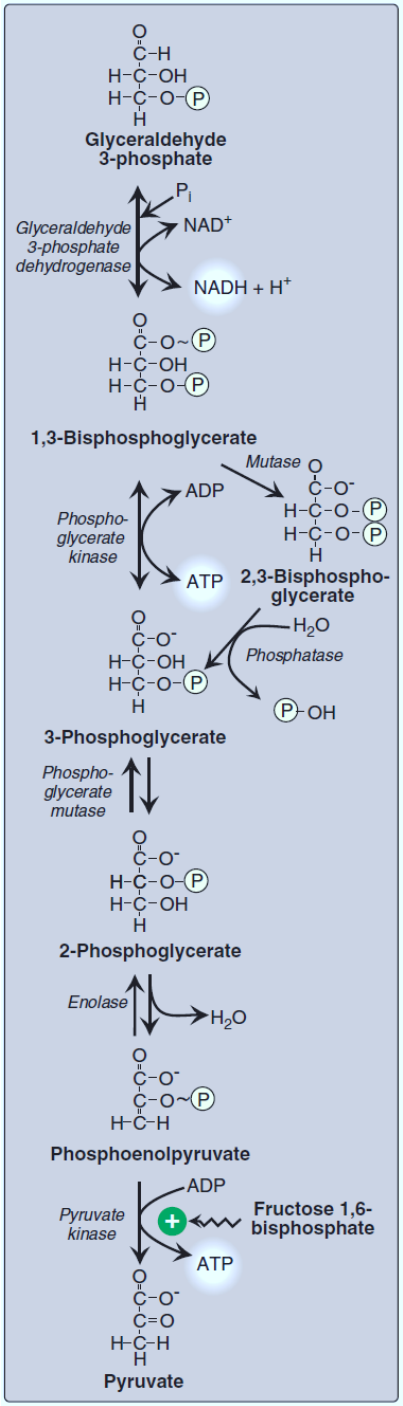
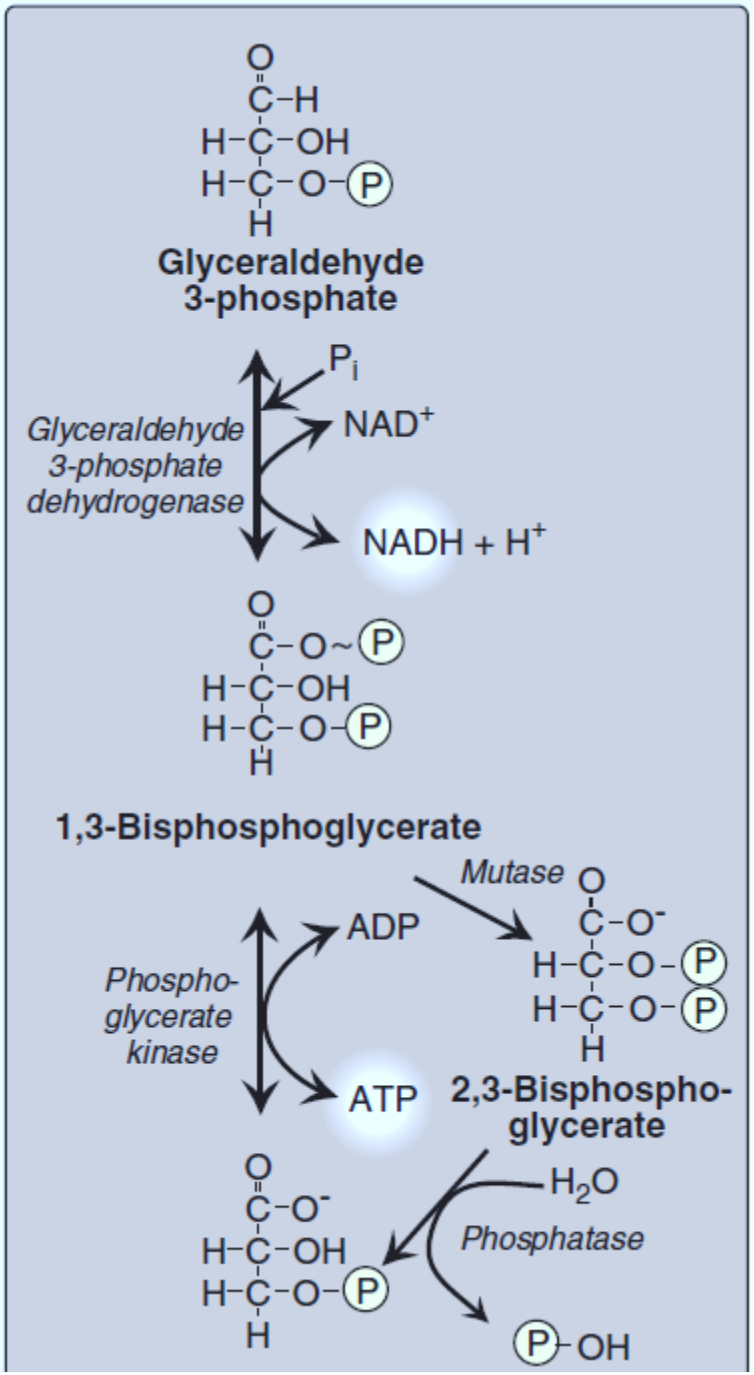
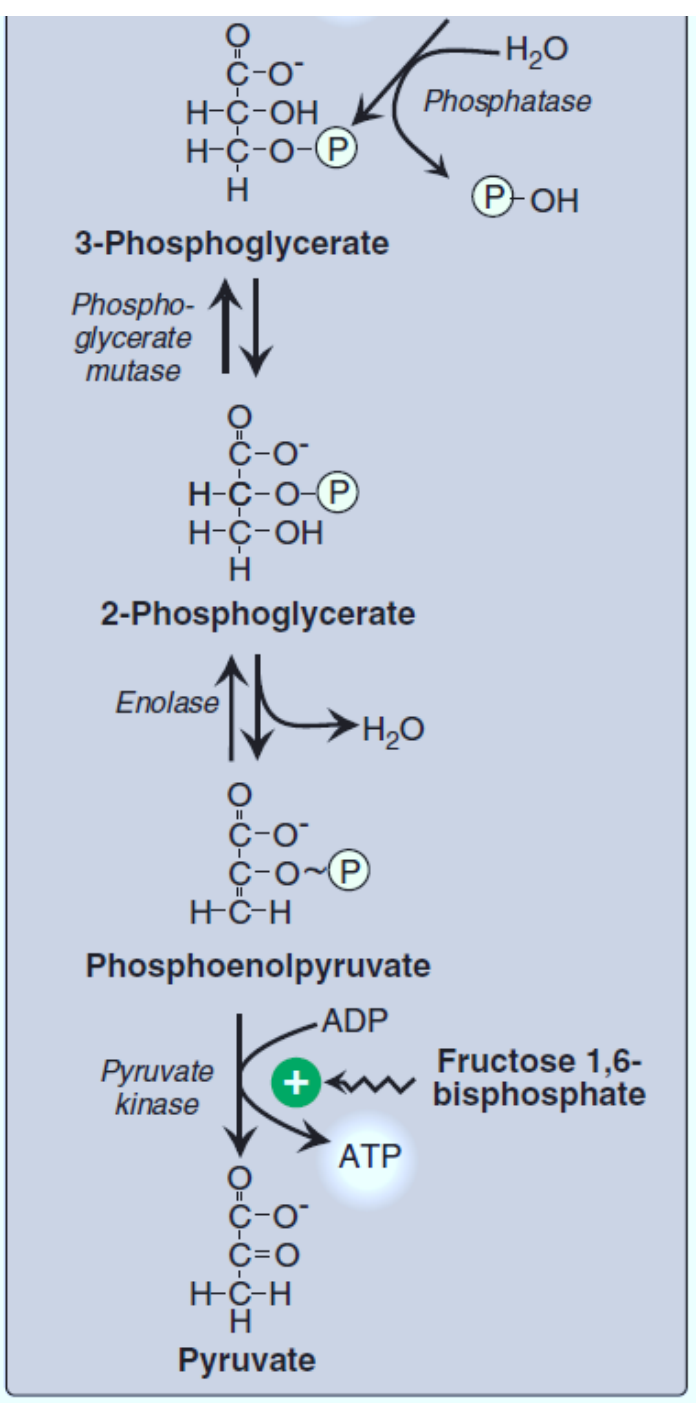



**Figure 2.18** Energy–generating phase: conversion of glyceraldehyde 3-phosphate to pyruvate. NAD(H) = nicotinamide adenine dinucleotide; P = phosphate; Pi = inorganic phosphate.


Glyceraldehyde 3-phosphate
Pi
NAD+
Glyceraldehyde 3-phosphate dehydrogenase
NADH + H+
1,3-Bisphosphoglycerate
Mutase
ADP
Phospho-glycerate kinase
ATP
2,3-Bisphospho-glycerate
H2O
Phosphatase
P-OH


H2O
Phosphatase
P-OH
3-Phosphoglycerate
Phospho-glycerate mutase
2-Phosphoglycerate
Enolase
H2O
Phosphoenolpyruvate
ADP
Pyruvate kinase
Fructose 1,6-bisphosphate
ATP
Pyruvate

# 1. Synthesis of 1,3-bisphosphoglycerate:

- The oxidation of the aldehyde group of glyceraldehyde 3-phosphate to a carboxyl group is coupled to the attachment of $P_i$ to the carboxyl group.
- The high-energy phosphate group at carbon 1 of 1,3-BPG conserves much of the free energy produced by the oxidation of glyceraldehyde 3-phosphate.
- The energy of this high-energy phosphate drives the synthesis of ATP in the next reaction of glycolysis.

# 2. Mechanism of arsenic poisoning :

- The toxicity of arsenic is due primarily to the inhibition by trivalent arsenic (arsenite) of enzymes such as the *pyruvate dehydrogenase* complex, which require lipoic acid as a coenzyme.
- However, pentavalent arsenic (arsenate) can prevent net ATP and NADH production by glycolysis without inhibiting the pathway itself. It does so by competing with $P_i$ as a substrate for *glyceraldehyde 3-phosphate dehydrogenase*, forming a complex that spontaneously hydrolyzes to form 3-phosphoglycerate (Figure 2.18).
- By bypassing the synthesis of and phosphate transfer from 1,3- BPG, the cell is deprived of energy usually obtained from the glycolytic pathway.
- [Note: Arsenate also competes with $P_i$ on the $F_1$ domain of *ATP synthase*, resulting in formation of ADP-arsenate that is rapidly hydrolyzed.]

## 3. Synthesis of 2,3-bisphosphoglycerate in red blood cells:

- Some of the 1,3-BPG is converted to 2,3-BPG by the action of *bisphosphoglycerate mutase* (see Figure 2.18).
- 2,3-BPG, which is found in only trace amounts in most cells, is present at high concentration in red blood cells (RBCs ) and serves to increase $O_2$ delivery.
- 2,3-BPG is hydrolyzed by a *phosphatase* to 3-phosphoglycerate, which is also an intermediate in glycolysis (see Figure 2.18).
- In the RBC, glycolysis is modified by inclusion of these "shunt" reactions.

# G. Synthesis of 3-phosphoglycerate producing ATP

- When 1,3-BPG is converted to 3-phosphoglycerate, the high-energy phosphate group of 1,3-BPG is used to synthesize ATP from ADP (see Figure 2.18).
- This reaction is catalyzed by *phosphoglycerate kinase*, which, unlike most other *kinases*, is physiologically reversible.
- Because two molecules of 1,3-BPG are formed from each glucose molecule, this *kinase* reaction replaces the two ATP molecules consumed by the earlier formation of glucose 6-phosphate and fructose 1,6-bisphosphate.
- [Note: This is an example of substrate-level phosphorylation, in which the energy needed for the production of a high-energy phosphate comes from a substrate rather than from the electron transport chain.]

## H. Shift of the phosphate group

- The shift of the phosphate group from carbon 3 to carbon 2 of phosphoglycerate by *phosphoglycerate mutase* is freely reversible (see Figure 8.18).

## I. Dehydration of 2-phosphoglycerate

- The dehydration of 2-phosphoglycerate by *enolase* redistributes the energy within the substrate, resulting in the formation of phosphoenolpyruvate (PEP), which contains a high-energy enol phosphate (see Figure 8.18).
- The reaction is reversible despite the high-energy nature of the product.
- [Note: Fluoride inhibits *enolase*, and water fluoridation reduces lactate production by mouth bacteria, decreasing dental caries.]

# J. Formation of pyruvate producing ATP

- The conversion of PEP to pyruvate is catalyzed by *pyruvate kinase* (PK), the third irreversible reaction of glycolysis.
- The high-energy enol phosphate in PEP is used to synthesize ATP from ADP and is another example of substrate-level phosphorylation (see Figure 8.18).

1. **Feedforward regulation:** PK is activated by fructose 1,6-bisphosphate, the product of the *phosphofructokinase-1* reaction. This feedforward (instead of the more usual feedback) regulation has the effect of linking the two *kinase* activities: increased *phosphofructokinase* activity results in elevated levels of fructose 1,6-bisphosphate, which activates PK.

## 2. Covalent modulation of pyruvate kinase:

- Phosphorylation by a *cAMP-dependent protein kinase* leads to inactivation of the hepatic isozyme of *PK* (Figure 2.19).
- When blood glucose levels are low, elevated glucagon increases the intracellular level of cAMP, which causes the phosphorylation and inactivation of *PK* in the liver only. Therefore, PEP is unable to continue in glycolysis and, instead, enters the gluconeogenesis pathway.
- This, in part, explains the observed inhibition of hepatic glycolysis and stimulation of gluconeogenesis by glucagon.
- Dephosphorylation of *PK* by a *phosphatase* results in reactivation of the enzyme.

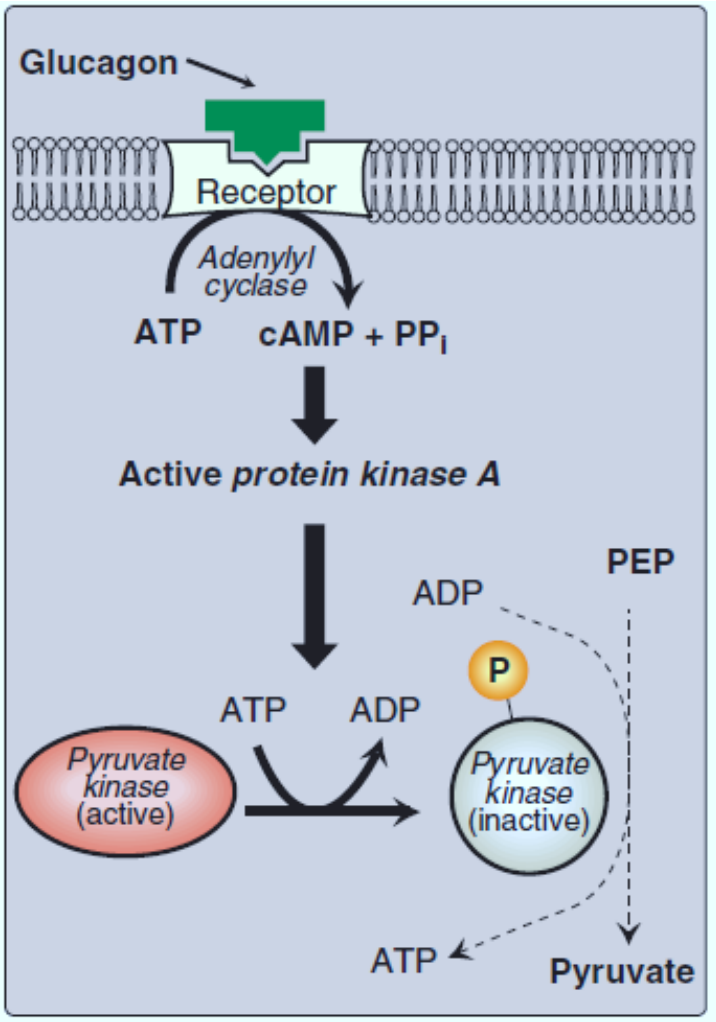



**Figure 2.19** Covalent modification of hepatic pyruvate kinase results in inactivation of the enzyme. cAMP = cyclic AMP; PEP = phosphoenolpyruvate; P = phosphate; PPi = pyrophosphate.

## 3. Pyruvate kinase deficiency:

- Mature RBCs lack mitochondria and are, therefore, completely dependent on glycolysis for ATP production.
- ATP is required to meet the metabolic needs of RBCs and to fuel the ion pumps necessary for the maintenance of the flexible, biconcave shape that allows them to squeeze through narrow capillaries.
- The anemia observed in glycolytic enzyme deficiencies is a consequence of the reduced rate of glycolysis, leading to decreased ATP production.
- The resulting alterations in the RBC membrane lead to changes in cell shape and, ultimately, to phagocytosis by cells of the reticuloendothelial system, particularly macrophages of the spleen.

- The premature death and lysis of RBCs result in hemolytic anemia.
- Among patients exhibiting the rare genetic defects of glycolytic enzymes, the majority has a deficiency in *PK*.
- The effects of *PK* deficiency are restricted to RBCs and include mild-to-severe nonspherocytic hemolytic anemia, with the severe form requiring regular transfusions.
- [Note: Hepatic *PK* is encoded by the same gene as the RBC isozyme. Liver cells show no effect, however, because they have mitochondria and can generate ATP by oxidative phosphorylation.]
- Severity depends both on the degree of enzyme deficiency (generally 5–35% of normal levels) and on the extent to which RBCs compensate by synthesizing increased levels of 2,3-BPG.

- Almost all individuals with *PK* deficiency have a mutant enzyme that shows abnormal properties such as altered kinetics (Figure 2.20).
- Individuals heterozygous for *PK* deficiency have resistance to the most severe forms of malaria.
- The tissue-specific express ion of PK in RBCs and the liver is the result of differential promoter utilization in transcription of the gene that encodes both isozymes.

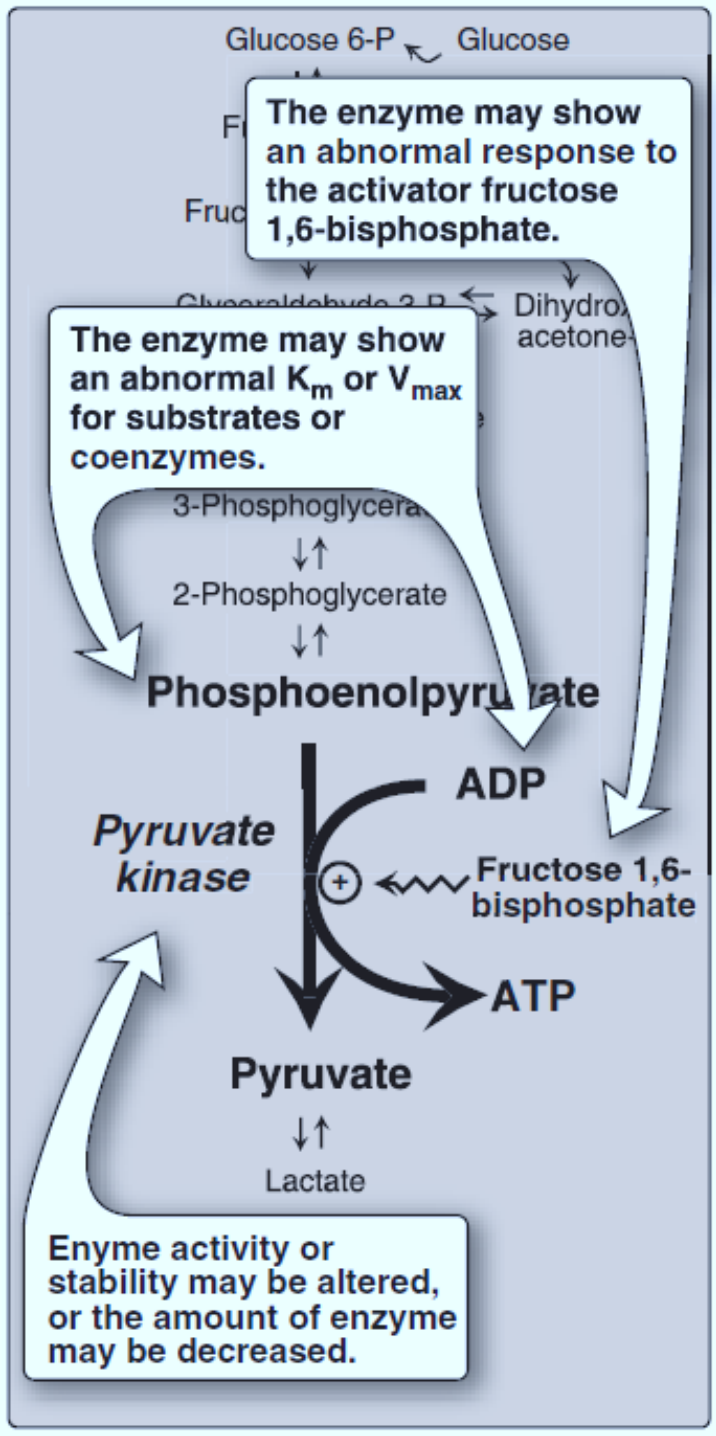



**Figure 2.20** Alterations observed with various mutant forms of *pyruvate kinase*. $K_m$ = Michaelis constant; $V_{max}$ = maximal velocity.

# K. Reduction of pyruvate to lactate

- Lactate, formed by the action of *lactate dehydrogenase*, is the final product of anaerobic glycolysis in eukaryotic cells (Figure 2.21).
- The formation of lactate is the major fate for pyruvate in the lens and cornea of the eye, kidney medulla, testes, leukocytes, and RBCs, because these are all poorly vascularized and/or lack mitochondria.
- [Note: Lactate produced in muscle enters the circulation, is picked up by liver through facilitated diffusion, and is oxidized to pyruvate. Pyruvate is used by liver to make glucose.]

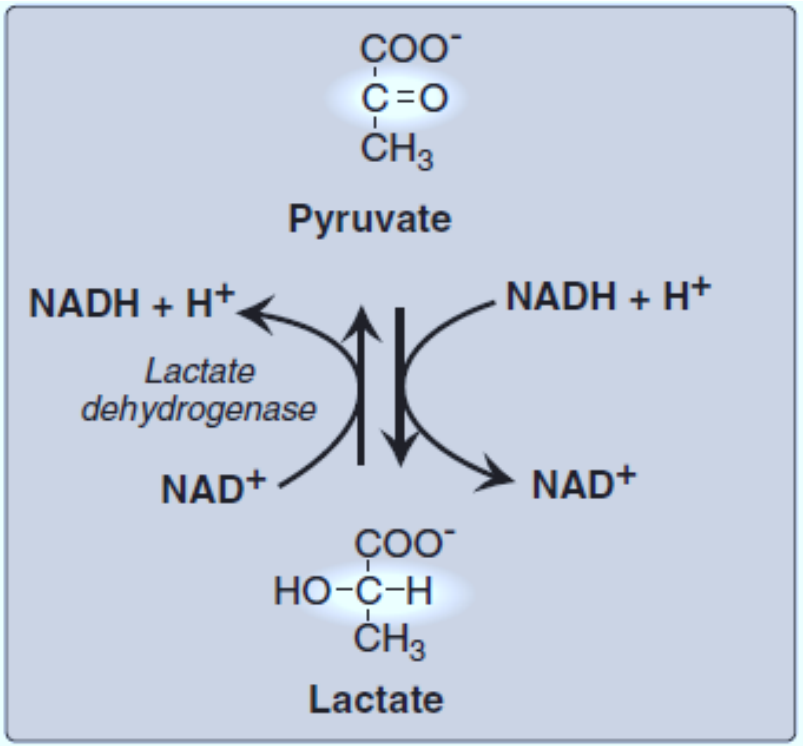



**Figure 2.21** Interconvers ion of pyruvate and lactate. NAD(H) = nicotinamide adenine dinucleotide.

# 1. Lactate formation in muscle:

- In exercising skeletal muscle, NADH production (by glyceraldehyde 3-phosphate dehydrogenase and by the three $NAD^+$-linked dehydrogenases of the TCA cycle) exceeds the oxidative capacity of the respiratory chain.
- This results in an elevated $NADH/NAD^+$ ratio, favoring reduction of pyruvate to lactate.
- Therefore, during intense exercise, lactate accumulates in muscle, causing a drop in the intracellular pH, potentially resulting in cramps.
- Much of this lactate eventually diffuses into the bloodstream and can be used by the liver to make glucose.

# 2. Lactate utilization:

- The direction of the lactate dehydrogenase reaction depends on the relative intracellular concentrations of pyruvate and lactate and on the ratio of NADH/$NAD^+$ in the cell.
- For example, in the liver and heart, the ratio of NADH/$NAD^+$ is lower than in exercising muscle.
- These tissues oxidize lactate (obtained from the blood) to pyruvate.
- In the liver, pyruvate is either converted to glucose by gluconeogenesis or oxidized in the TCA cycle.
- Heart muscle exclusively oxidizes lactate to $CO_2$ and $H_2O$ via the TCA cycle.

# 3. Lactic acidosis:

- Elevated concentrations of lactate in the plasma, termed lactic acidosis (a type of metabolic acidosis), occur when there is a collapse of the circulatory system, such as in myocardial infarction, pulmonary embolism, and uncontrolled hemorrhage, or when an individual is in shock.

- The failure to bring adequate amounts of oxygen to the tissues results in impaired oxidative phosphorylation and decreased ATP synthesis.

- To survive, the cells rely on anaerobic glycolysis for generating ATP, producing lactic acid as the end product.

- [Note: Production of even meager amounts of ATP may be life-saving during the period required to reestablish adequate blood flow to the tissues.]
- The excess oxygen required to recover from a period when the availability of oxygen has been inadequate is termed the "oxygen debt."

The oxygen debt is often related to patient morbidity or mortality. In many clinical situations, measuring the blood levels of lactic acid allows the rapid, early detection of oxygen debt in patients and the monitoring of their recovery.

# L. Energy yield from glycolysis

- Despite the production of some ATP during glycolysis, the end product, pyruvate or lacta te, still contains most of the energy originally contained in glucose. The TCA cycle is required to release that energy completely.

1. **Anaerobic glycolysis:** Two molecules of ATP are generated for each molecule of glucose converted to two molecules of lactate (Figure 2.22). There is no net production or consumption of NADH.

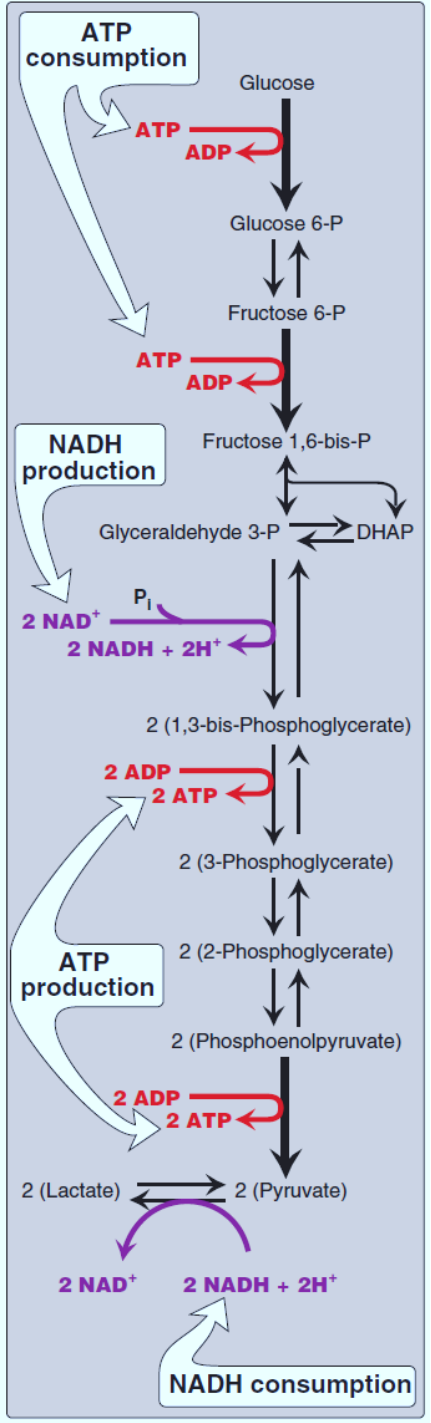



**Figure 2.22** Summary of anaerobic glycolysis. Reactions involving the production or consumption of ATP or NADH are indicated. The three irreversible reactions of glycolysis are shown with thick arrows. DHAP = dihydroxyacetone phosphate; NAD(H) = nicotinamide adenine dinucleotide; P = phosphate.

## 2. Aerobic glycolysis:

- The direct consumption and formation of ATP is the same as in anaerobic glycolysis (that is, a net gain of two ATP per molecule of glucose).
- Two molecules of NADH are also produced per molecule of glucose.
- Ongoing aerobic glycolysis requires the oxidation of most of this NADH by the electron transport chain, producing approximately three ATP for each NADH molecule entering the chain.
- [Note: NADH cannot cross the inner mitochondrial membrane, and substrate shuttles are required.]

## نشاط (1/5/2) نشاط فردي

**In the glycolysis, glucose 6-phosphate is converted to what, and by what enzyme?**

## نشاط (2/5/2) نشاط فردي

**What is special about PFK-1?**

**AL-RASHEED UNIVERSITY COLLEGE**

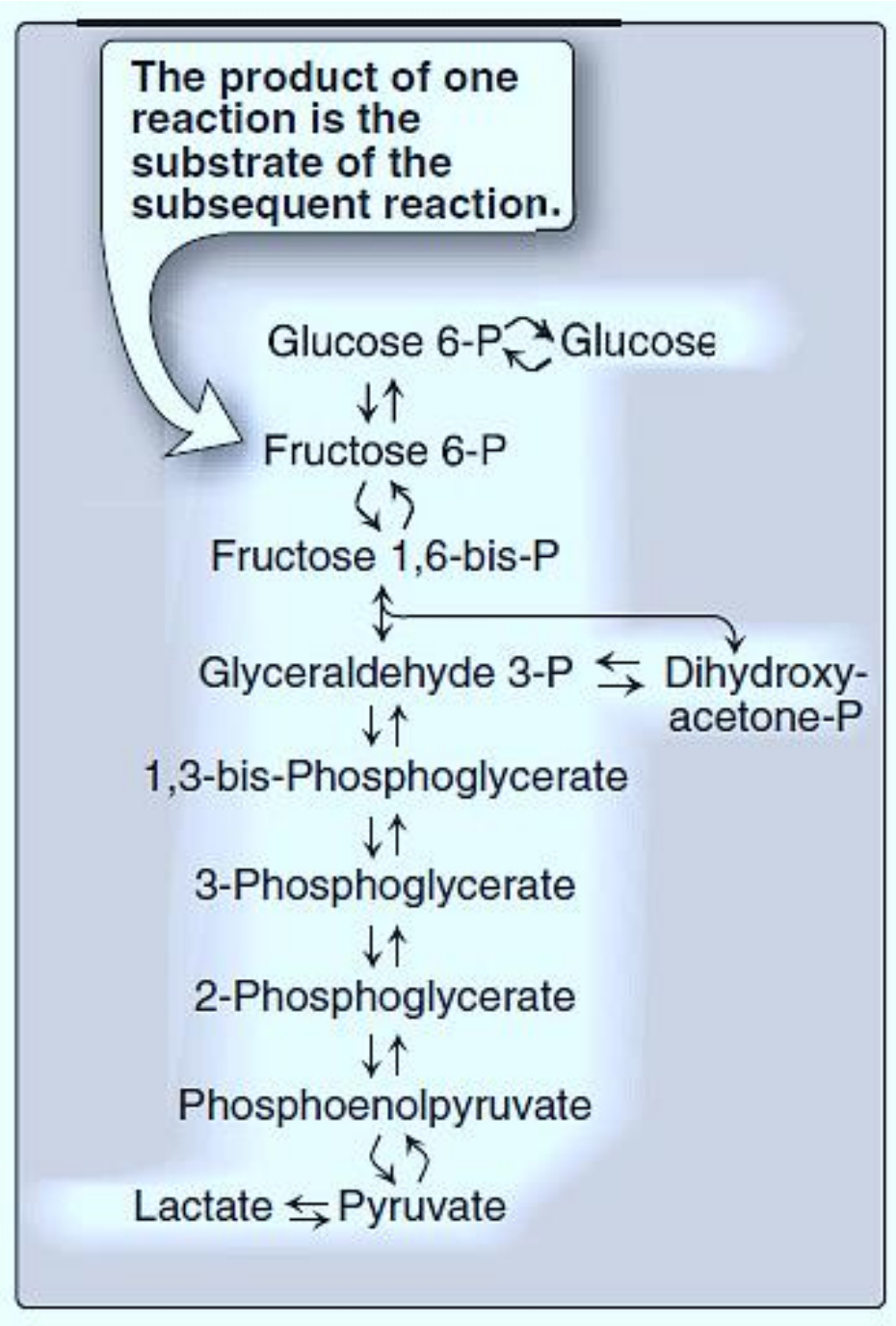
**DEPARTMENT OF MEDICAL LABORATORY TECHNIQUES**

# Introduction to Metabolism and Glycolysis

## Lecture 10

***Prepared By***

***Dr. Kutaiba I. Alzand & Dr. Rusul H. Hamza***



## الوحدة الثانية - المحاضرة السادسة - الزمن: 90 دقيقة

## أهداف المحاضرة السادسة:

**يتوقع في نهاية المحاضرة أن يكون الطالب قادراً على:**

By the end of the lecture, the student should be able to:

1. Explain the hormonal regulation of glycolysis
2. Describe the oxidative decarboxylation of pyruvate
3. Describe the carboxylation of pyruvate to oxaloacetate
4. Explain the reduction of pyruvate to ethanol (microorganisms)

# موضوعات المحاضرة الخامسة:

- **HORMONAL REGULATION OF GLYCOLYSIS**
- **ALTERNATE FATES OF PYRUVATE**
  - Oxidative decarboxylation of pyruvate
  - Carboxylation of pyruvate to oxaloacetate
  - Reduction of pyruvate to ethanol (microorganisms)
- **CHAPTER SUMMARY**

# VI. HORMONAL REGULATION OF GLYCOLYSIS

- The regulation of glycolysis by allosteric activation or inhibition, or the covalent phosphorylation/dephosphorylation of rate-limiting enzymes, is short-term (that is, they influence glucose consumption over periods of minutes or hours).
- Superimposed on these moment-to-moment effects are s lower, and often more profound, hormonal influences on gene expression, or the amount of enzyme protein synthesized.
- These effects can result in 10-fold to 20-fold increases in enzyme activity that typically occur over hours to days .
- Although the current focus is on glycolysis, reciprocal changes occur in the rate-limiting enzymes of gluconeogenesis.

- Regular consumption of meals rich in carbohydrate or administration of insulin initiates an increase in the amount of *glucokinase*, *phosphofructokinase*, and PK in the liver (Figure 2.23).
- These changes reflect an increase in gene transcription, resulting in increased enzyme synthesis. High activity of these three enzymes favors the conversion of glucose to pyruvate , a characteristic of the absorptive state.
- Conversely, gene transcription and synthesis of *glucokinase*, *phosphofructokinase*, and PK are decreased when plasma glucagon is high and insulin is low (for example, as seen in fasting or diabetes).

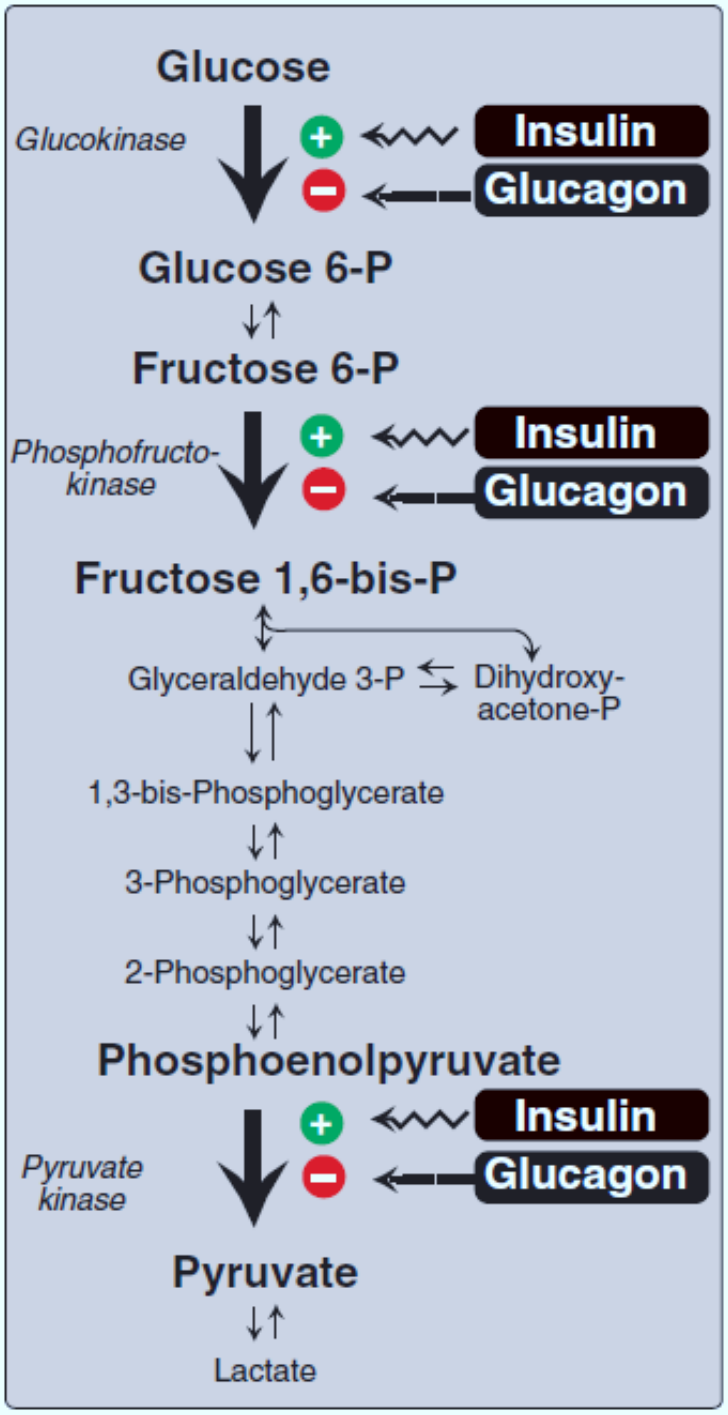



**Figure 2.23** Effect of insulin and glucagon on the synthes is of key enzymes of glycolysis in liver.

# VII. ALTERNATE FATES OF PYRUVATE

## A. Oxidative decarboxylation of pyruvate

- Oxidative decarboxylation of pyruvate by the *pyruvate dehydrogenase* complex is an important pathway in tissues with a high oxidative capacity such as cardiac muscle (Figure 2.24).
- Pyruvate dehydrogenase irreversibly converts pyruvate, the end product of glycolysis, into acetyl CoA, a major fuel for the TCA cycle and the building block for fatty acid synthesis.

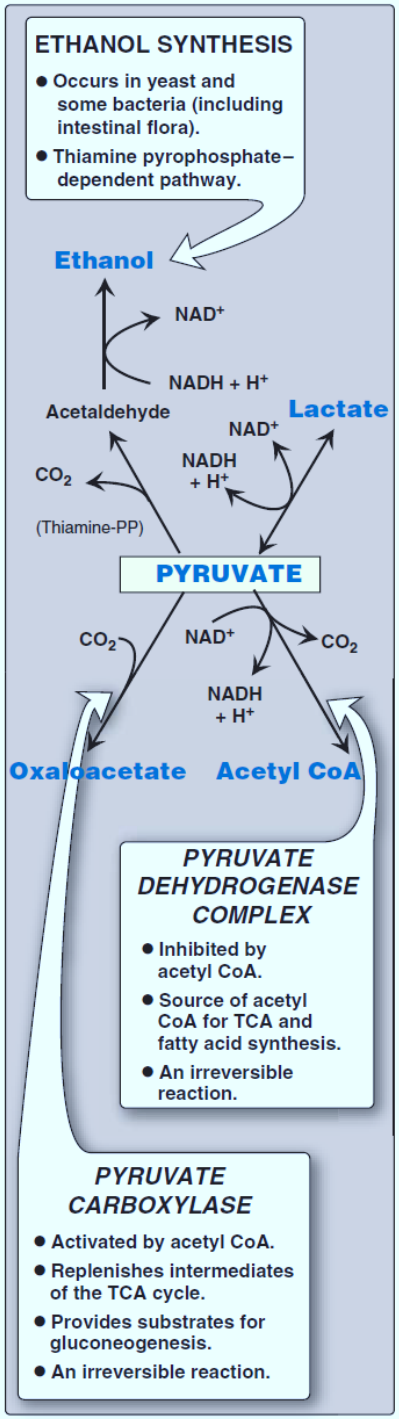
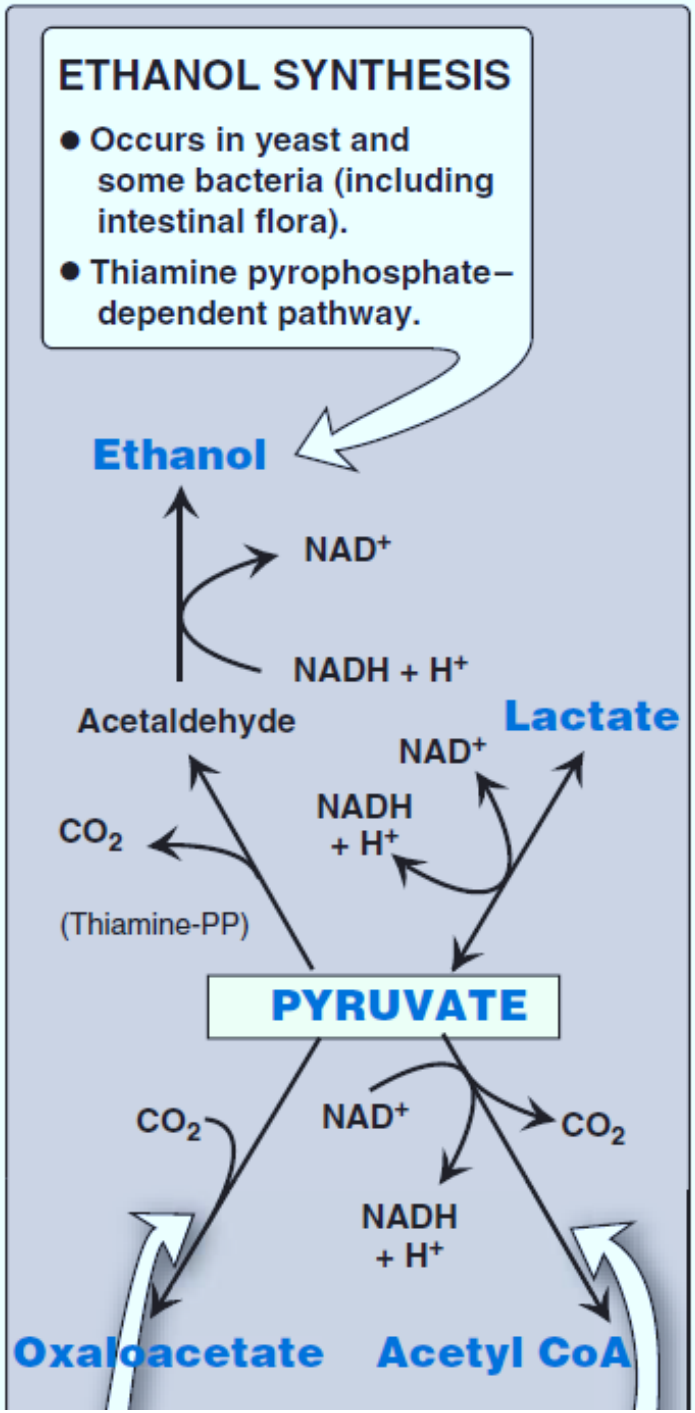
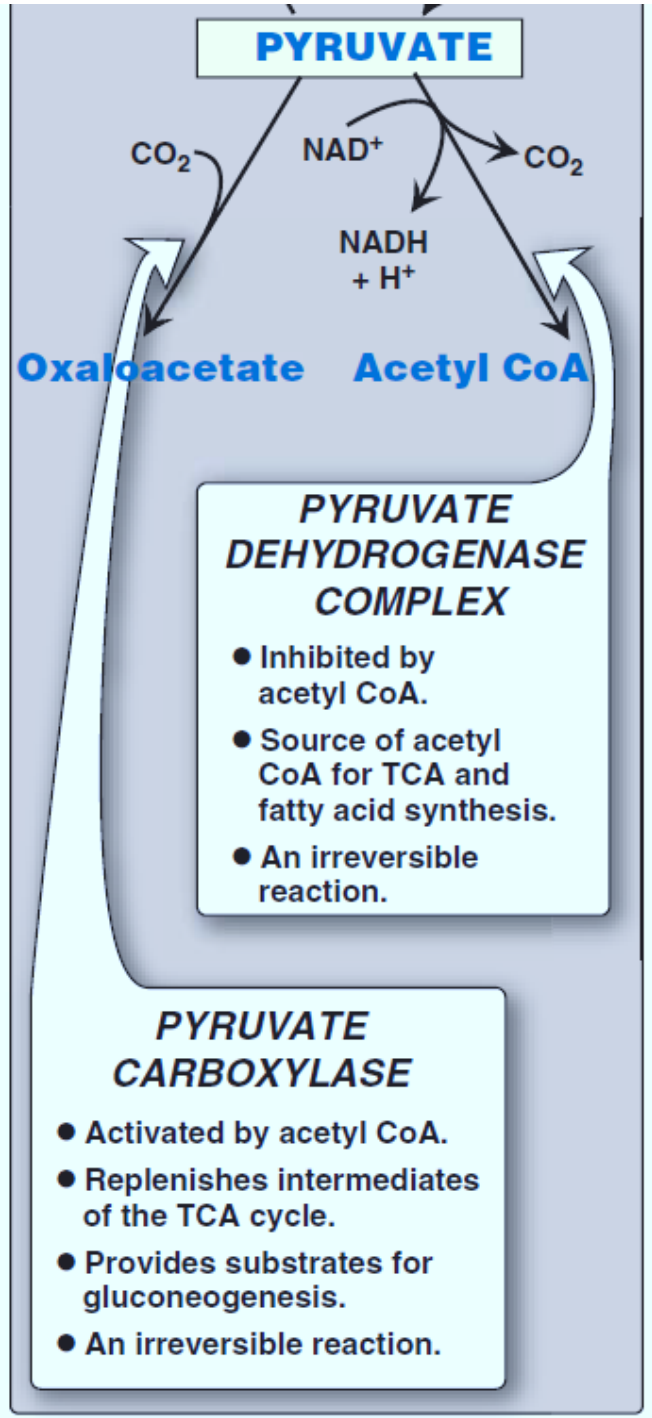



**Figure 2.24** Summary of the metabolic fates of pyruvate. TPP = thiamine pyrophosphate. TCA = trica rboxylic acid; NAD(H) = nicotinamide adenine dinucleotide; CoA = coenzyme A.


ETHANOL SYNTHESIS
● Occurs in yeast and some bacteria (including intestinal flora).
● Thiamine pyrophosphate–dependent pathway.
Ethanol
NAD+
NADH + H+
Acetaldehyde
Lactate
NAD+
NADH + H+
CO2
(Thiamine-PP)
PYRUVATE
CO2
NAD+
CO2
NADH + H+
Oxaloacetate
Acetyl CoA


PYRUVATE
CO2
NAD+
CO2
NADH + H+
Oxaloacetate
Acetyl CoA
PYRUVATE DEHYDROGENASE COMPLEX
● Inhibited by acetyl CoA.
● Source of acetyl CoA for TCA and fatty acid synthesis.
● An irreversible reaction.
PYRUVATE CARBOXYLASE
● Activated by acetyl CoA.
● Replenishes intermediates of the TCA cycle.
● Provides substrates for gluconeogenesis.
● An irreversible reaction.

## B. Carboxylation of pyruvate to oxaloacetate

- Carboxylation of pyruvate to oxaloacetate by *pyruvate carboxylase* is a biotin-dependent reaction (see Figure 2.24).
- This reaction is important because it replenishes the TCA cycle intermediates and provides substrate for gluconeogenesis.

## C. Reduction of pyruvate to ethanol (microorganisms)

- The convers ion of pyruvate to ethanol occurs by the two reactions summarized in Figure 2.24.
- The decarboxylation of pyruvate by *pyruvate decarboxylase* occurs in yeast and certain other microorganisms but not in humans.
- The enzyme requires thiamine pyrophosphate as a coenzyme and catalyzes are action similar to that described for pyruvate dehydrogenase.

# VIII. CHAPTER SUMMARY

- Most pathways can be classified as either **catabolic** (**degrade** complex molecules to a few simple products) or **anabolic** (**synthesize** complex end products from simple precursors).
- **Catabolic reactions** also **capture chemical energy** in the form of **ATP** from the degradation of energy rich molecules.
- **Anabolic reactions require energy**, which is generally provided by the hydrolysis of ATP.
- The rate of a metabolic pathway can respond to **regulatory signals** such as **allosteric activators** or **inhibitors** that arise from **within the cell**.
- Signaling **between cells** provides for the integration of metabolism. The most important route of this communication is **chemical signaling** (for example, by **hormones** or **neurotransmitters**).

- **Second messenger molecules** transduce a chemical signal (hormone or neurotransmitter) to appropriate intracellular responders.
- *Adenylyl cyclase* is a cell membrane enzyme that synthesizes **cyclic AMP (cAMP)** in response to chemical signals, such a s the hormones **glucagon** and **epinephrine**.
- Following binding of a hormone to its **cell- surface receptor**, a GTP-dependent regulatory protein (**G protein**) is activated that, in turn, activates *adenylyl cyclase*.
- The cAMP produced activates a *protein kinase*, which phosphorylates a cadre of enzymes, causing their activation or deactivation.
- Phosphorylation is reversed by protein phosphatases.
- **Aerobic glycolysis**, in which **pyruvate** is the end product, occurs in cells with mitochondria and an adequate supply of oxygen (Figure 2.25).

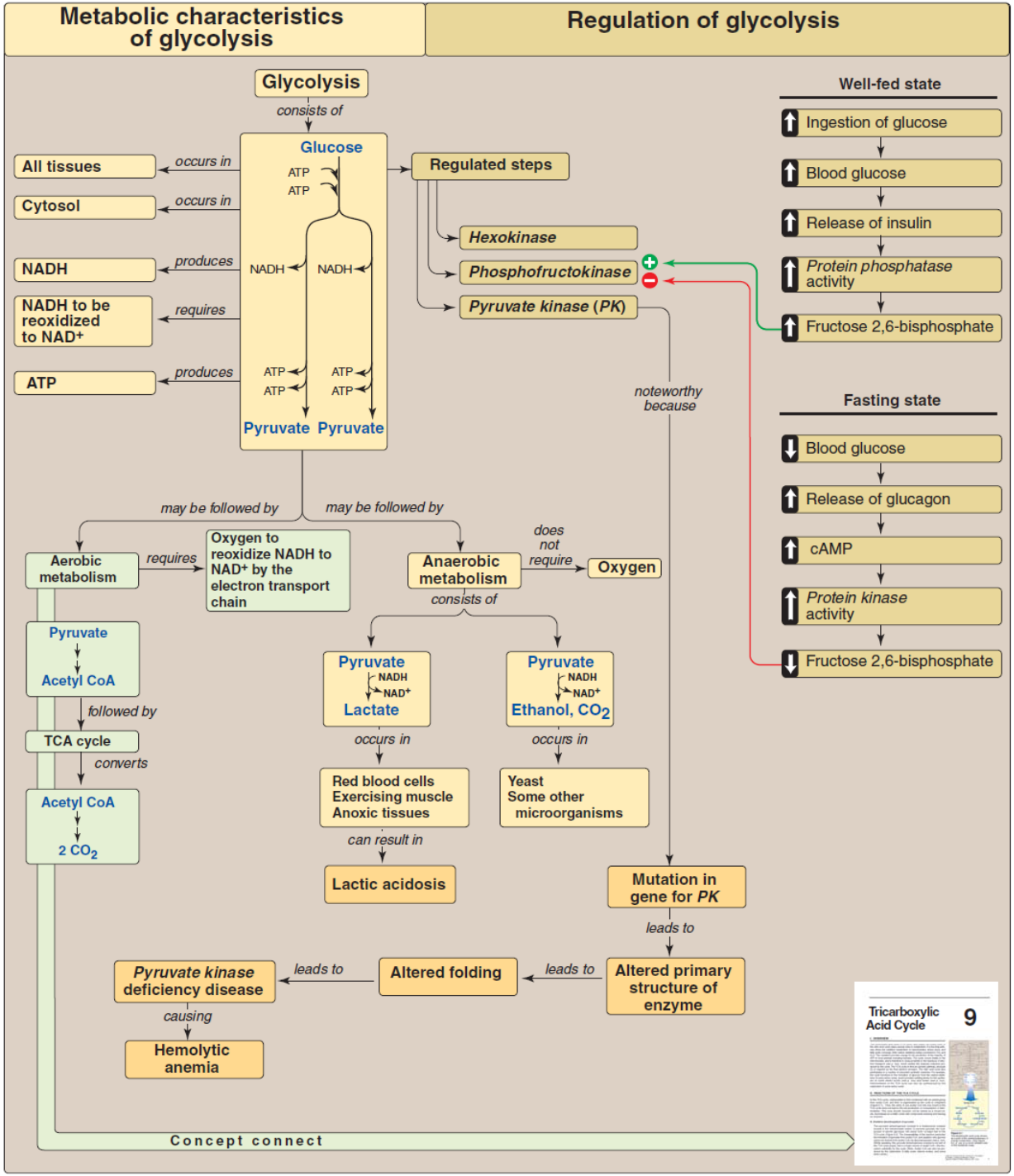
- **Anaerobic glycolysis**, in which **lactic acid** is the end product, occurs in cells that lack mitochondria and in cells deprived of sufficient oxygen.
- Glucose is transported across membranes by one of 14 **glucose transporter isoforms** (**GLUTs**).
- **GLUT-1** is abundant in **erythrocytes** and the **brain**, **GLUT-4** (which is **insulin dependent**) is found in **muscle** and **adipose tissue**, and **GLUT-2** is found in the **liver**, **kidney**, and **β cells** of the pancreas.
- The conversion of glucose to pyruvate (**glycolysis**) occurs in two stages: an **energy–investmentphase** in which phosphorylated intermediates are synthesized at the expense of ATP, and an **energy-generation phase**, in which ATP is produced.
- In the energy-investment phase, glucose is phosphorylated by hexokinase (found in **most tissues** ) or glucokinase (a hexokinase found in **liver cells** and the **β cells** of the pancreas).

- Hexokinase has a **high affinity (low Km)** and a **low Vmax** for glucose and is **inhibited** by **glucose 6-phosphate**.
- Glucokinase has a **high Km** and a **high Vmax** for glucose. It is indire**e**ctly **inhibited** by **fructose 6-phosphate** and **activated** by **glucose**.
- The **transcription** of the gene for glucokinase is **enhanced by insulin**.
- Glucose 6-phosphate is isomerized to fructose 6-phosphate, which is phosphorylated to **fructose 1,6-bisphosphate** by phosphofructokinase-1 (PFK-1).
- This enzyme is **allosterically inhibited** by **ATP** and **citrate** and **activated** by **AMP**.
- **Fructose 2,6-bisphosphate**, whose synthesis by phosphofructokinase-2 (PFK-2) is **activated** by **insulin**, is the most potent allosteric activator of PFK-1.

- A total of **two ATP are used** during this phase of glycolysis.
- Fructose 1,6-bisphosphate is cleaved to form two trioses that are further metabolized by the glycolytic pathway, forming pyruvate. During these reactions, **four ATP** and **two NADH are produced** from ADP and NAD+.
- The final step in pyruvate synthesis from phosphoenolpyruvate is catalyzed by pyruvate kinase (PK).
- This enzyme is **allosterically activated** by **fructose 1,6-bisphosphate** and **hormonally activated** by **insulin** and **inhibited** in the liver by **glucagon** via the **cAMP pathway**.
- PK **deficiency** accounts for the majority of all inherited defects in glycolytic enzymes. Effects are restricted to **erythrocytes** and present as mild to severe **chronic, nonspherocytic hemolytic anemia**.

- In **anaerobic glycolysis**, NADH is reoxidized to NAD+ by the **convers ion of pyruvate to lactate**.
- This occurs in cells, such as **erythrocytes**, that have few or no mitochondria, and in tissues, such as **exercising muscle**, where production of NADH exceeds the oxidative capacity of the respiratory chain.
- Elevated concentrations of lactate in the plasma (**lactic acidosis**) occur when there is a **collapse of the circulatory system** or when an individual is in **shock**.
- Pyruvate can be 1) **oxidatively decarboxylated** by pyruvate dehydrogenase, producing **acetyl coenzyme A**; 2) **carboxylated** to **oxaloacetate** (a tricarboxylic acid cycle intermediate) by pyruvate carboxylase; or 3) **reduced** by microorganisms to **ethanol** by pyruvate decarboxylase.

**Figure 2.25** Key concept map for glycolysis. NAD(H) = nicotinamide adenine dinucleotide; cAMP = cyclic adenosine monophosphate; CoA = coenzyme A; TCA = tricarboxylic acid.



## نشاط (1/6/2) نشاط فردي

**Draw the glycolysis net reaction**

## نشاط (2/6/2) نشاط فردي

**What are the alternate fates of pyruvate?**