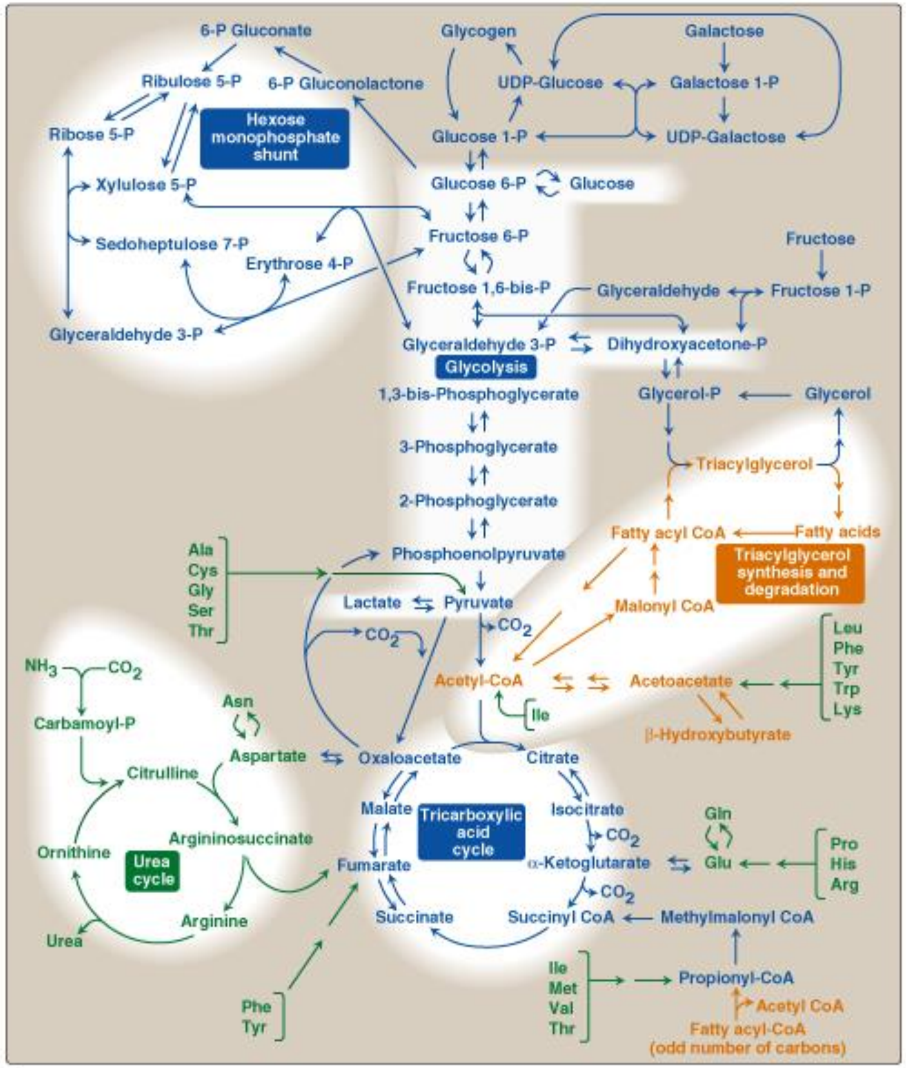

Figure 8.2

Important reactions of intermediary metabolism. Several important pathways to be discussed in later chapters are highlighted. Curved reaction arrows ( ) indicate forward and reverse reactions that are catalyzed by different enzymes. The straight arrows ( ) indicate forward and reverse reactions that are catalyzed by the same enzyme. Key: Blue text = intermediates of carbohydrate metabolism; brown text = intermediates of lipid metabolism; green text = intermediates of protein metabolism.

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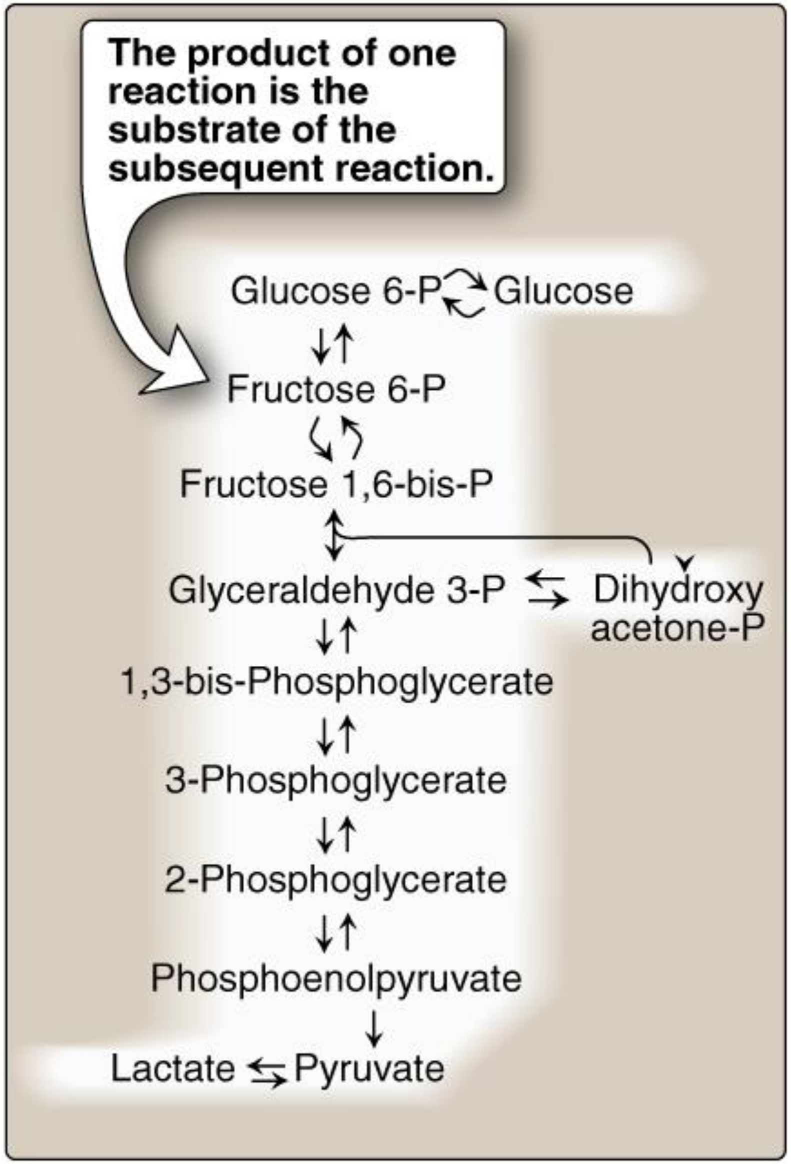

Figure 8.1

Glycolysis, an example of a metabolic pathway.

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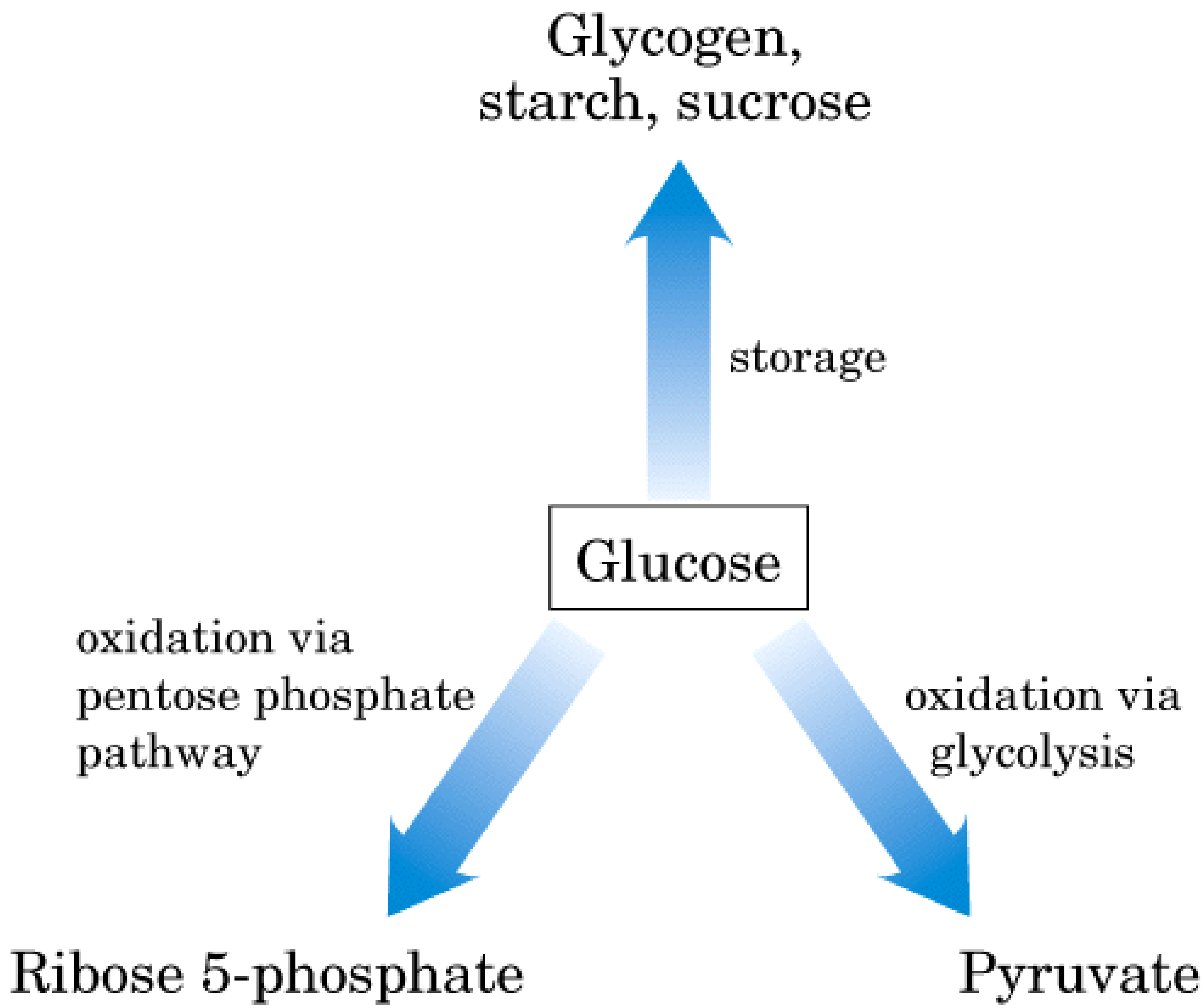
Glycogen,
starch, sucrose
storage
Glucose
oxidation via
pentose phosphate
pathway
oxidation via
glycolysis
Ribose 5-phosphate
Pyruvate

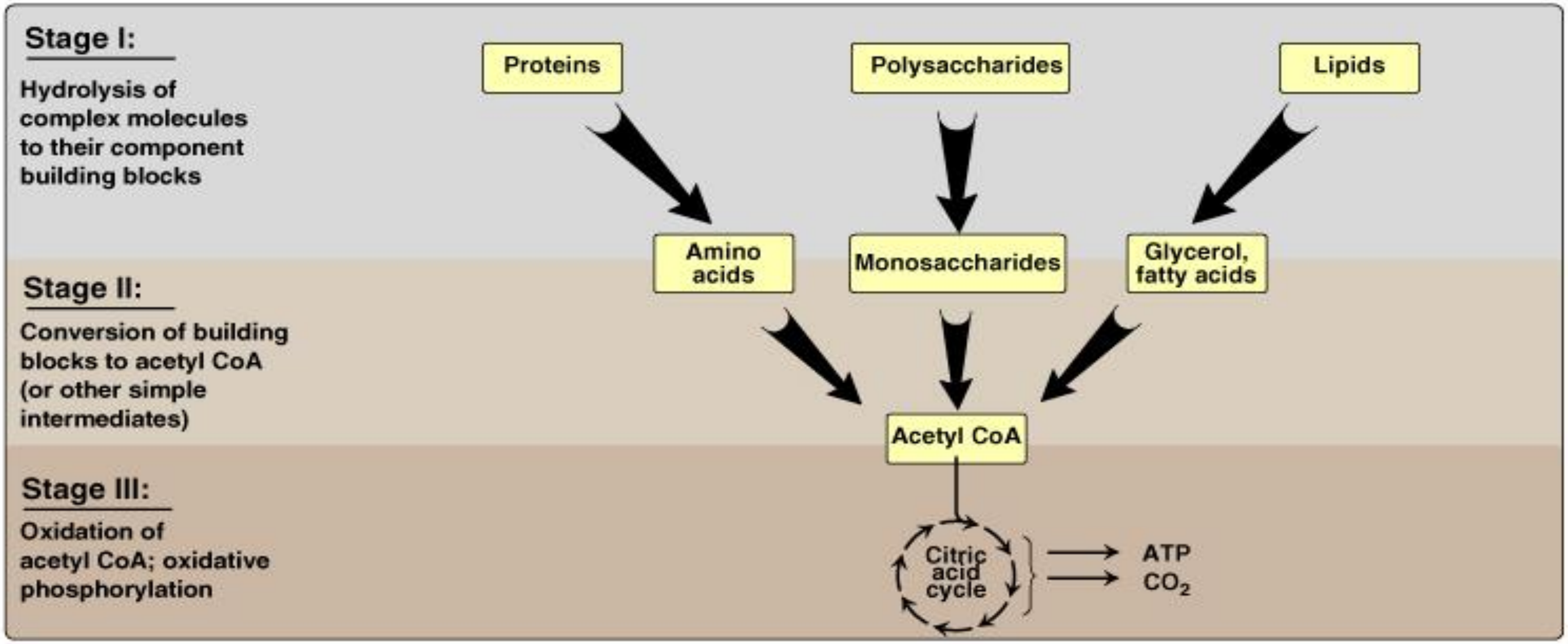

Figure 8.3
Three stages of catabolism.

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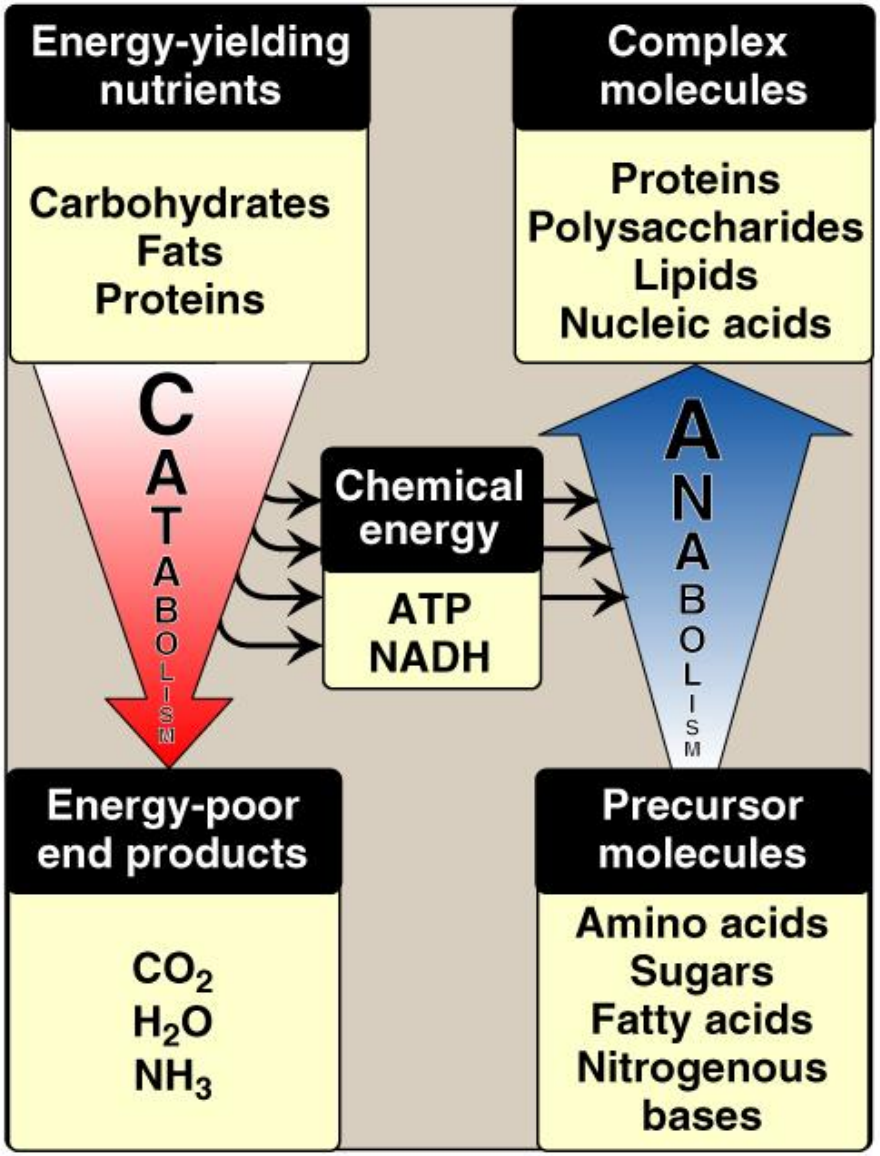

Figure 8.4
Comparison of catabolic and anabolic pathways.

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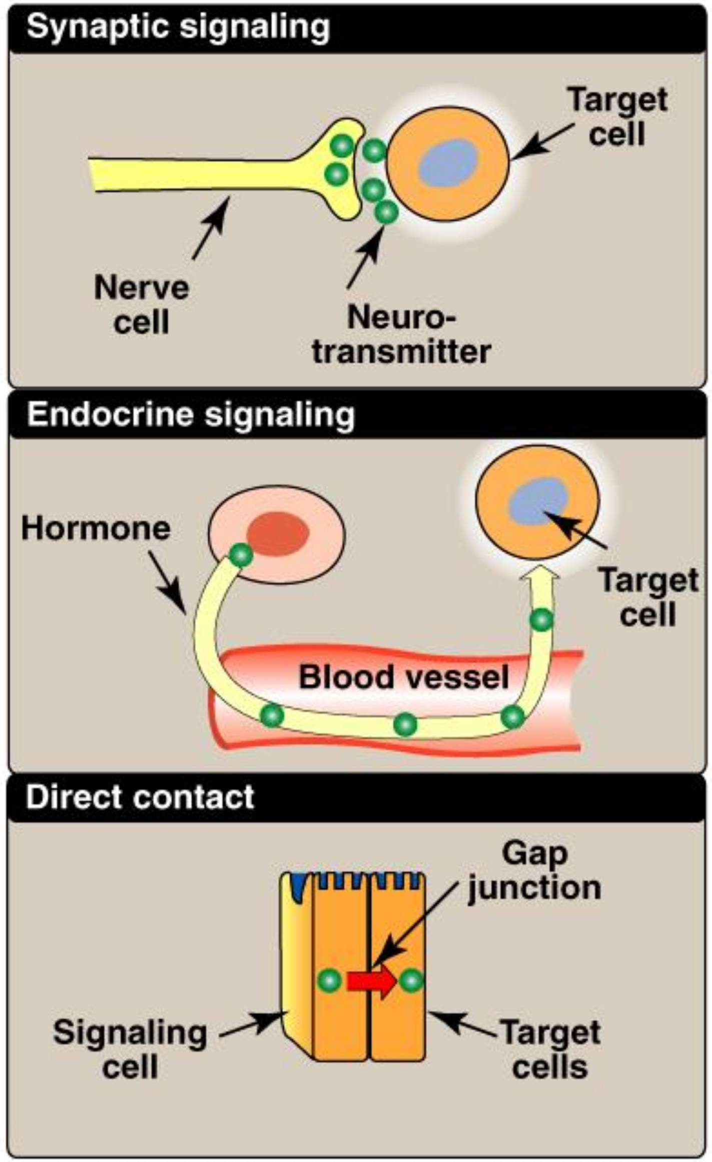

Figure 8.5

Some commonly used mechanisms for transmission of regulatory signals between cells.

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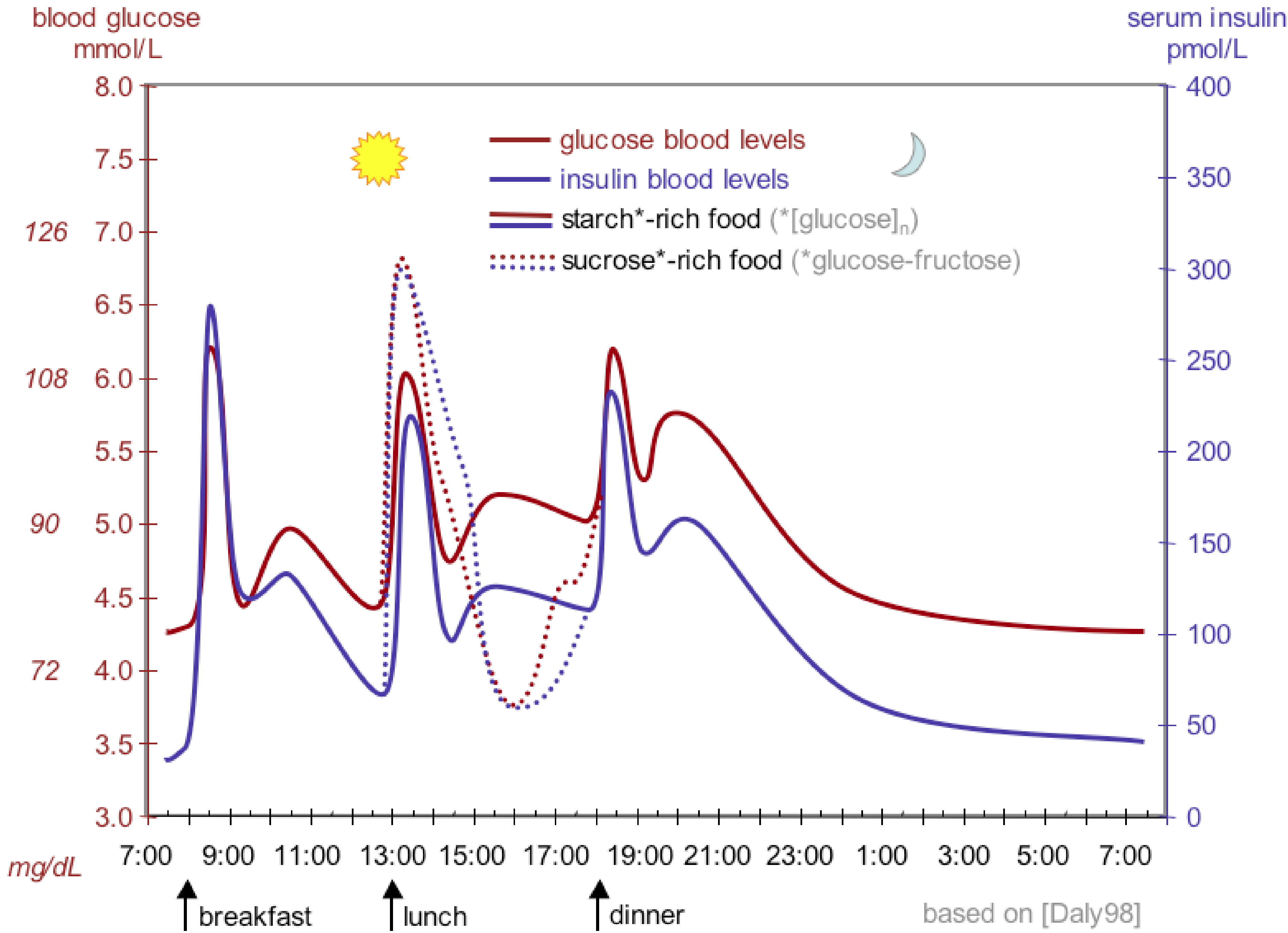
What are the levels of glucose in saliva? Is there any correlation between saliva glucose and blood glucose? How can we use saliva glucose levels for health prevention?

Over 80 million Americans and 300 million individuals worldwide are estimated to have diabetes or prediabetes. Already an epidemic, this number is expected to double by 2030 according to the CDC.

To function, your brain cells need two times more fuel than other cells in your body. This is why eating the recommended amount of carbohydrates daily is so important

Thinking and memory drain glucose from the brain at a higher rate than any other brain functions.


blood glucose
mmol/L
serum insulin
pmol/L
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
126
108
90
72
mg/dL
400
350
300
250
200
150
100
50
0
glucose blood levels
insulin blood levels
starch*-rich food (*[glucose]n)
sucrose*-rich food (*glucose-fructose)
7:00
9:00
11:00
13:00
15:00
17:00
19:00
21:00
23:00
1:00
3:00
5:00
7:00
breakfast
lunch
dinner
based on [Daly98]

The mean normal blood glucose level in humans is about 5.5 mM (5.5 mmol/L or 100 mg/dL); this level fluctuates throughout the day. Glucose levels are lowest in the morning, (termed "the fasting level"), and rise after meals for an hour or two by a few millimolar. The normal blood glucose level (tested while fasting) for non-diabetics, should be between 70 and 100 mg/dL). Blood sugar levels for those without diabetes and who are not fasting should be below 125 mg/dL. The blood glucose target range for diabetics, according to the American Diabetes Association, should be 70–130 (mg/dL) before meals, and less than 160 mg/dL after meals.

A persistently high level is referred to as hyperglycemia; low levels are referred to as hypoglycemia. Diabetes mellitus is characterized by persistent hyperglycemia from any of several causes, and is the most prominent disease related to failure of blood sugar regulation. Intake of alcohol causes an initial surge in blood sugar, and later tends to cause levels to fall.

The average levels of glucose in saliva is around 0.0017 mmol/l in the normal population and about 0.022 mmol/l in diabetes patients. The global prevalence of diabetes is around 6.4% in adult population. We expect around 438 million people with diabetes for the year 2030.

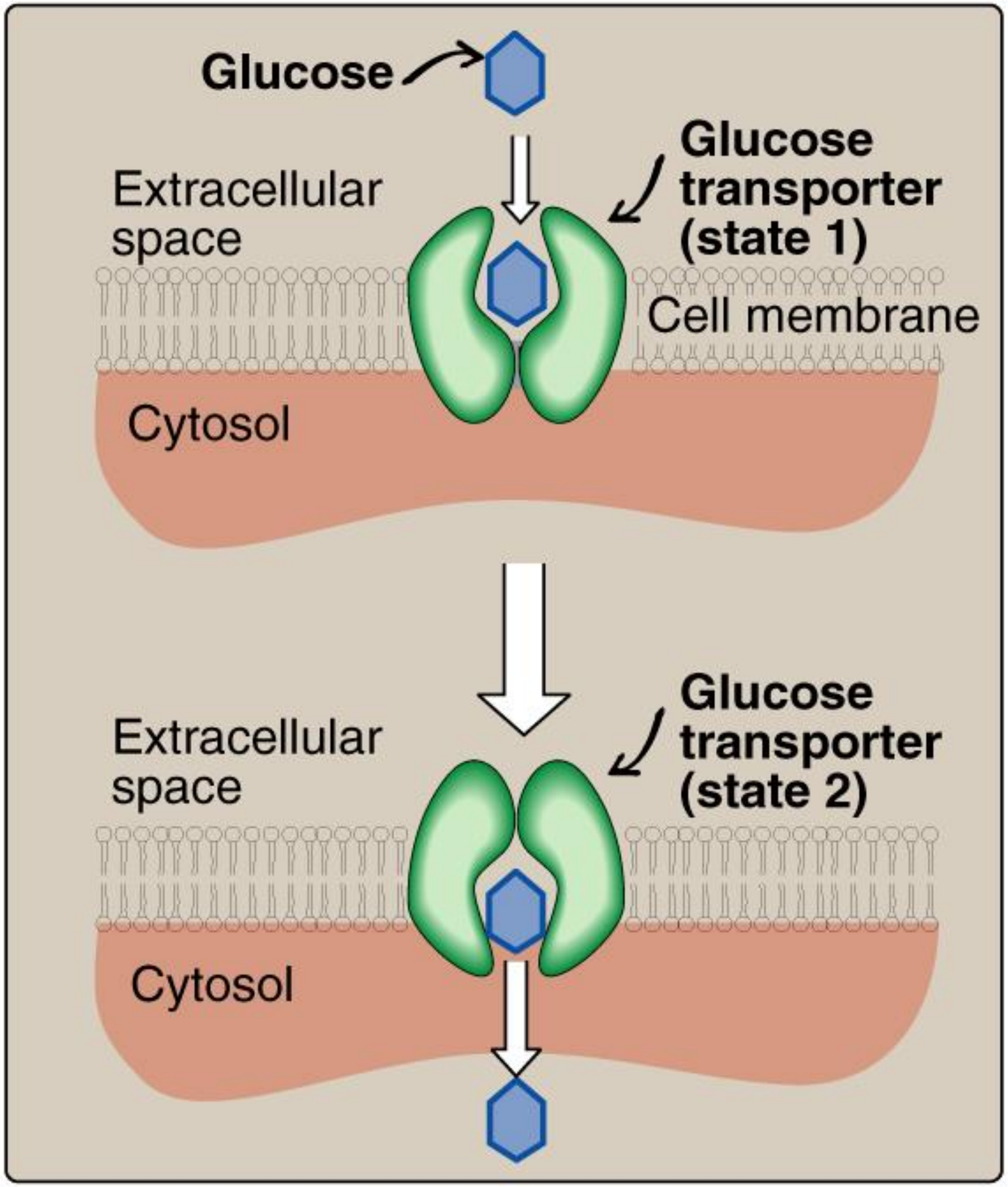


Figure 8.10
Schematic representation of the facilitated transport of glucose through a cell membrane.

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Glucose can not diffuse directly into cell, there are two mechanisms. Facilitated transport (glucose transporters GLUT1-5) Tissue specific GLUT 4 (abundant in adipose tissue and skeletal muscle) insulin regulated. Gradient dependent. GLUT 1,3&4 uptake from blood

Cotransport carrier mediated concentration gradient with Na+ monosaccharide (occurs in epithelial cells of the intestine, renal tubes.

GLUT1-14 isoforms

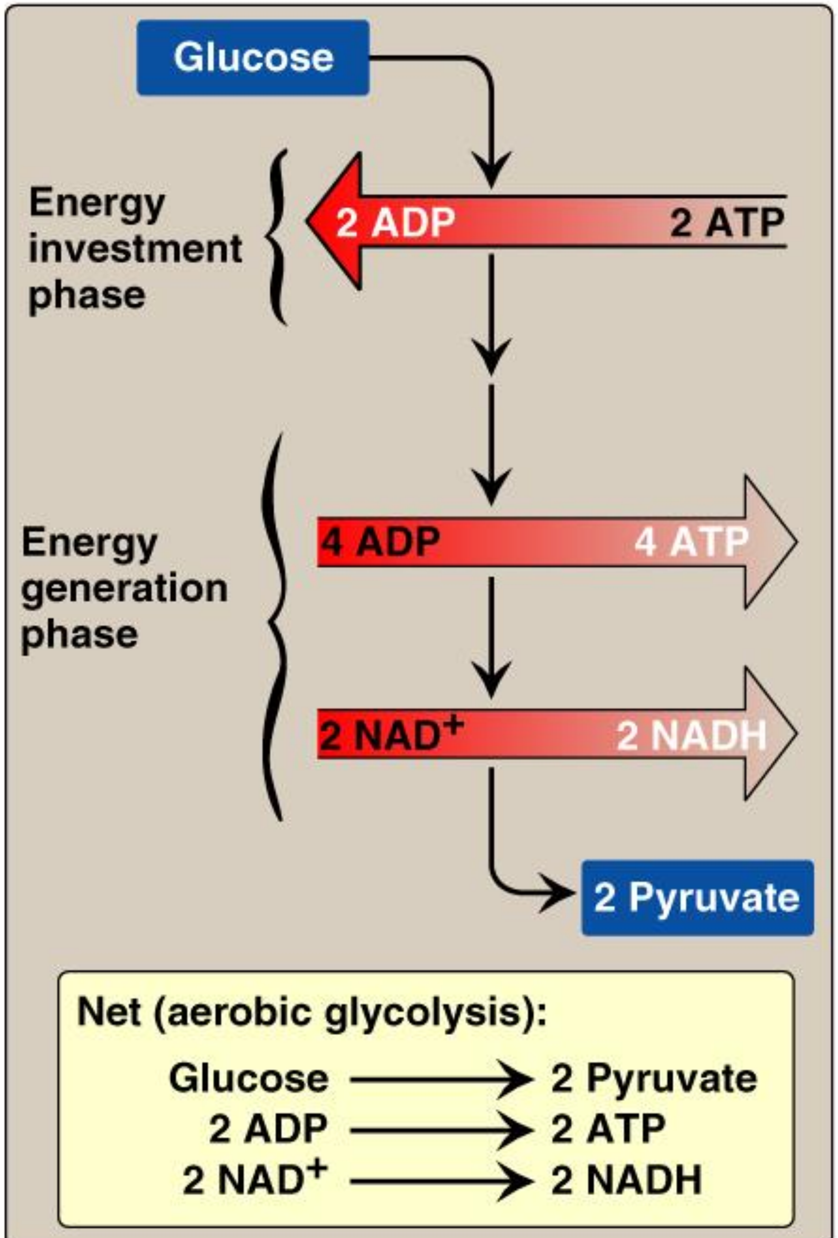

Figure 8.11
Two phases of aerobic glycolysis.

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GLUT1 erythrocytes, brain

GLUT2 liver, kidney, B cells

Transports from cell to blood

GLUT3 in neurons

GLUT4 adipose skeletal musc

GLUT5 transporter fructose, small intestine and testes

Glycolysis occurs in two stages. First five reactions are energy investment Formation of fructose at the expense of ATP. Stage 2 is an energy generation stage net of 2 ATP and 2NADH.

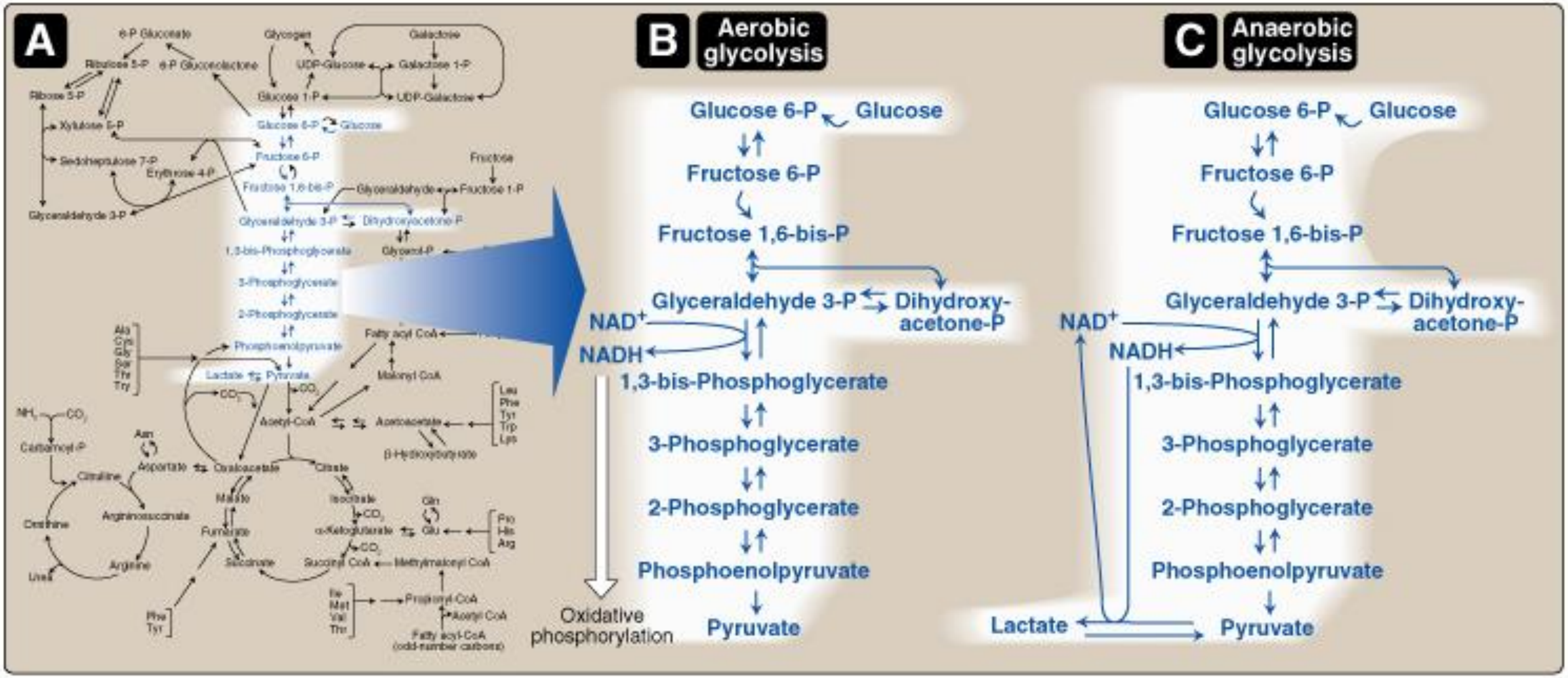


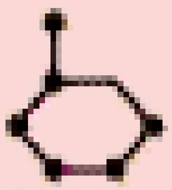
Figure 8.9

A. Glycolysis shown as one of the essential pathways of energy metabolism. B. Reactions of aerobic glycolysis. C. Reactions of anaerobic glycolysis.

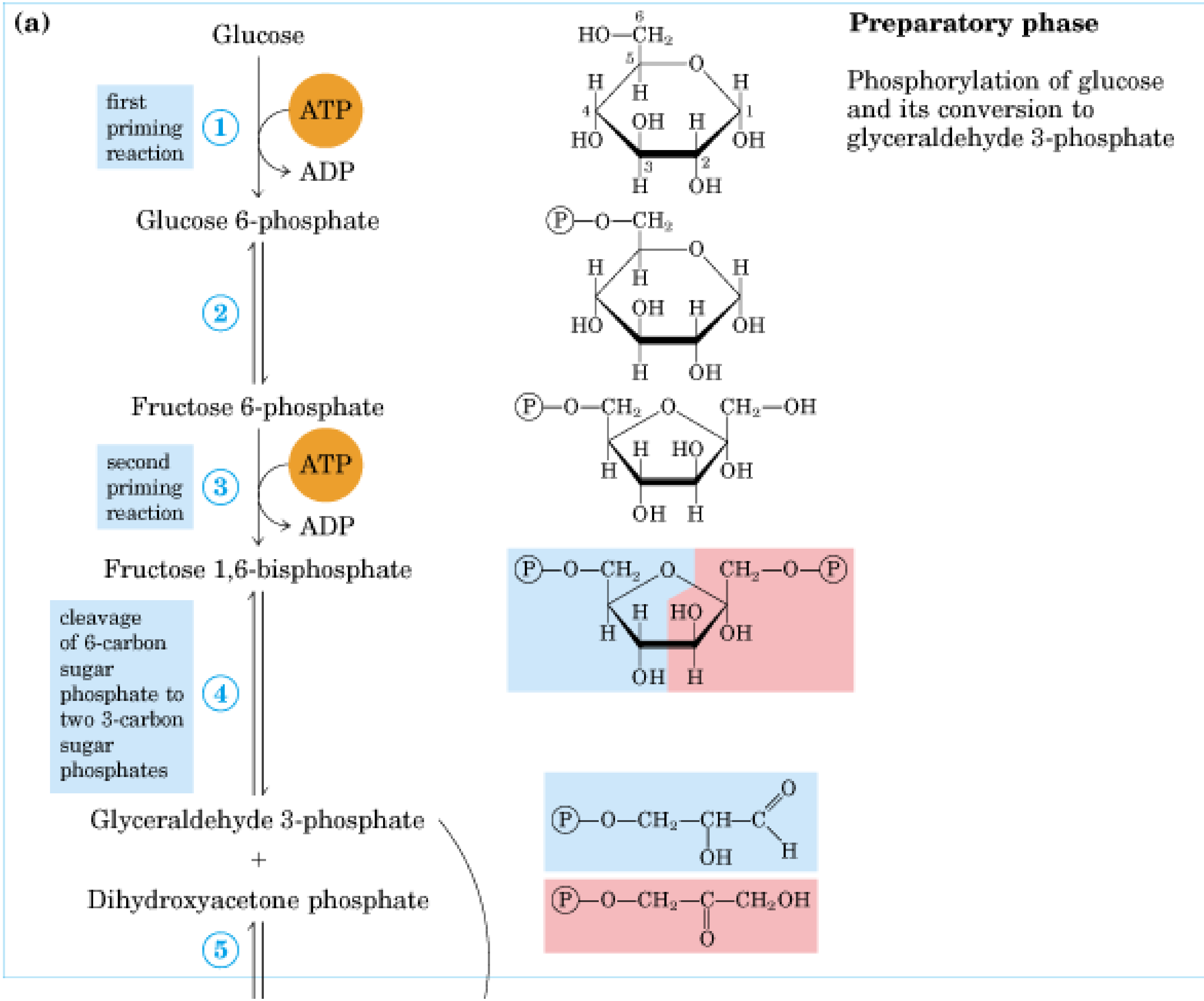
Glycolysis employed by all tissues, breakdown of Glucose to provide energy. Pyruvate is the end product in cells with mitochondria and a adequate supply of Oxygen. There are ten reactions in aerobic glycolysis because O_2 is required to re-oxidize NADH formed during oxidation of glyceraldehyde 3-P. In anaerobic glycolysis NADH reduces pyruvate to form lactate (there is no net formation of NADH) and therefore can occur in the absence of O_2 (red blood cells). Irreversible rxs (three)

GLYCOLYSIS

Mostly endergonic reactions

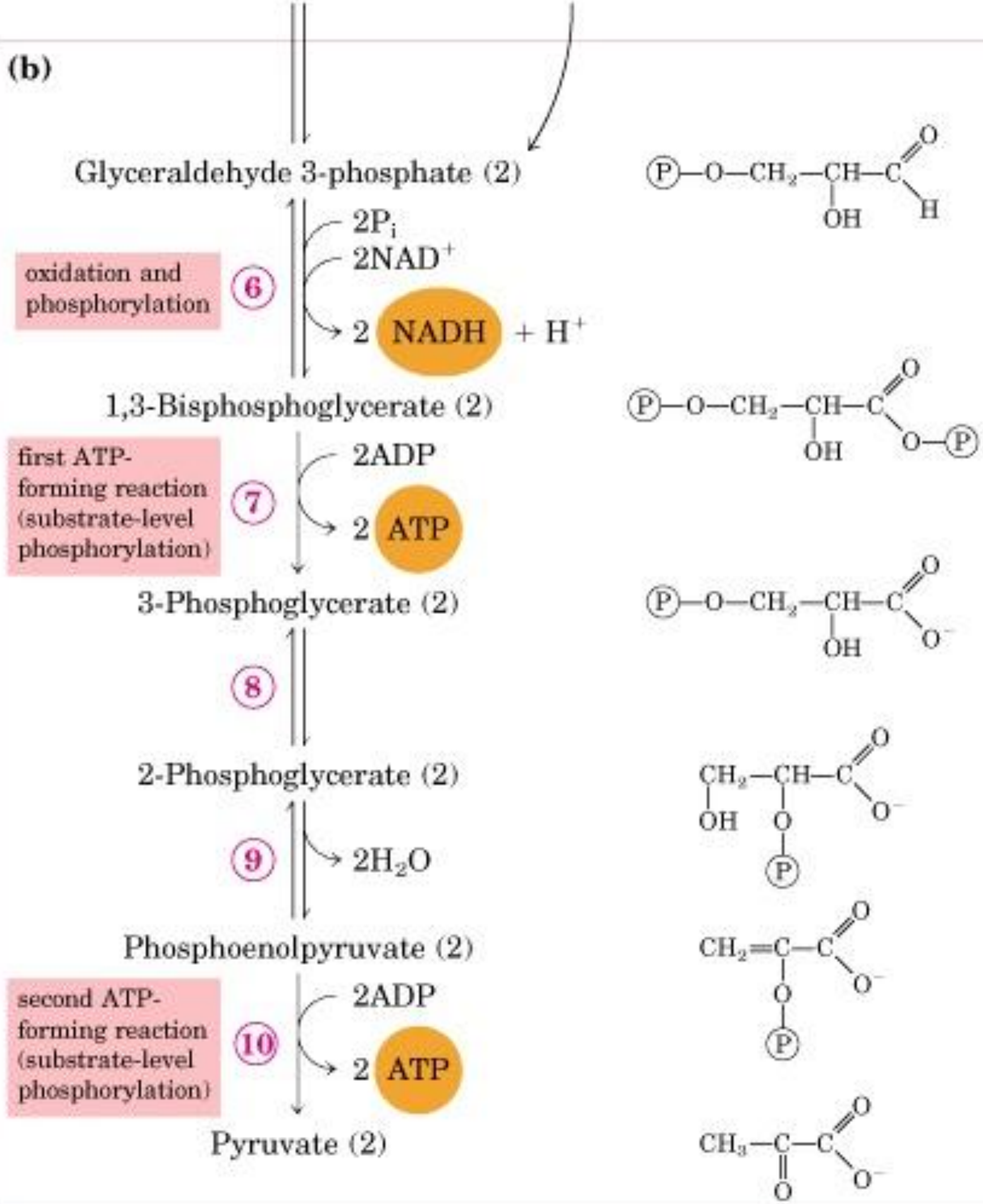


Glucose
(6 carbons)


(a)
Preparatory phase
Phosphorylation of glucose and its conversion to glyceraldehyde 3-phosphate
Glucose
first priming reaction
1
ATP
ADP
Glucose 6-phosphate
2
Fructose 6-phosphate
second priming reaction
3
ATP
ADP
Fructose 1,6-bisphosphate
cleavage of 6-carbon sugar phosphate to two 3-carbon sugar phosphates
4
Glyceraldehyde 3-phosphate
+
Dihydroxyacetone phosphate
5

Payoff phase

Oxidative conversion of glyceraldehyde 3-phosphate to pyruvate and the coupled formation of ATP and NADH



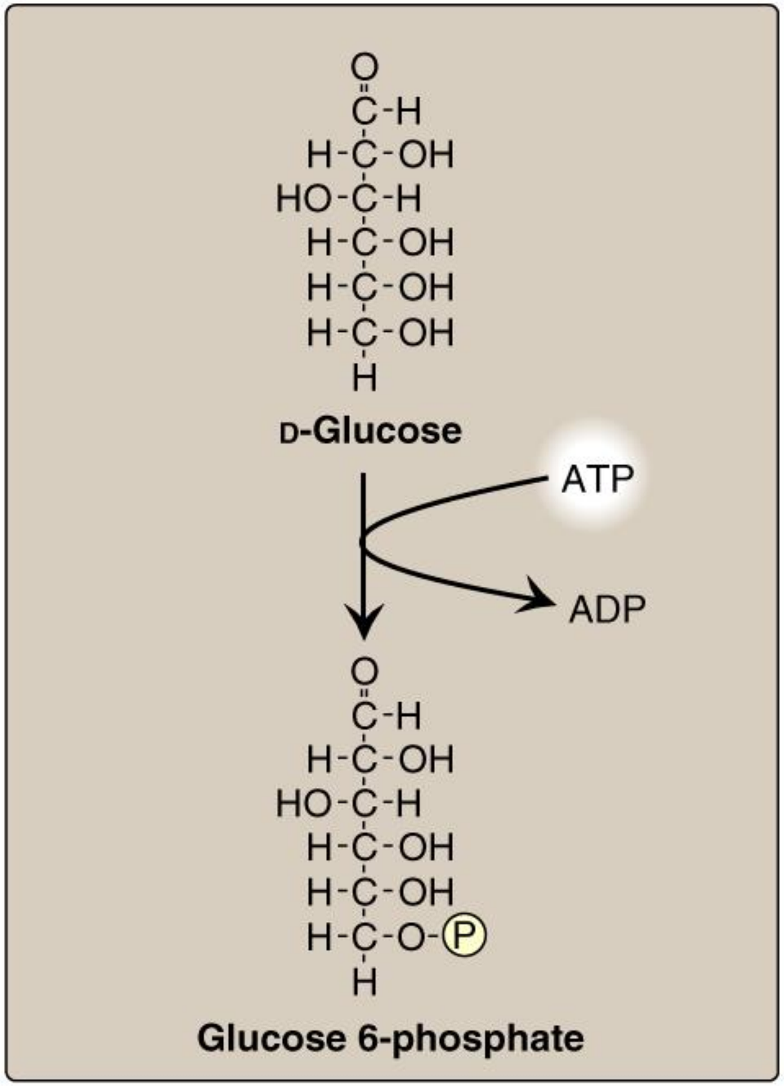


Figure 8.12

Energy investment phase: phosphorylation of glucose.

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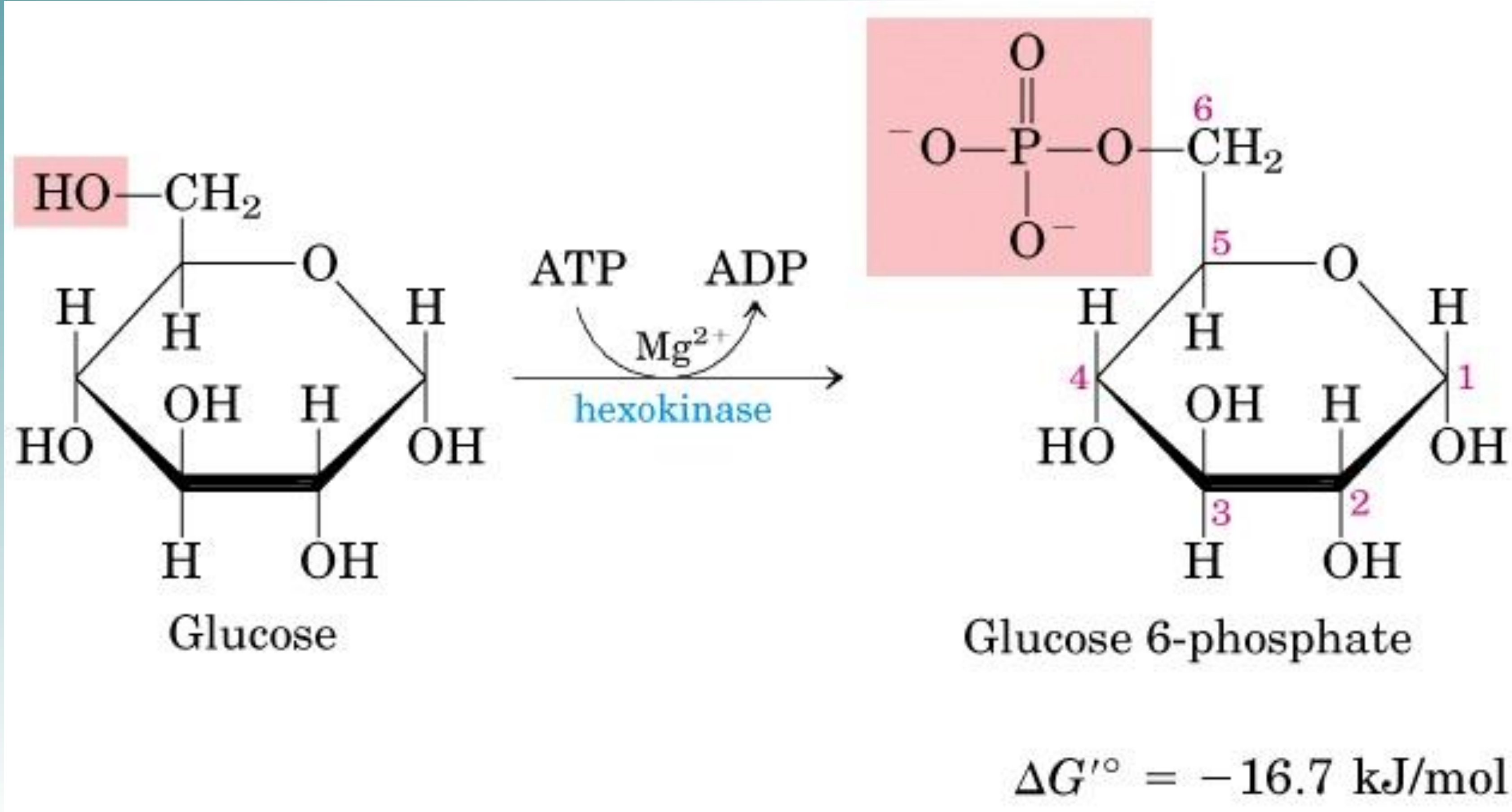
Irreversible phosphorylation reaction. Glucose 6-P does not diffuse out of cell. Commits glucose to further metabolism. Hexokinase catalyze phosphorylation of Glucose. Occurs in most tissues and is one of the three regulatory enzymes (pyruvate kinase and phosphofructokinase (PFK1)). Inhibited by reaction product, glucose 6-P.

Glucokinase: In liver and β cells in the pancreas. Requires higher glucose concentrations for half saturation. Active during carbohydrate rich meal minimizing hyperglycemia during absorptive periods. Increased by carbohydrates and Insulin. Not inhibited by Glucose 6-P.

First irreversible reaction



Phosphorylated on C-6, first ATP consumed

TABLE 7.1: PROPERTIES OF HEXOKINASE AND GLUCOKINASE

	Hexokinase	*Glucokinase*
Tissue distribution:	Most tissues	Liver and β-cells
K_m	Low (0.1 mmol/L = 2 mg%*)	High** (10 mmol/L = 200 mg%*)
V_m	Low	High
Inhibition by glucose 6-phosphate	Yes	No

*mg% = milligrams glucose per 100 mL plasma; normal fasting blood glucose is 70–90 mg% or about 5 mmol/L.

** The velocity of *glucokinase* shows a sigmoid dependence on glucose concentration and thus the term "half-saturation", rather than K_m, should be used in describing this enzyme.

Low Km = High affinity

High Vm = can phosphorylate large quantities of glucose.

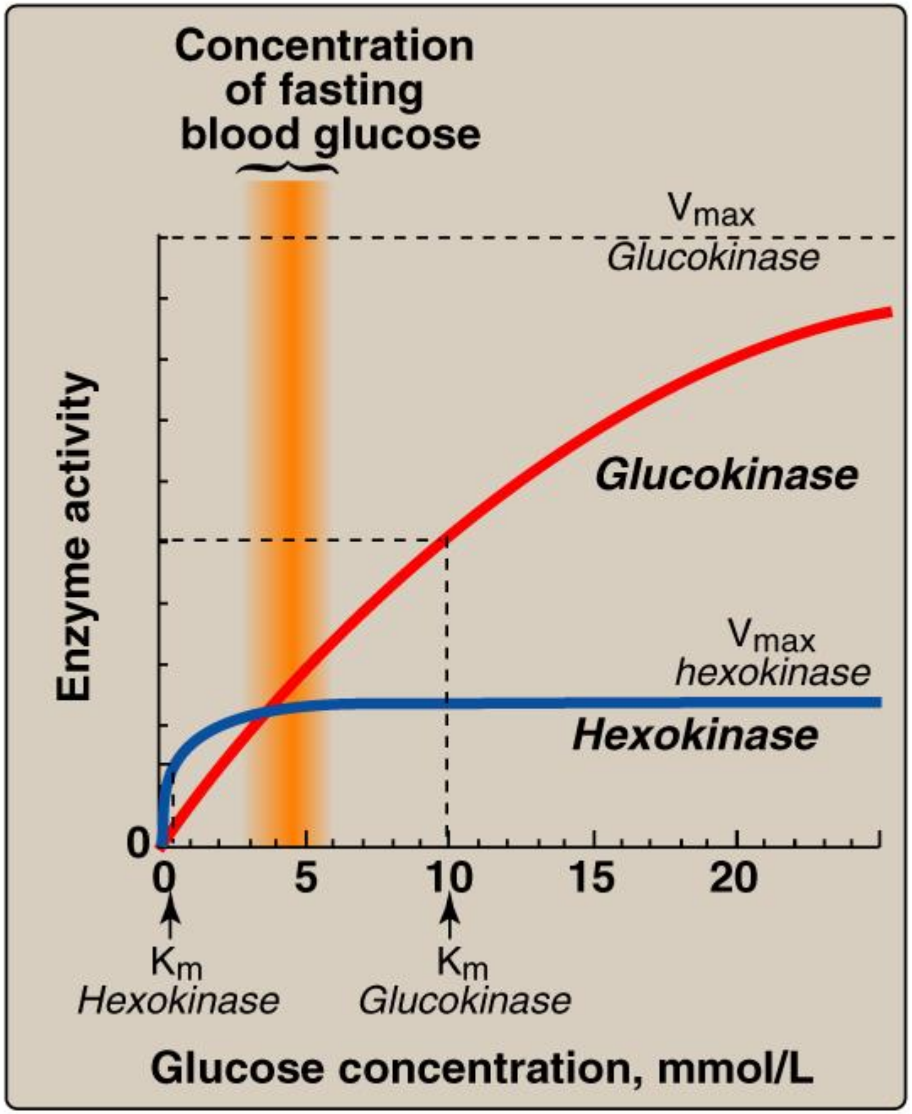


Figure 8.13
Effect of glucose concentration on the rate of phosphorylation catalyzed by *hexokinase* and *glucokinase*.

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Hexokinase are more efficient at low substrate conc while Glucokinase are more efficient at high substrate conc. Glucokinase are also known as hexokinase D it works like a glucose sensor for insulin release. Liver removes most glucose preventing it to enter the systemic circulation minimizing hyperglycemia. This enzyme is regulated by fructose 6-P and glucose.

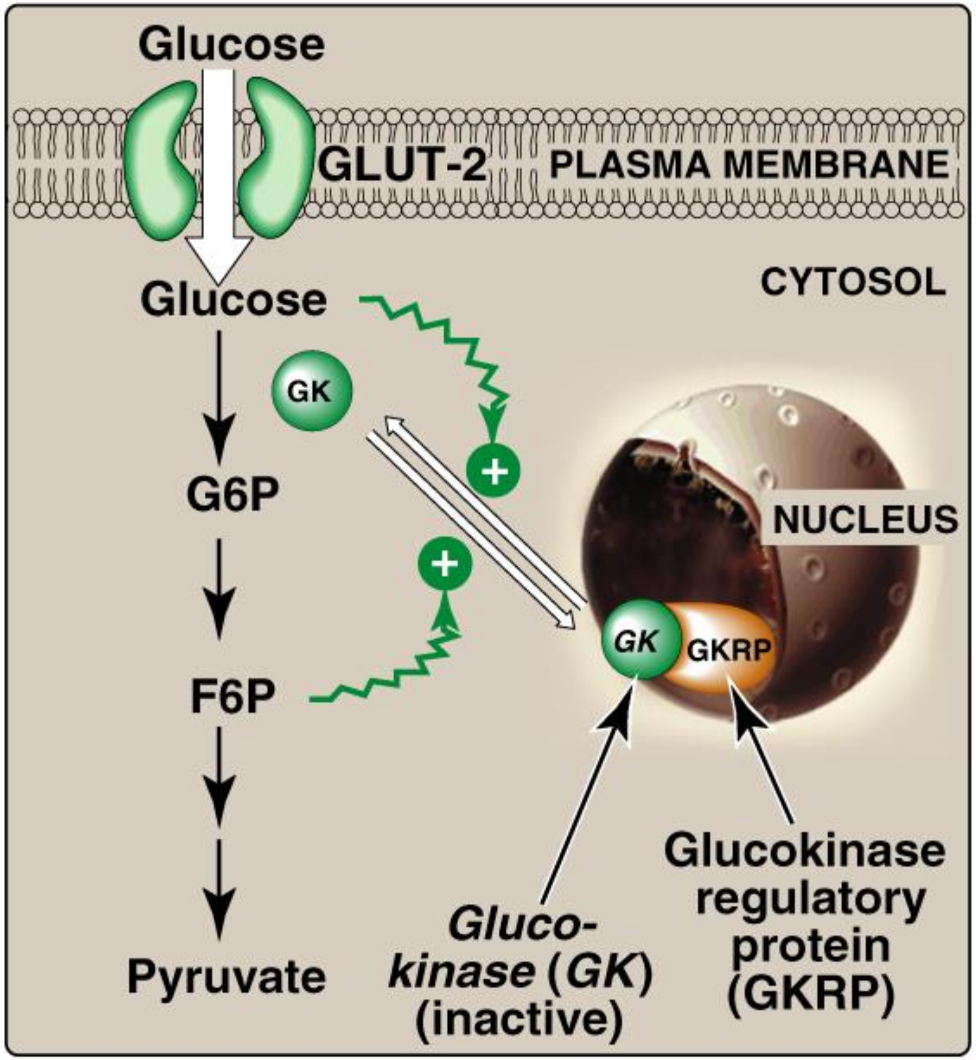

Figure 8.14

Regulation of *glucokinase* activity by glucokinase regulatory protein.

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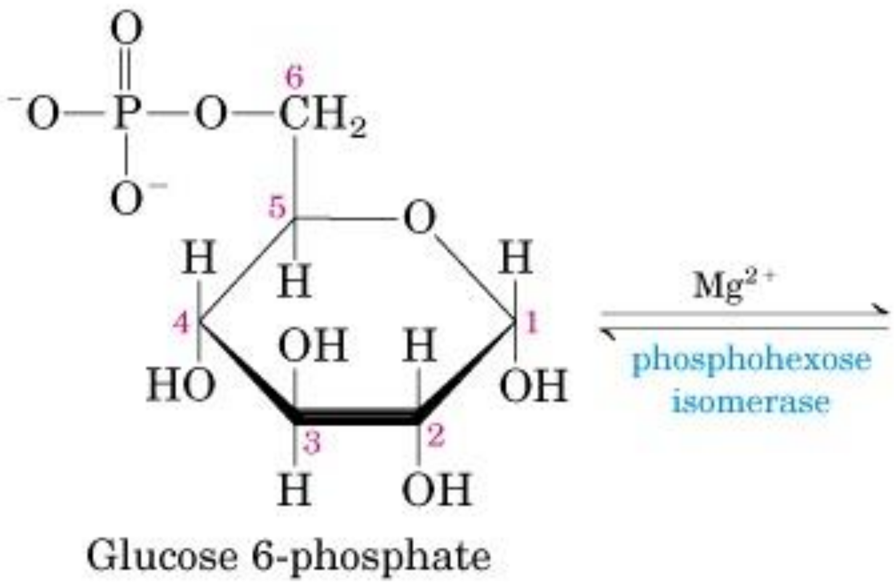
Second reaction



$\Delta G'^\circ = 1.7$ kJ/mol

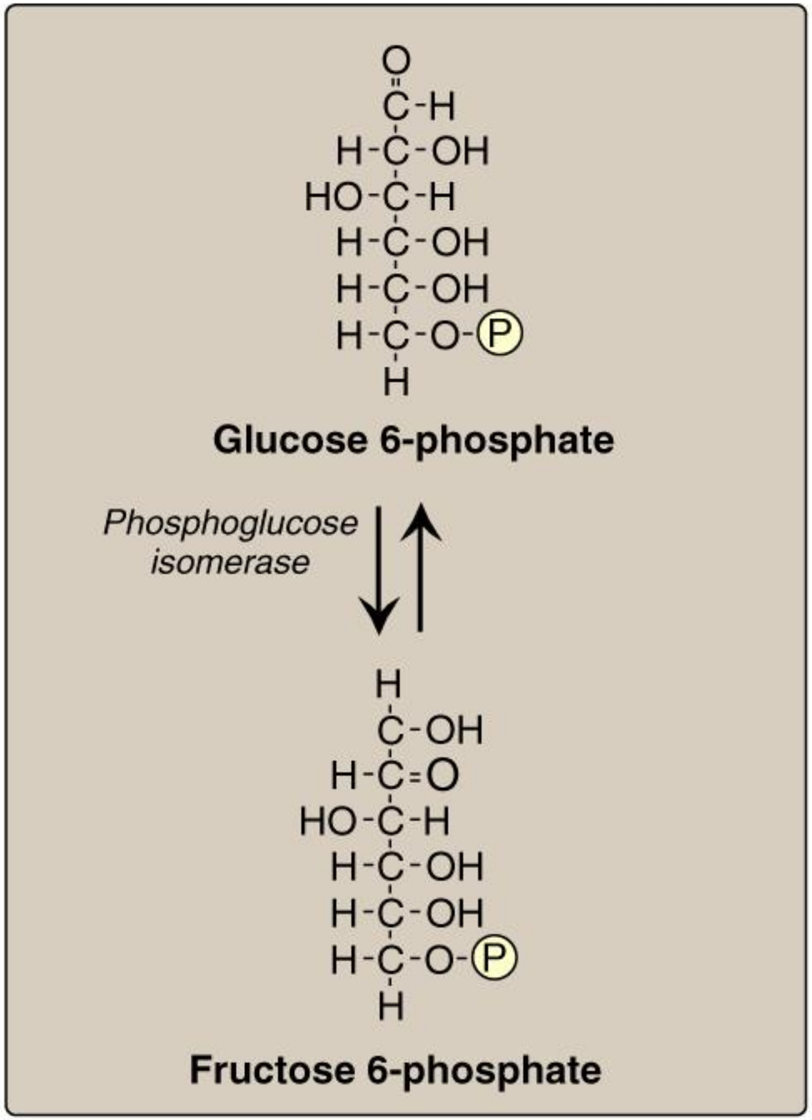


Figure 8.15

Isomerization of glucose 6-phosphate to fructose 6-phosphate.

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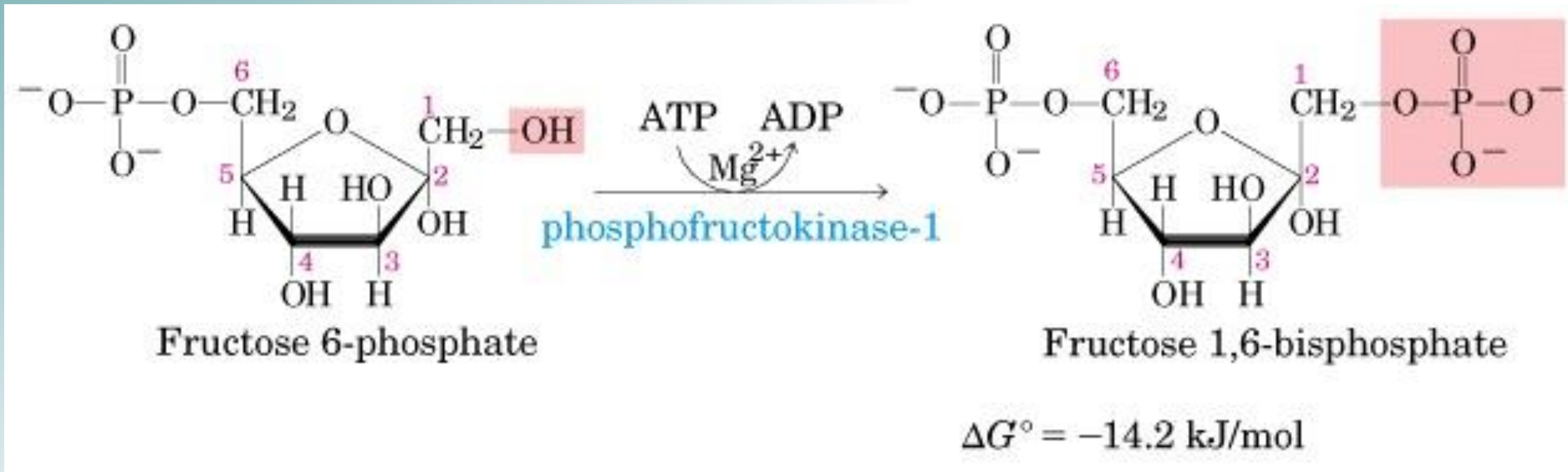
Third Reaction (second irreversible, rate limiting)



Phosphorylated on C-1, consumes ATP

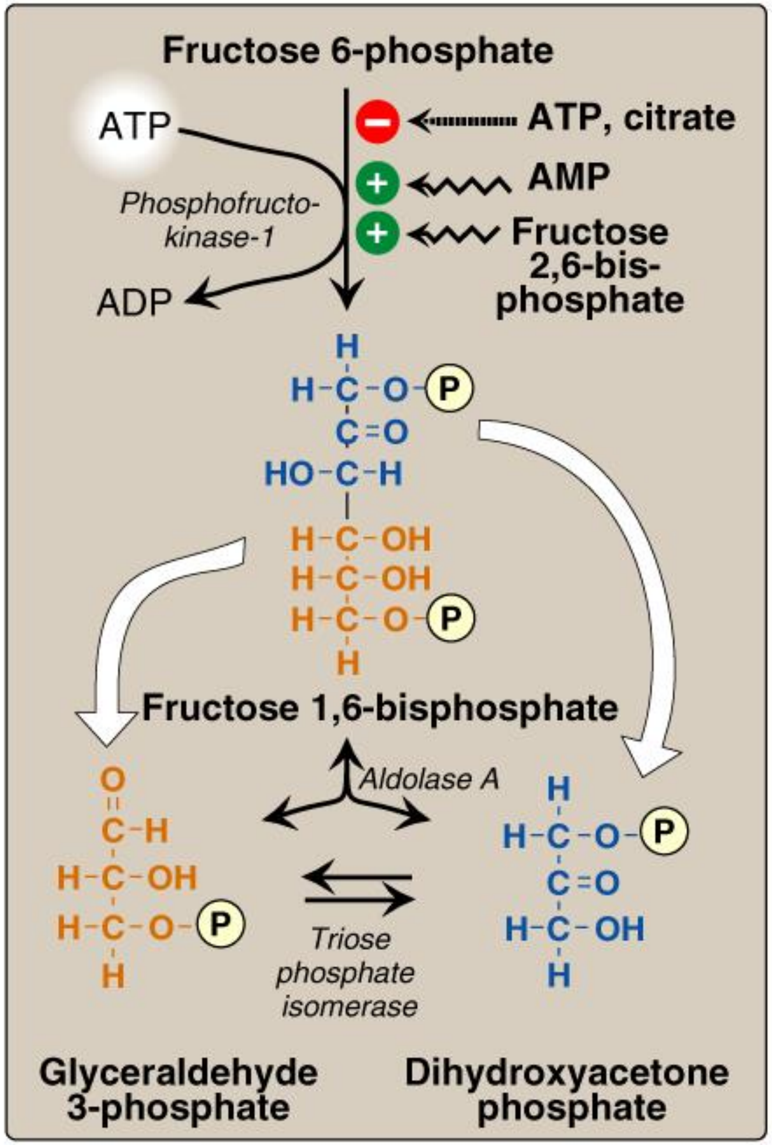


Figure 8.16

Energy investment phase (continued): Conversion of fructose 6-phosphate to triose phosphates.

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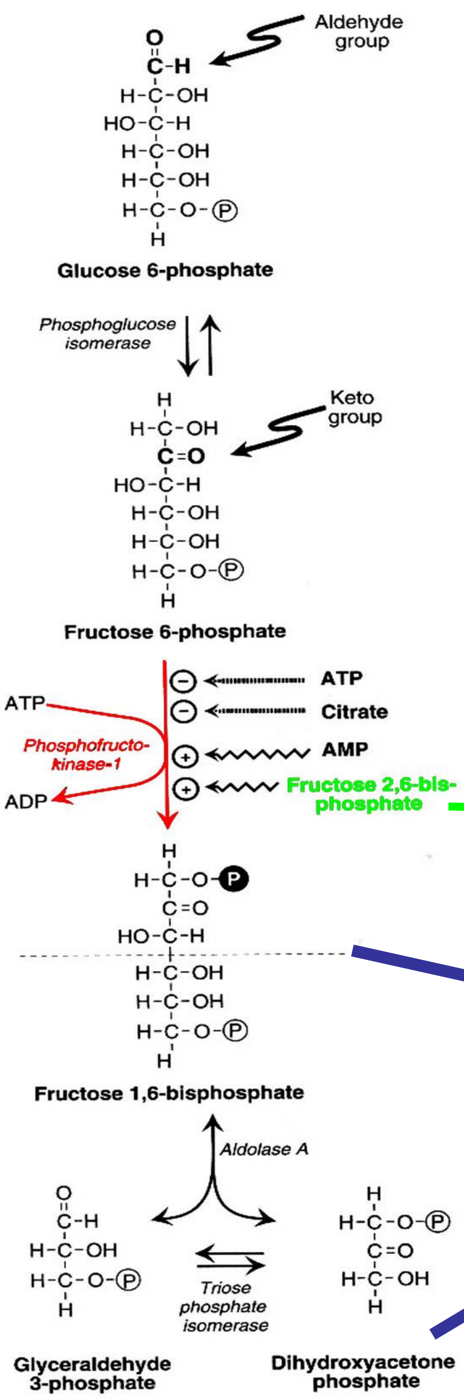
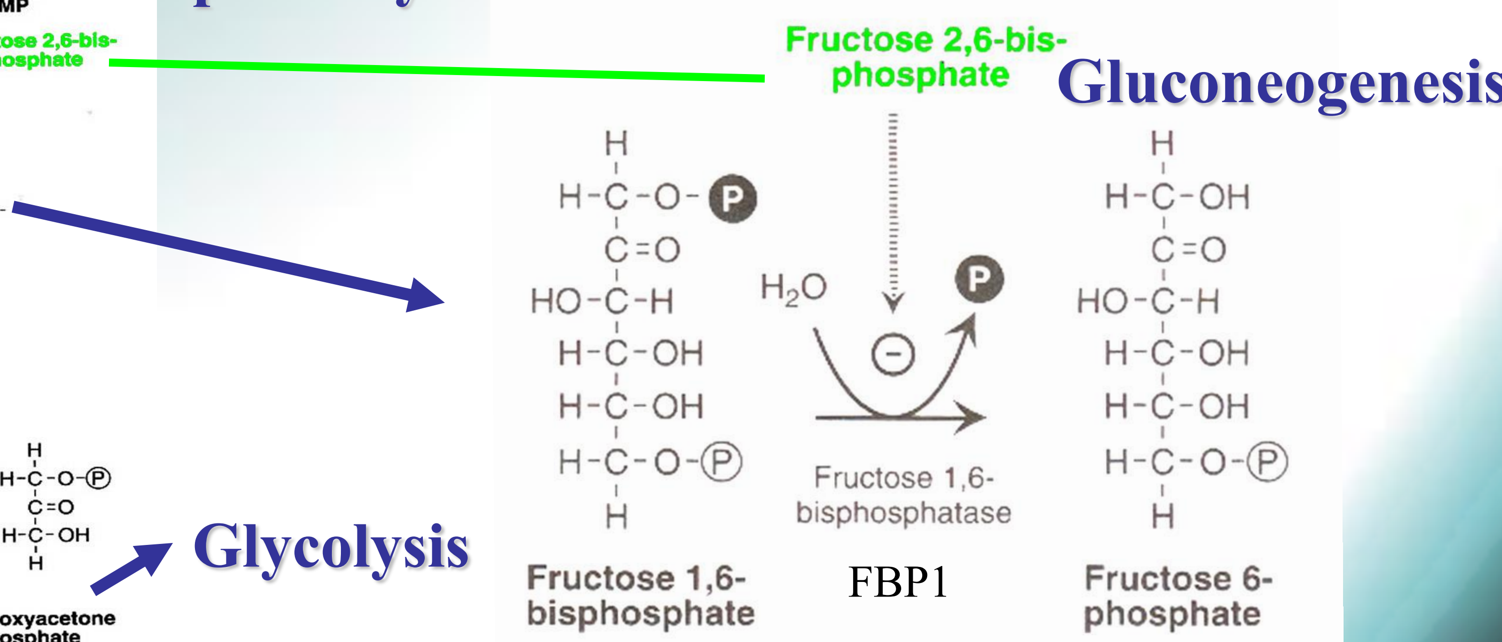
The irreversible phosphorylation reaction catalyzed by phosphofructokinase 1 (PFK-1) is the most important control point in glycolysis (rate limiting step). Controlled by the concentrations of ATP and fructose 6-P. Abundance of energy +ATP and citrate inhibit PFK-1. Induced by +AMP. The most potent activator of PFK-1 is fructose 2,6-biphosphate which also inhibit fructose 1,6 biphosphatase (dephosphorylation, page 101) ensuring that **both pathways** are not active at the same time.





Enzymes and receptors

Importance in metabolism

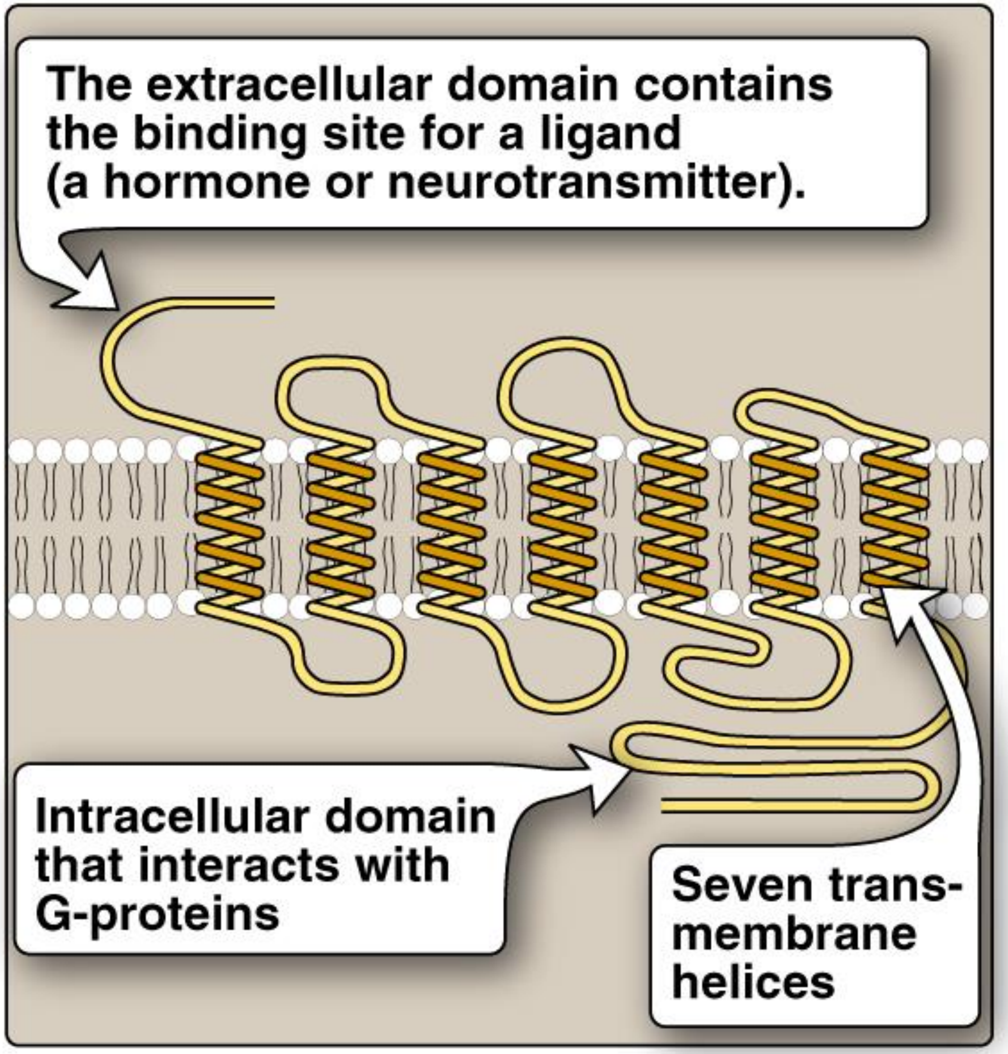


Figure 8.6
Structure of a typical membrane receptor.

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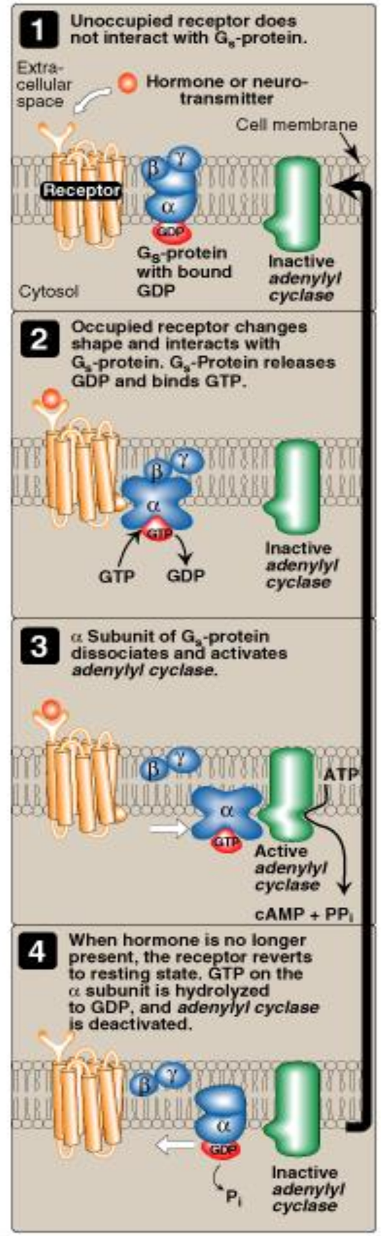
G proteins, trimeric

Gs and Gi

They have inherent GTPase activity causing rapid hydrolisis of GTP to GDP



cAMP-dependent protein kinase A see next slide

Figure 8.7

The recognition of chemical signals by certain membrane receptors triggers an increase (or, less often, a decrease) in the activity of adenylyl cyclase.

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Low levels of blood Glucose → High levels of Glucagon →

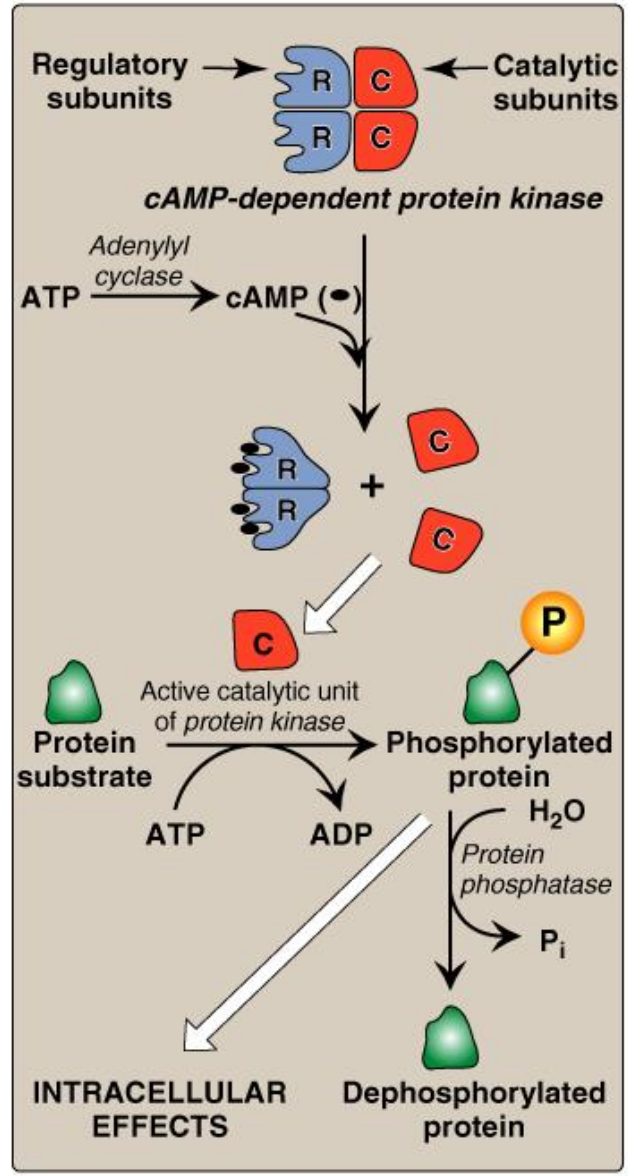

Figure 8.8
Actions of cAMP.

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Inhibition
Pyruvate Kinase
PFK2
(+)FBP-2

Protein kinase C not dependent on cAMP

Gluconeogenesis

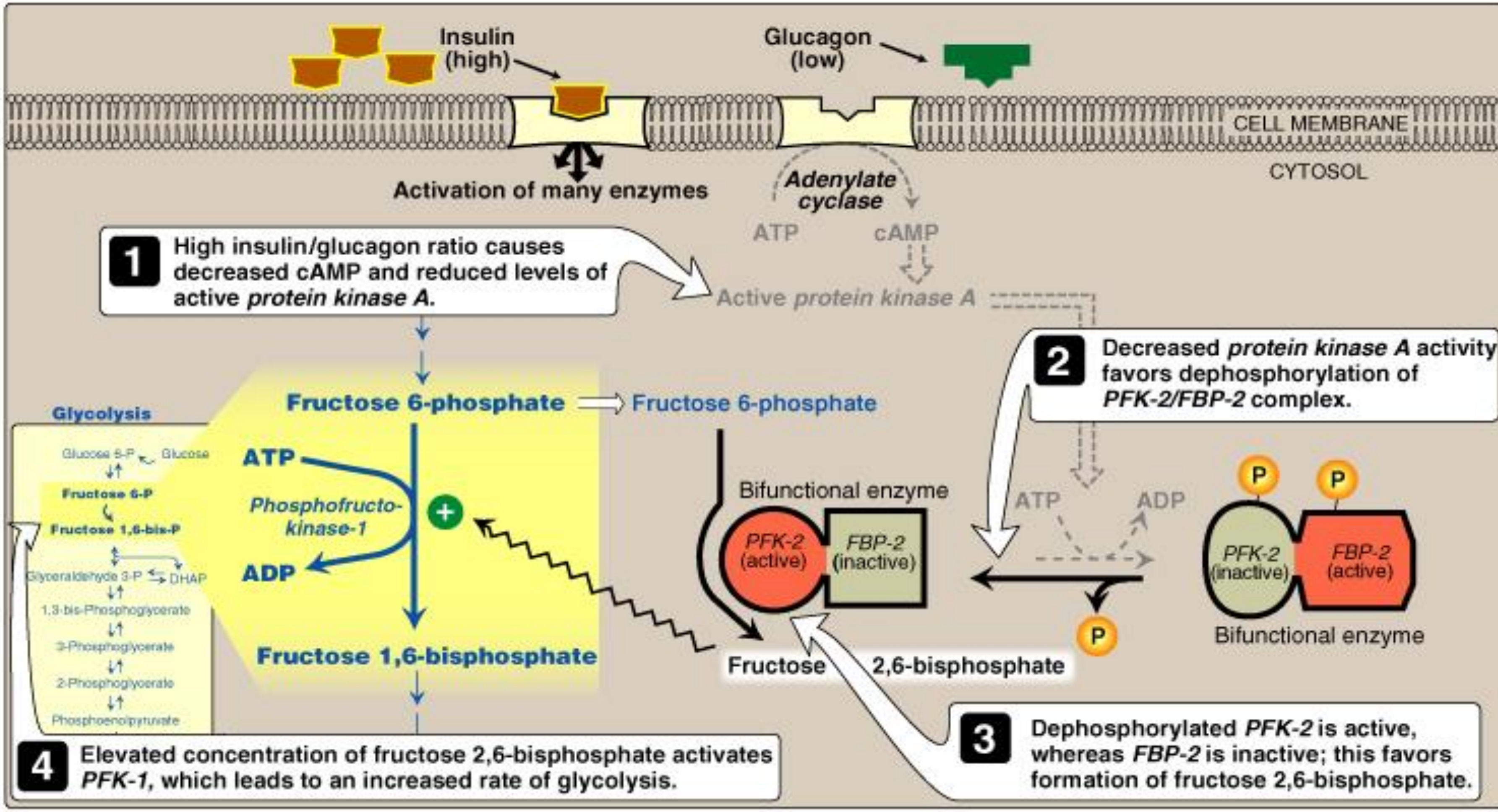

Figure 8.17

Effect of elevated insulin concentration on the intracellular concentration of fructose 2,6-bisphosphate in liver. *PFK-2 = phosphofructokinase-2; FBP-2 = Fructose bisphospate phosphatase-2.*

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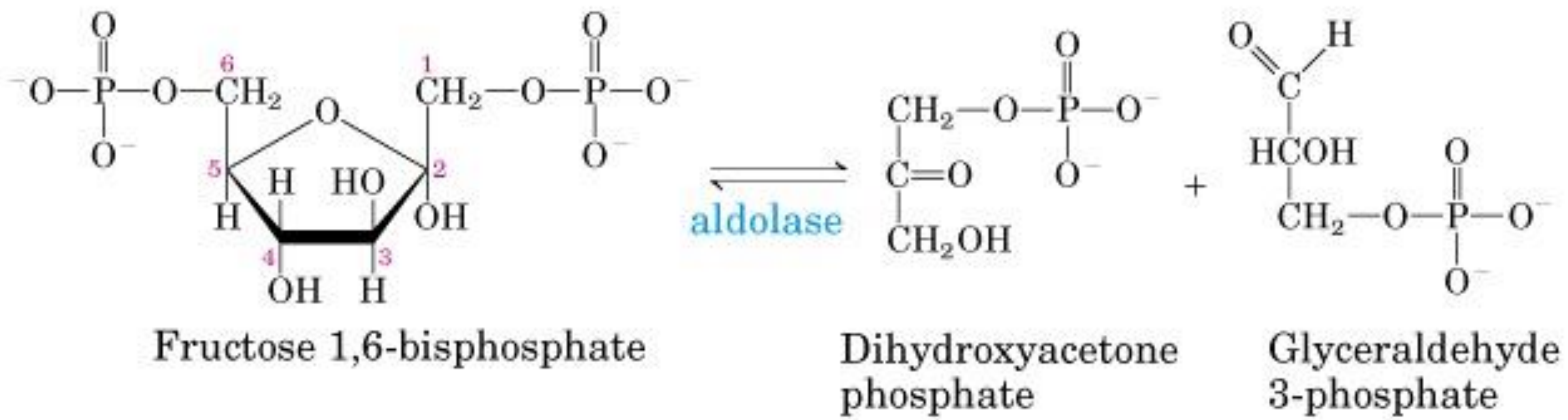
Fourth and Fifth reactions

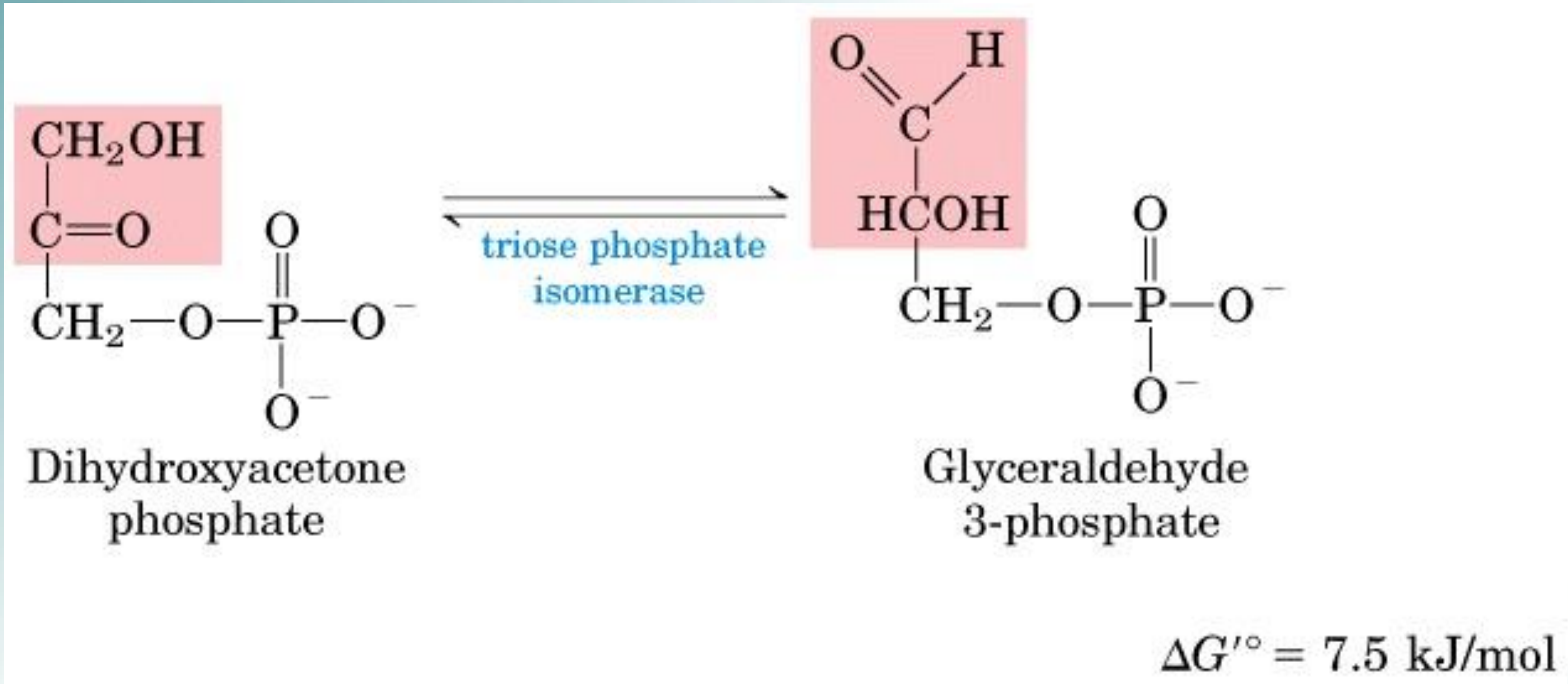


$\Delta G'^{\circ} = 23.8 kJ/mol$

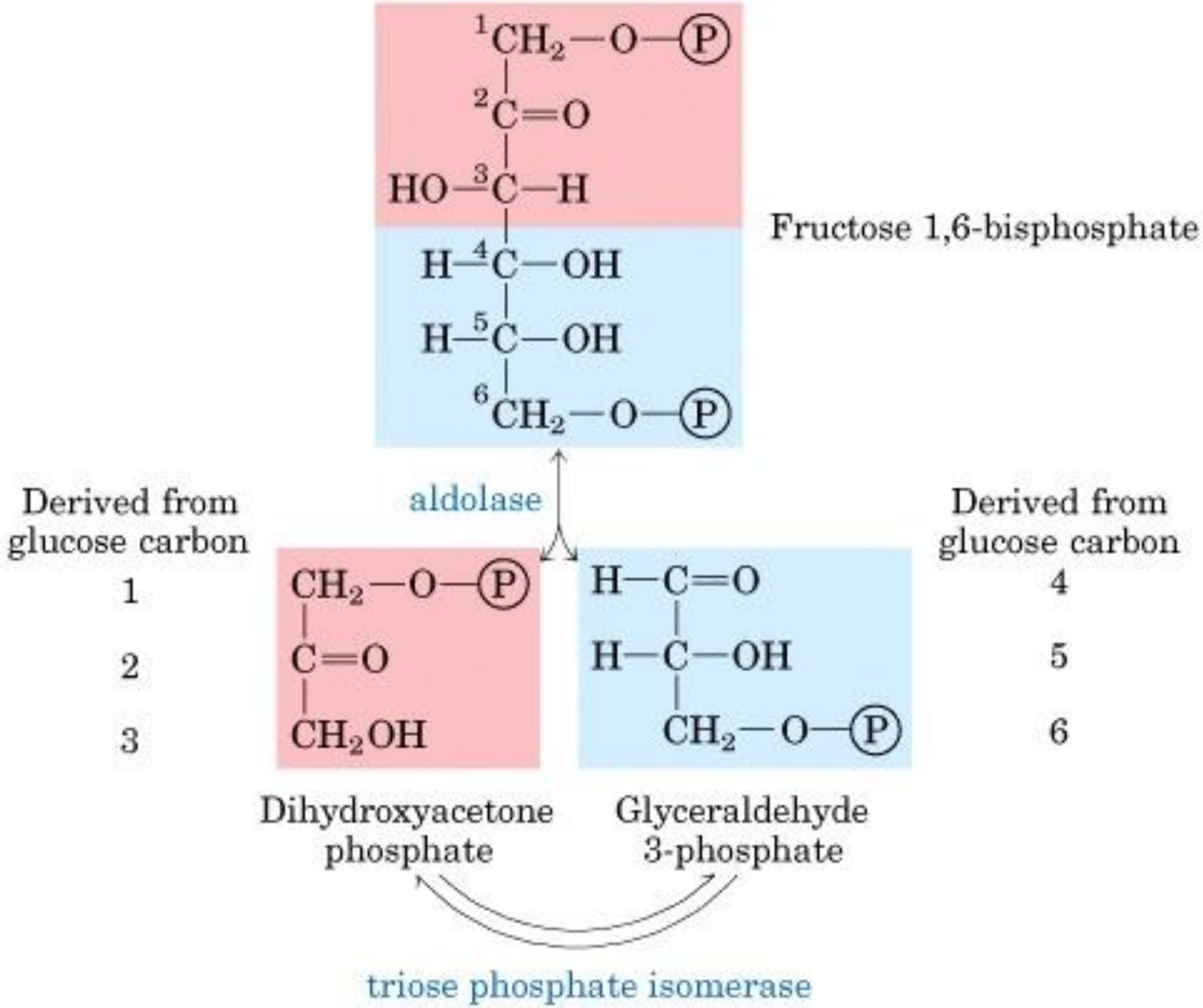
Gives this pathway its name lysis

Endergonic phase

Fifth reaction



End of Preparatory Phase



(a)

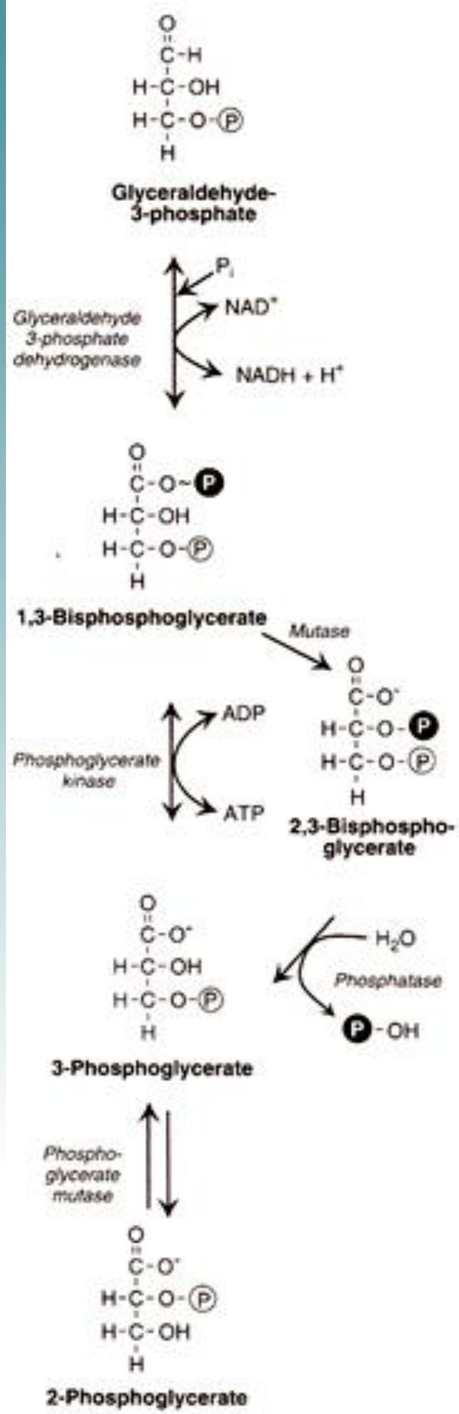
G3P the most complex functional group (carbonyl) is labeled C-1 vs C-4

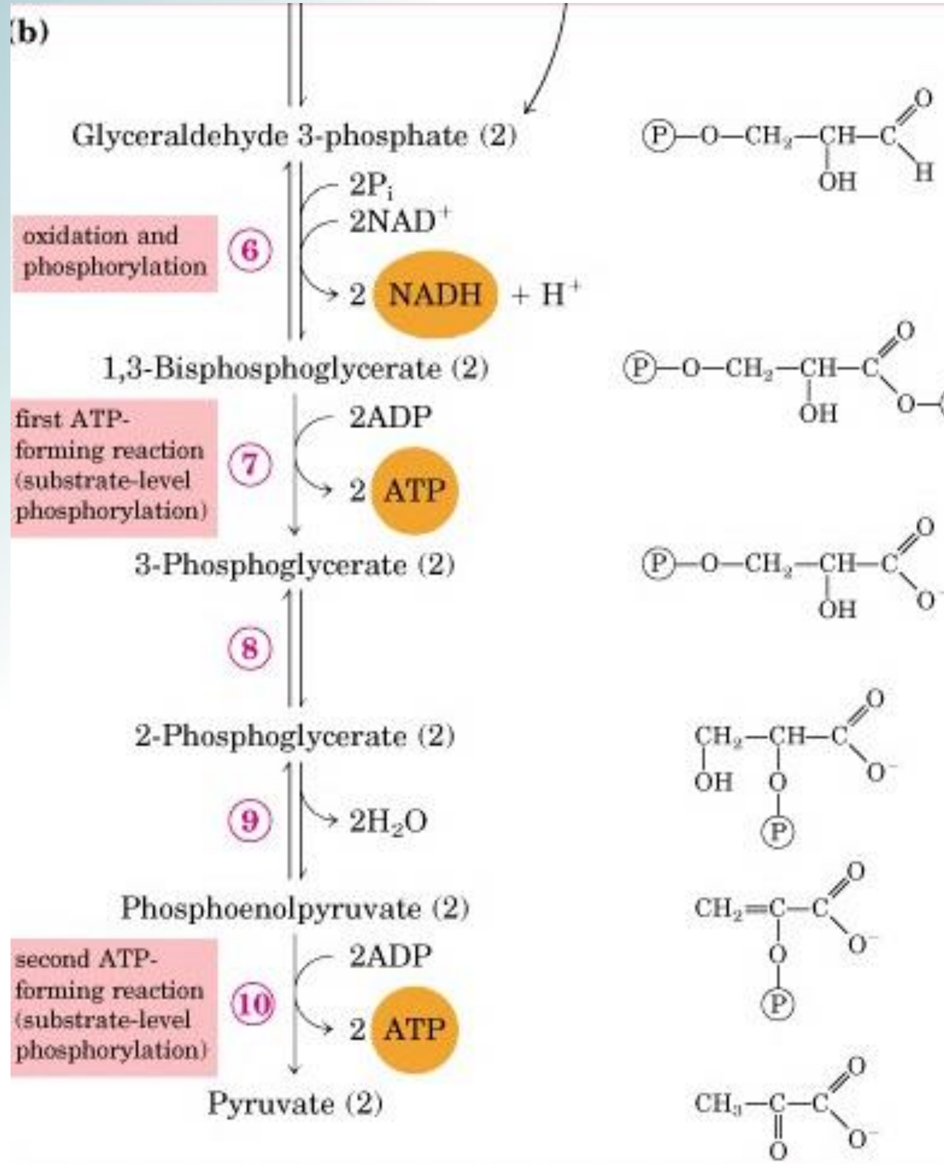
Lets summarize first 5 rx' s

GLYCOLYSIS

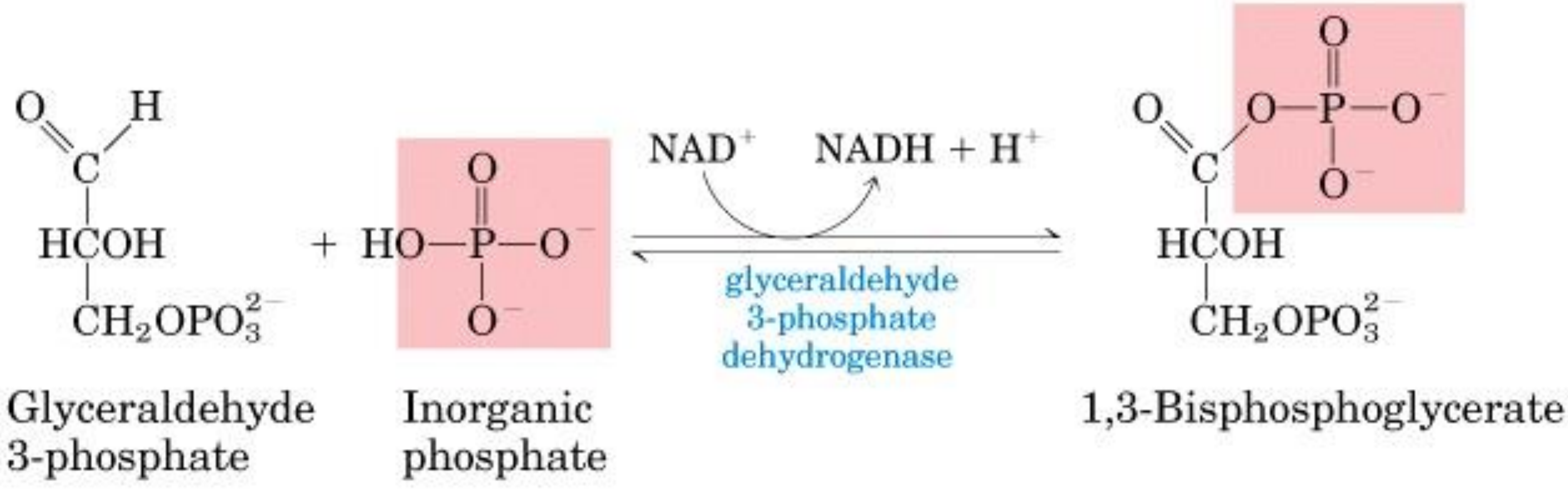
Mostly endergonic reactions	Mostly exergonic reactions

The payoff phase



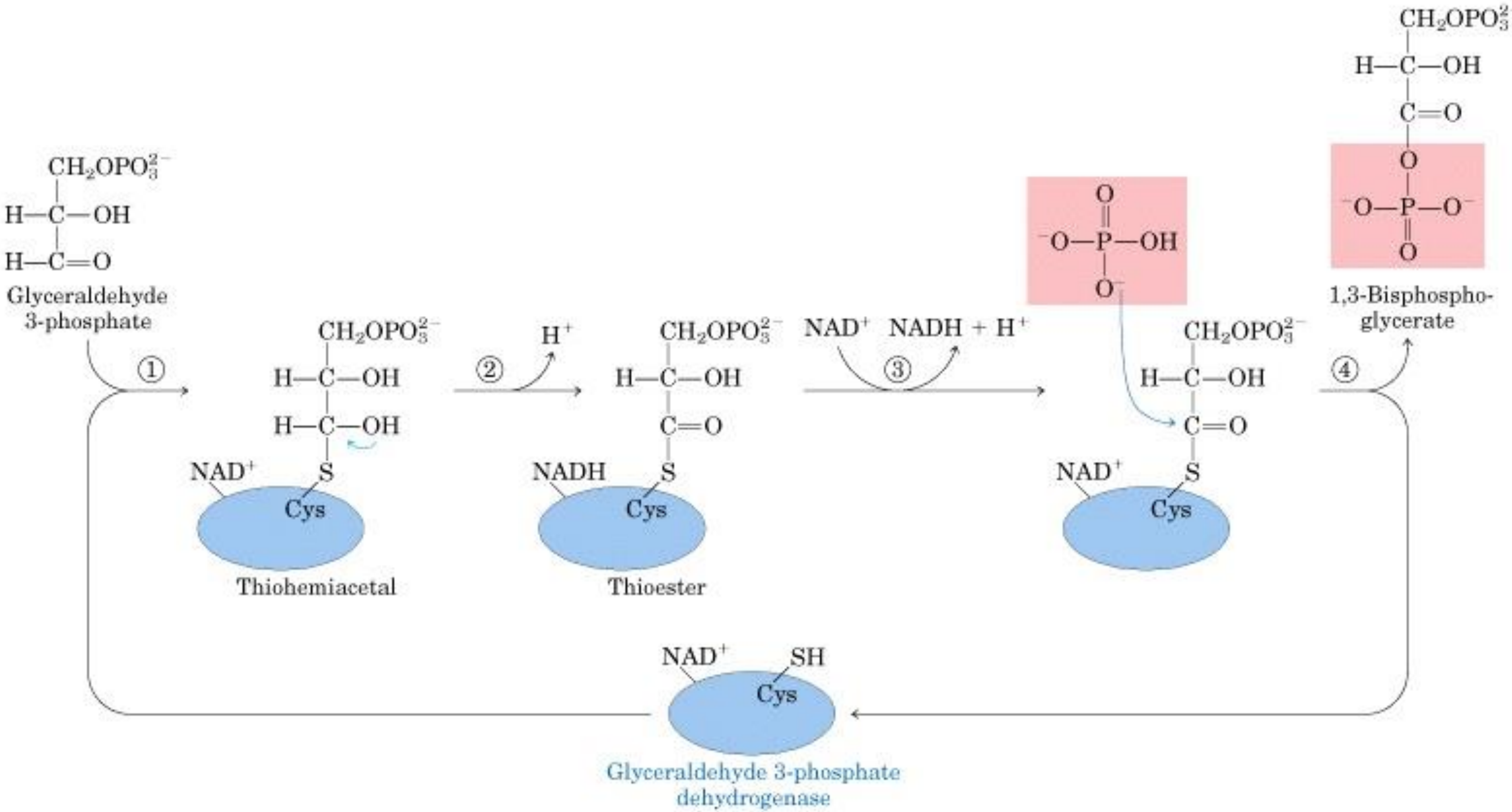


The Six reaction, first NADH formation

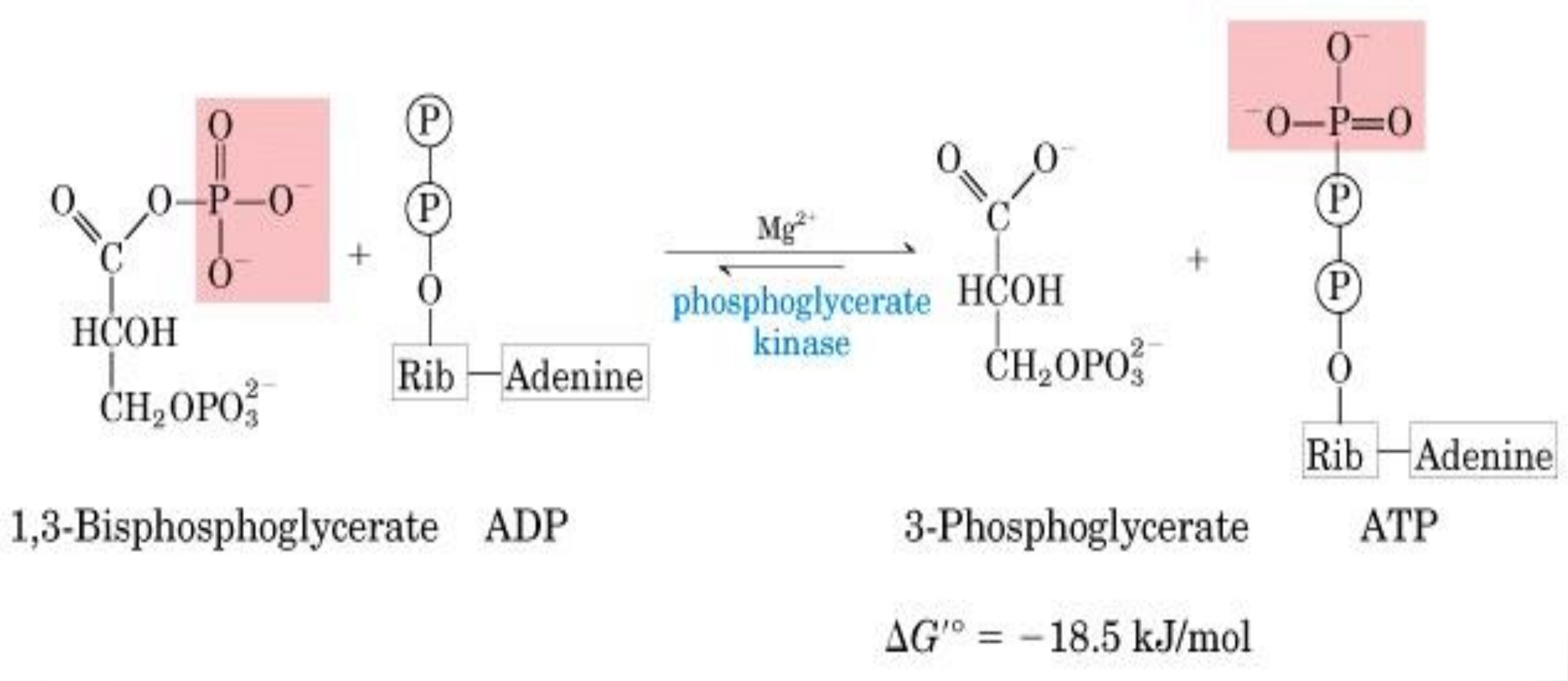


$\Delta G'^{\circ} = 6.3$ kJ/mol

Glycolysis would come to an end for lack of NAD$^+$ If NADH in this step were not continuously reoxidized.



Seventh reaction First ATP formed



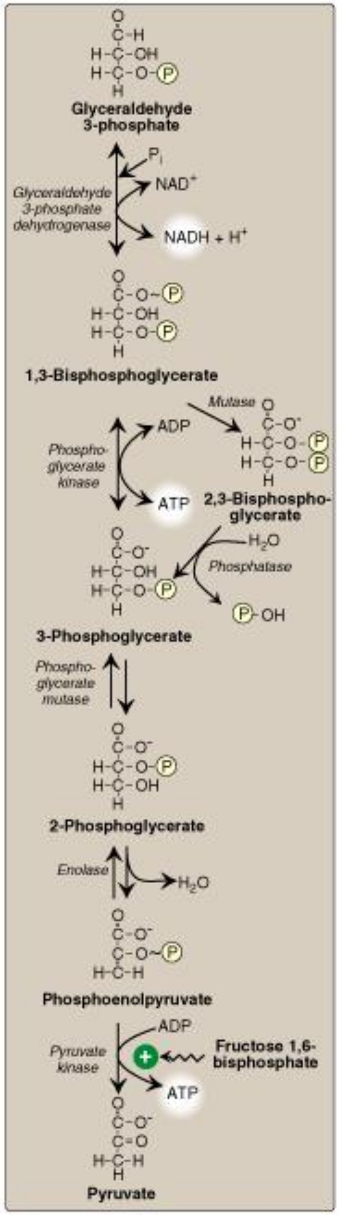


Figure 8.18
Energy-generating phase: conversion of glyceraldehyde 3-phosphate to pyruvate.

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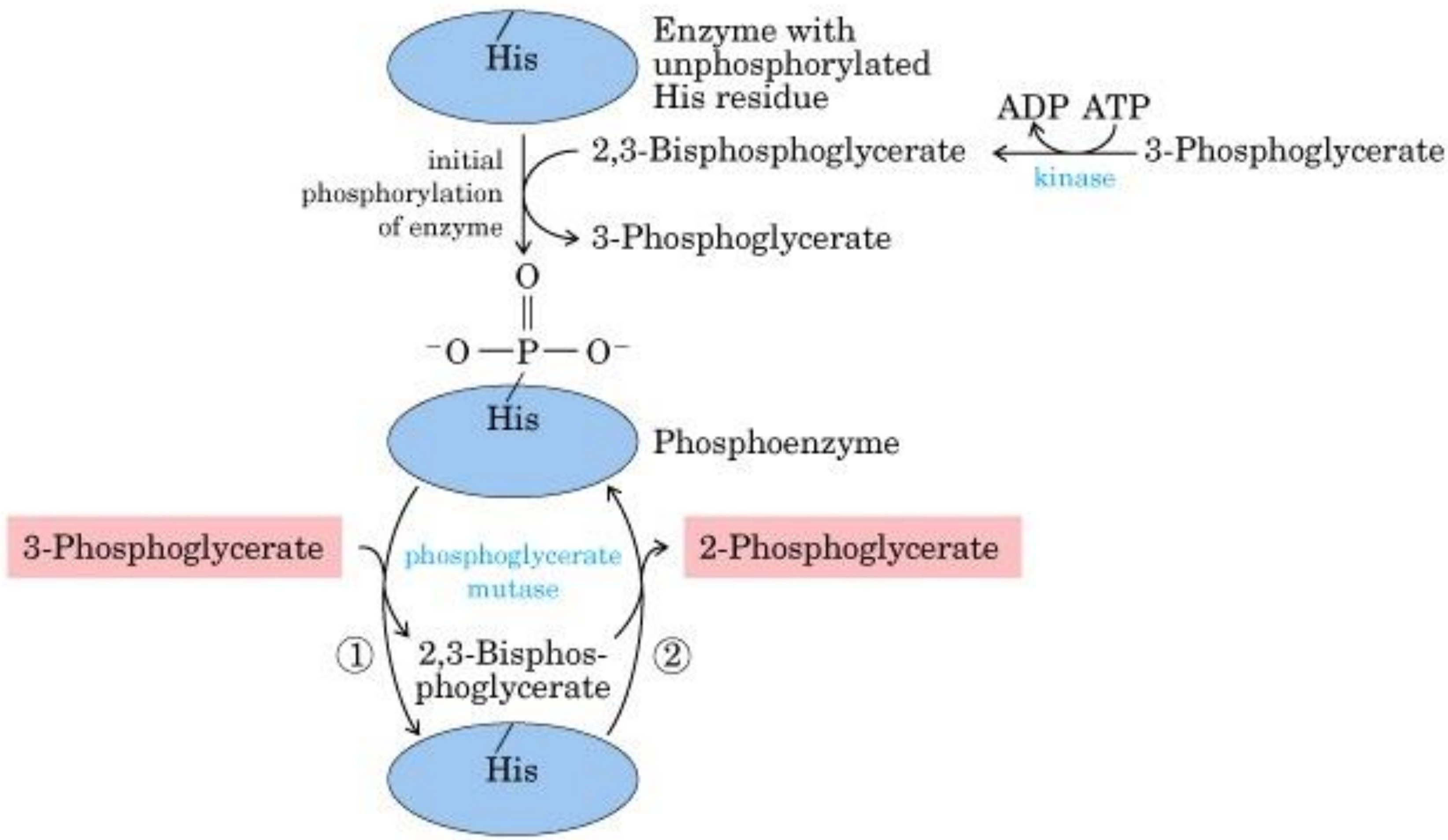
The formation of 1,3 bisphosphoglycerate is through a substrate level phosphorylation which is coupled directly to the oxidation of the substrate instead of oxidative phosphorylation via the electron transfer chain.

2,3-BPG is found in high concentration in red blood cells and in trace amount in other tissues. These shunt reactions are included in erythrocytes.

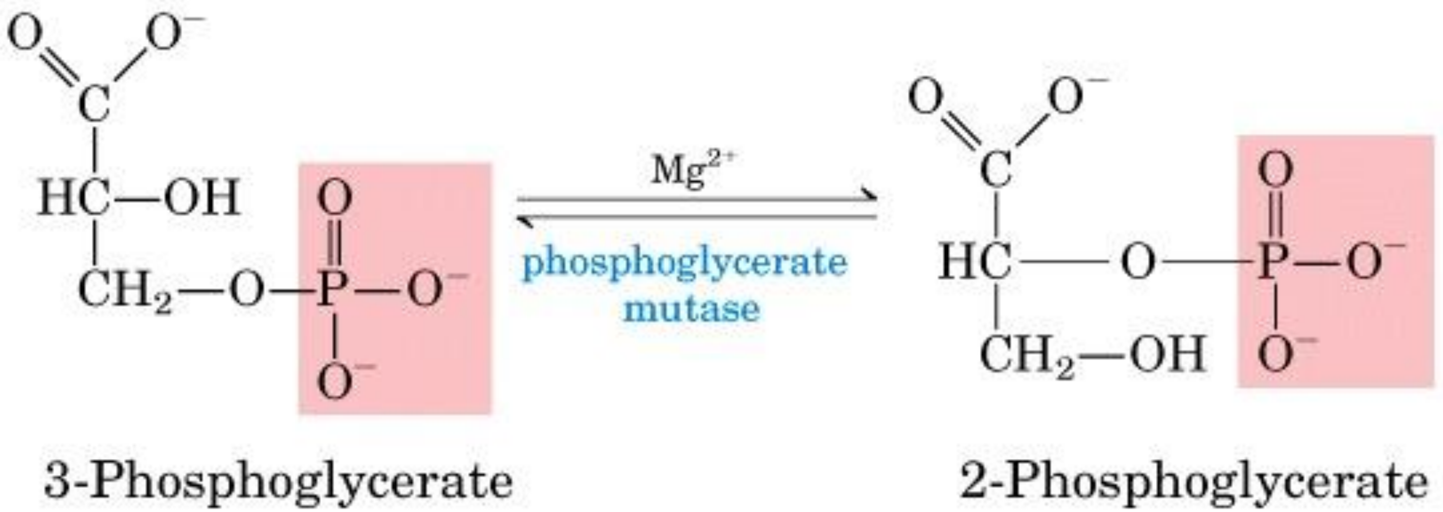
Most kinase rxs are irreversible, however, this kinase rx is reversible. Since there are two glyceraldehyde 3-P molecules two ATP molecules are formed (from each glucose molecule) replacing the two ATP molecules consumed in earlier rxs.

The next step is a shift of phosphate from carbon 3 to carbon 2 of phosphoglycerate.

Mutase needs to be phosphorylated

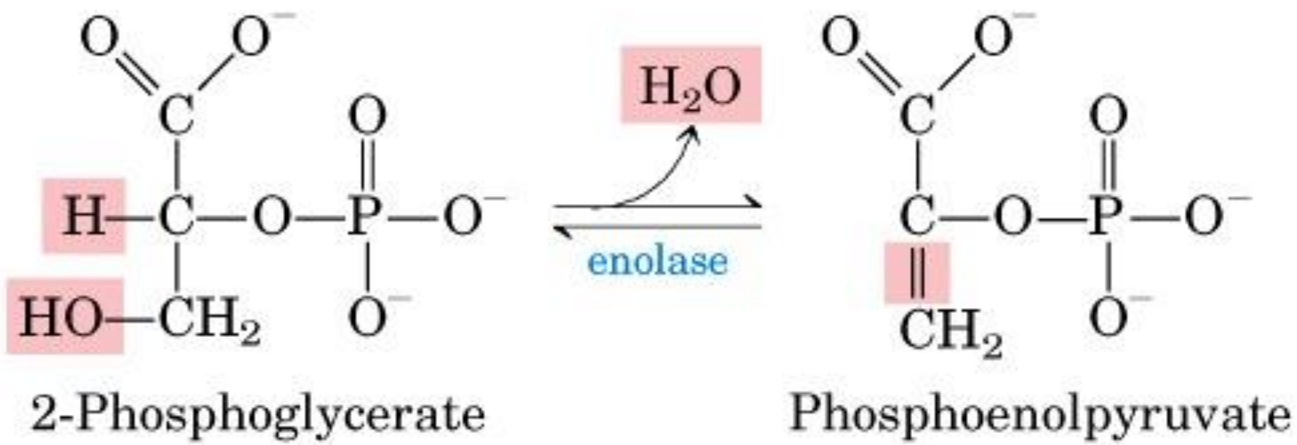


Eighth reaction shift in phosphate group to C-2



$\Delta G'^{\circ} = 4.4$ kJ/mol

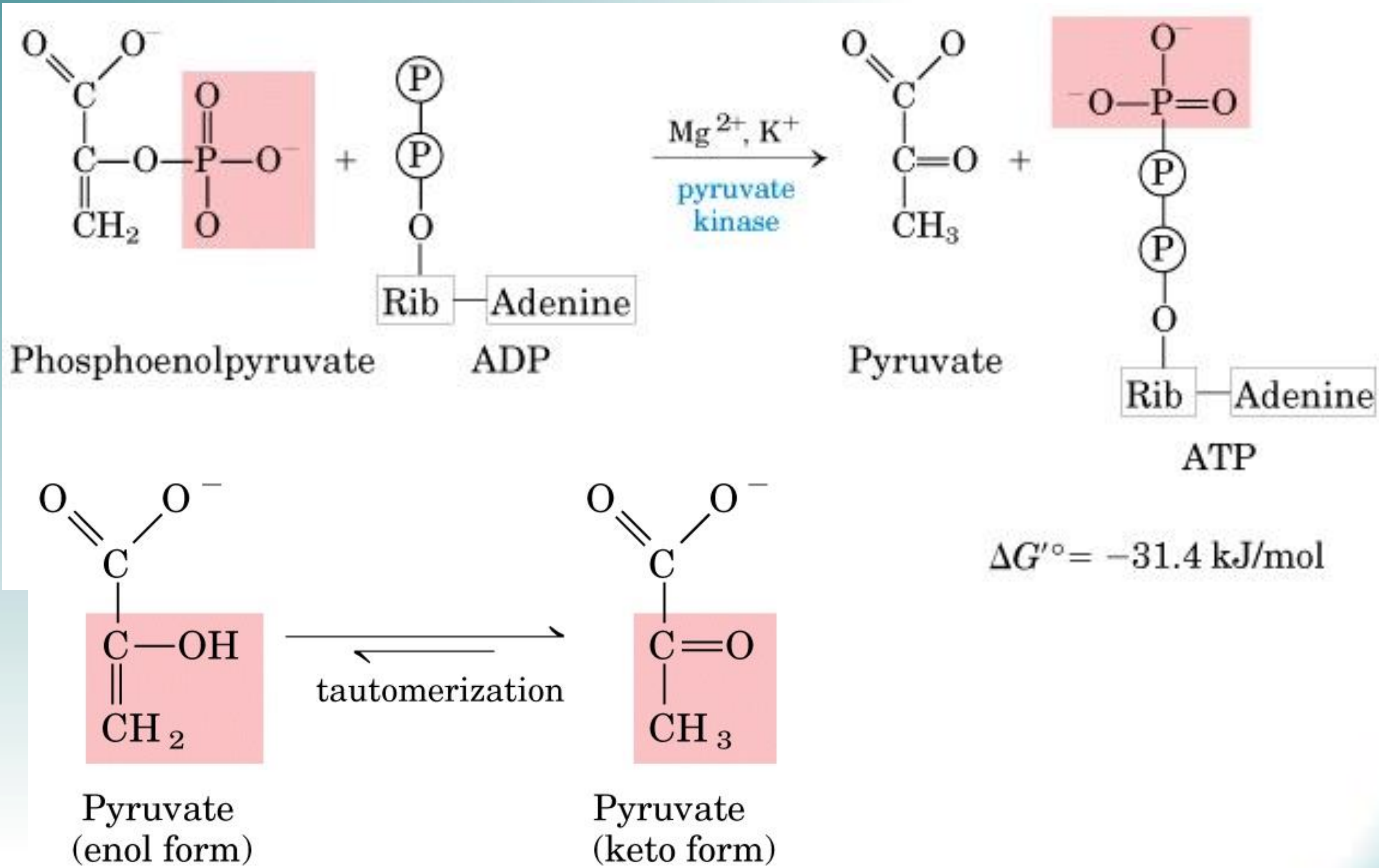
Ninth reaction



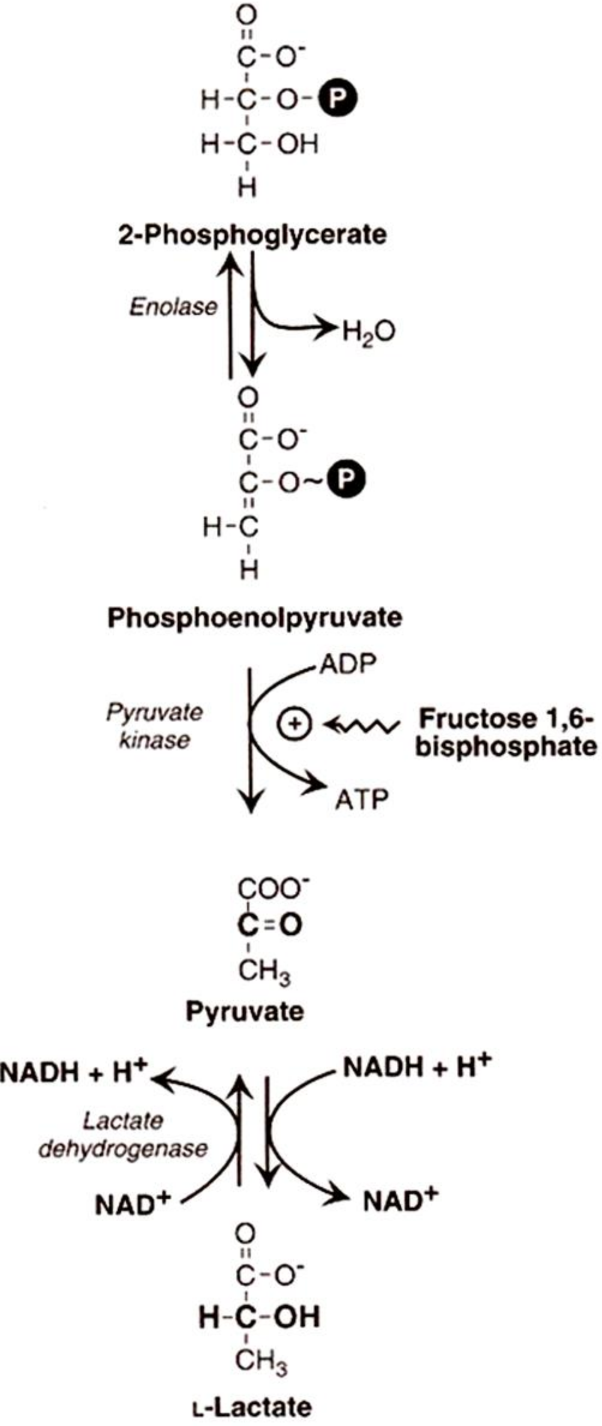
$\Delta G'^{\circ} = 7.5$ kJ/mol

(PEP)

Tenth and final reaction of the pathway, second ATP



Third and last irreversible reaction of the pathway. Exergonic phase



Dehydration to phosphoenolpyruvate (PEP)

The third irreversible rx of glycolysis the formation of pyruvate by pyruvate kinase (hexokinase and PFK-1) This is the second ATP forming rx of the oxidative stage of glycolysis. (another substrate level phosphorylation. In the liver pyruvate kinase is activated by fructose 1,6-bisphosphate.

Phosphorylation by cAMP-dependent protein kinase A due to increase in glucagon (low blood glucose) lead to inactivation of pyruvate kinase inhibiting glycolysis and inducing gluconogenesis (fig 8.7)

Reduction of pyruvate to lactate mainly occurs in red blood cells, lens and cornea, kidney medulla, testes and leucocytes. Lactate formation in muscle NADH/NAD elevated exceeds the capacity oxid.c

Low levels of Glucose in blood
Induce the secretion of Glucagon from B cells and activate protein Kinase A Which (-) PK and stops glycolysis.
Glycolytic enzyme deficiency due to a reduced rate of glycolysis and ATP formation in red blood cells 95% Show a deficiency in PK second to G6PD in hemolytic anemia.

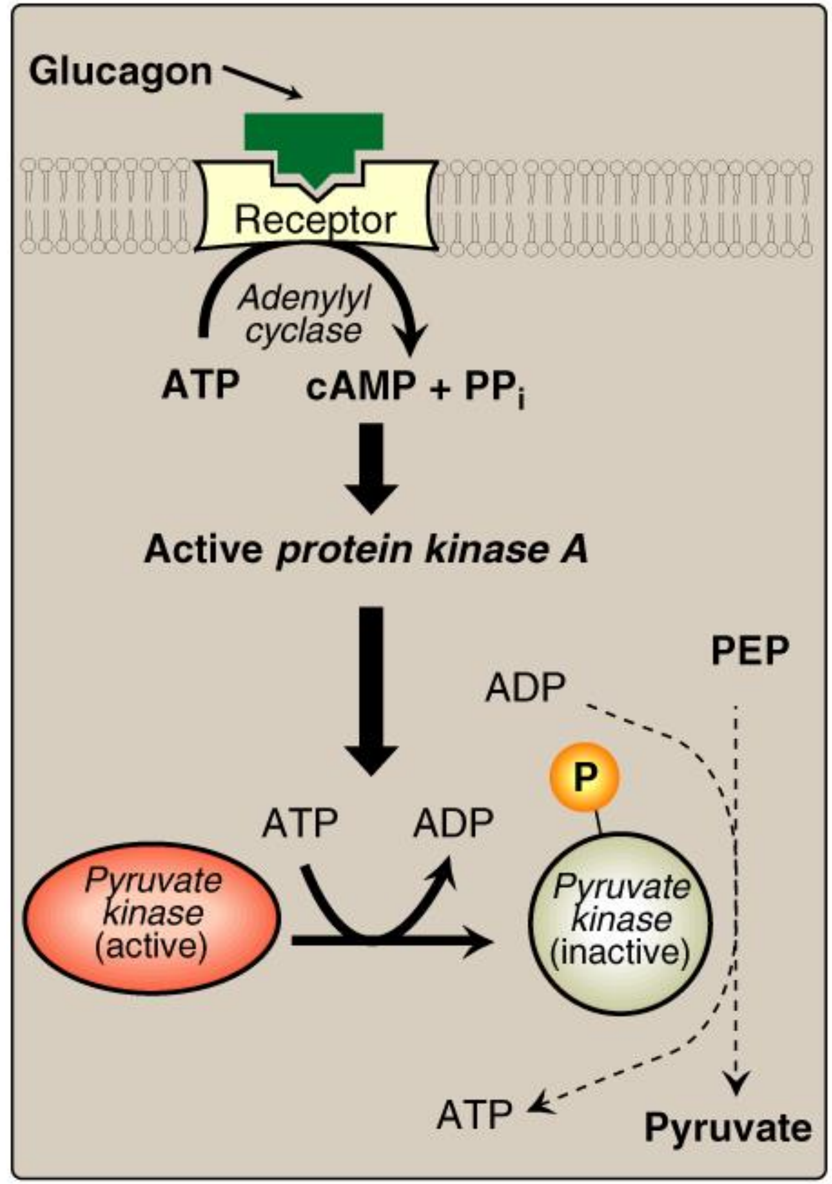


Figure 8.19

Covalent modification of *pyruvate kinase* results in inactivation of enzyme.

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GLYCOLYSIS	
Mostly endergonic reactions	Mostly exergonic reactions

Summary of Anaerobic Glycolisis

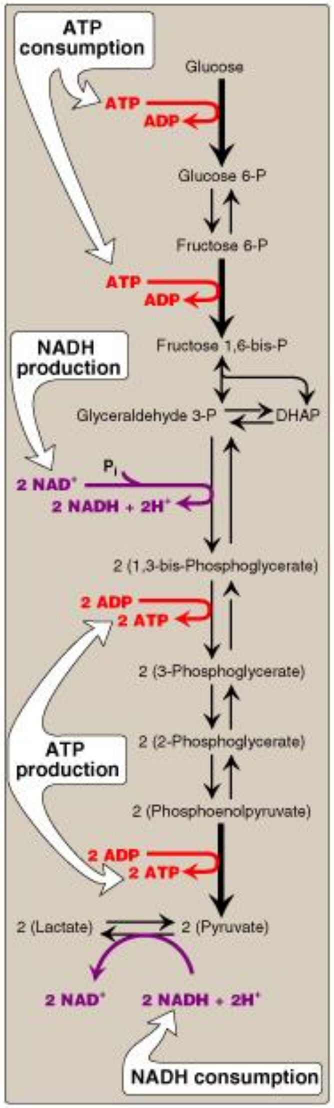

Figure 8.22
Summary of anaerobic glycolysis. Reactions involving the production or consumption of ATP or NADH are indicated. The irreversible reactions of glycolysis are shown with thick arrows. DHAP = dihydroxyacetone phosphate.

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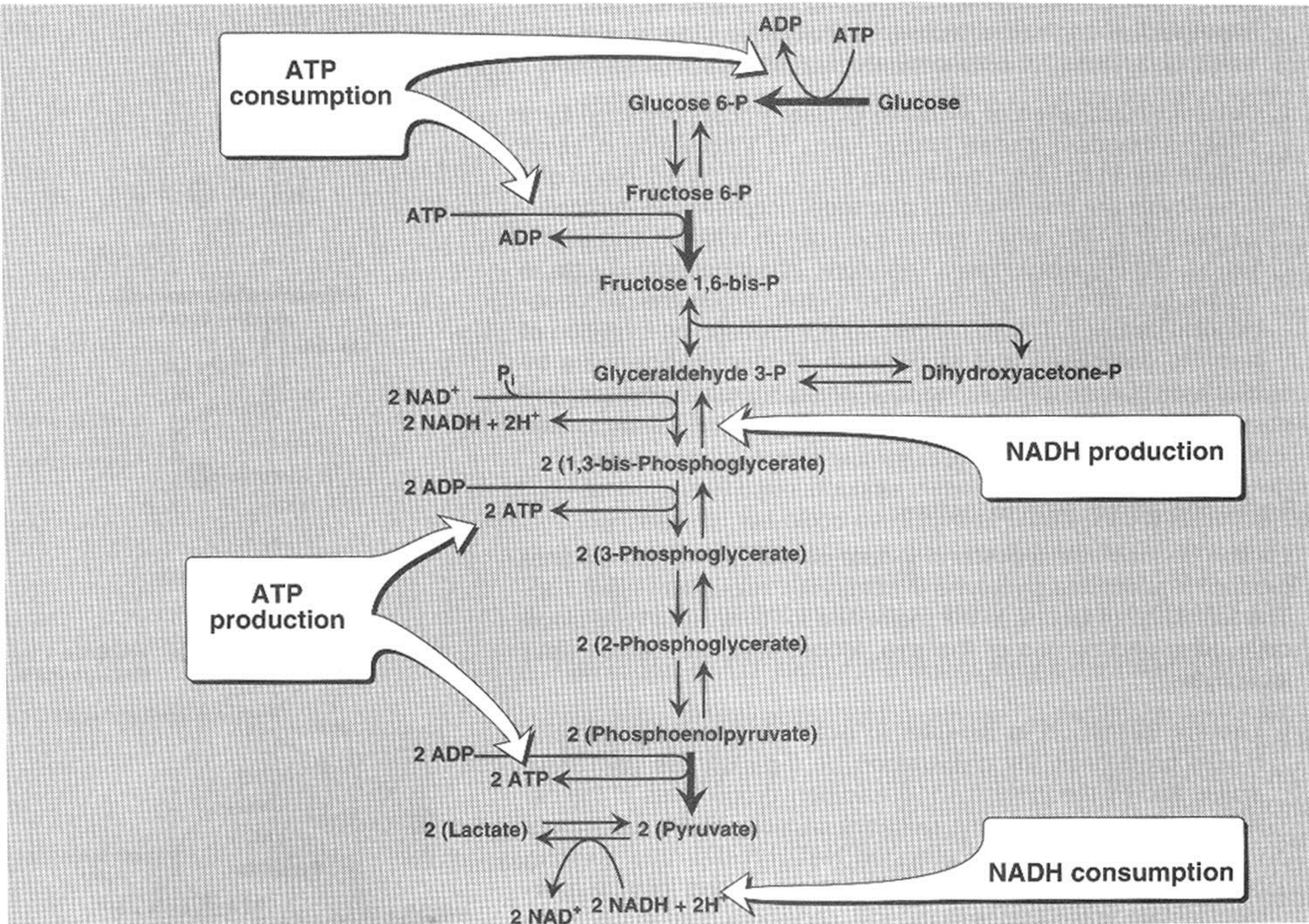
ATP consumption
ADP
ATP
Glucose 6-P
Glucose
Fructose 6-P
ATP
ADP
Fructose 1,6-bis-P
Glyceraldehyde 3-P
Dihydroxyacetone-P
P_i
2 NAD+
2 NADH + 2H+
NADH production
2 (1,3-bis-Phosphoglycerate)
2 ADP
2 ATP
2 (3-Phosphoglycerate)
ATP production
2 (2-Phosphoglycerate)
2 (Phosphoenolpyruvate)
2 ADP
2 ATP
2 (Lactate)
2 (Pyruvate)
NADH consumption
2 NAD+
2 NADH + 2H+

Summary

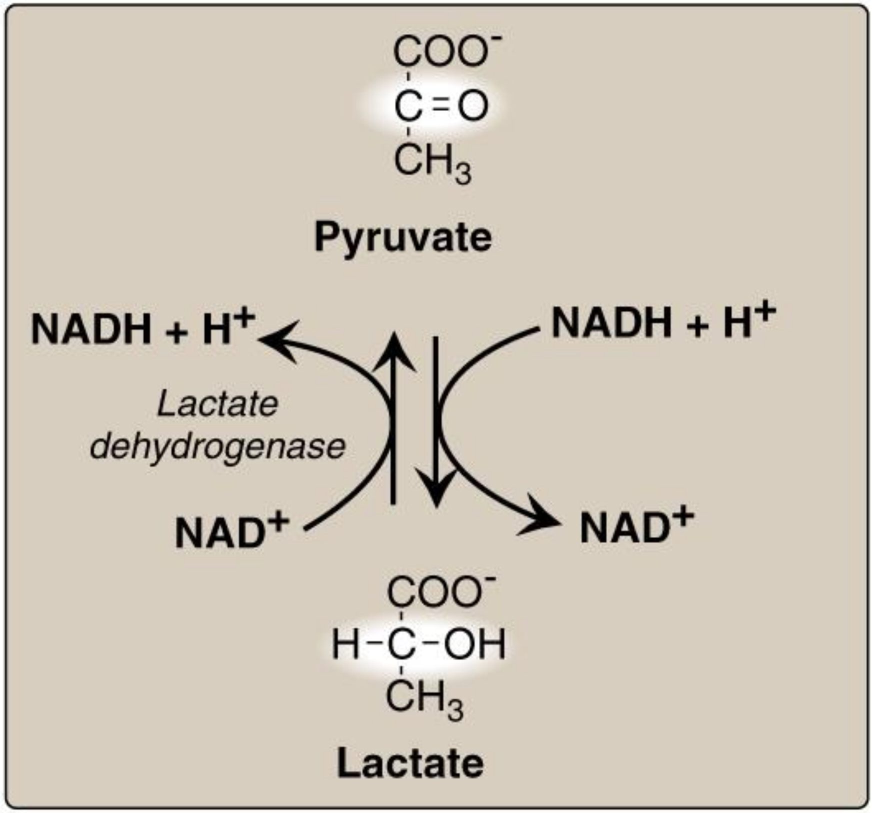

Figure 8.21

Interconversion of pyruvate and lactate.

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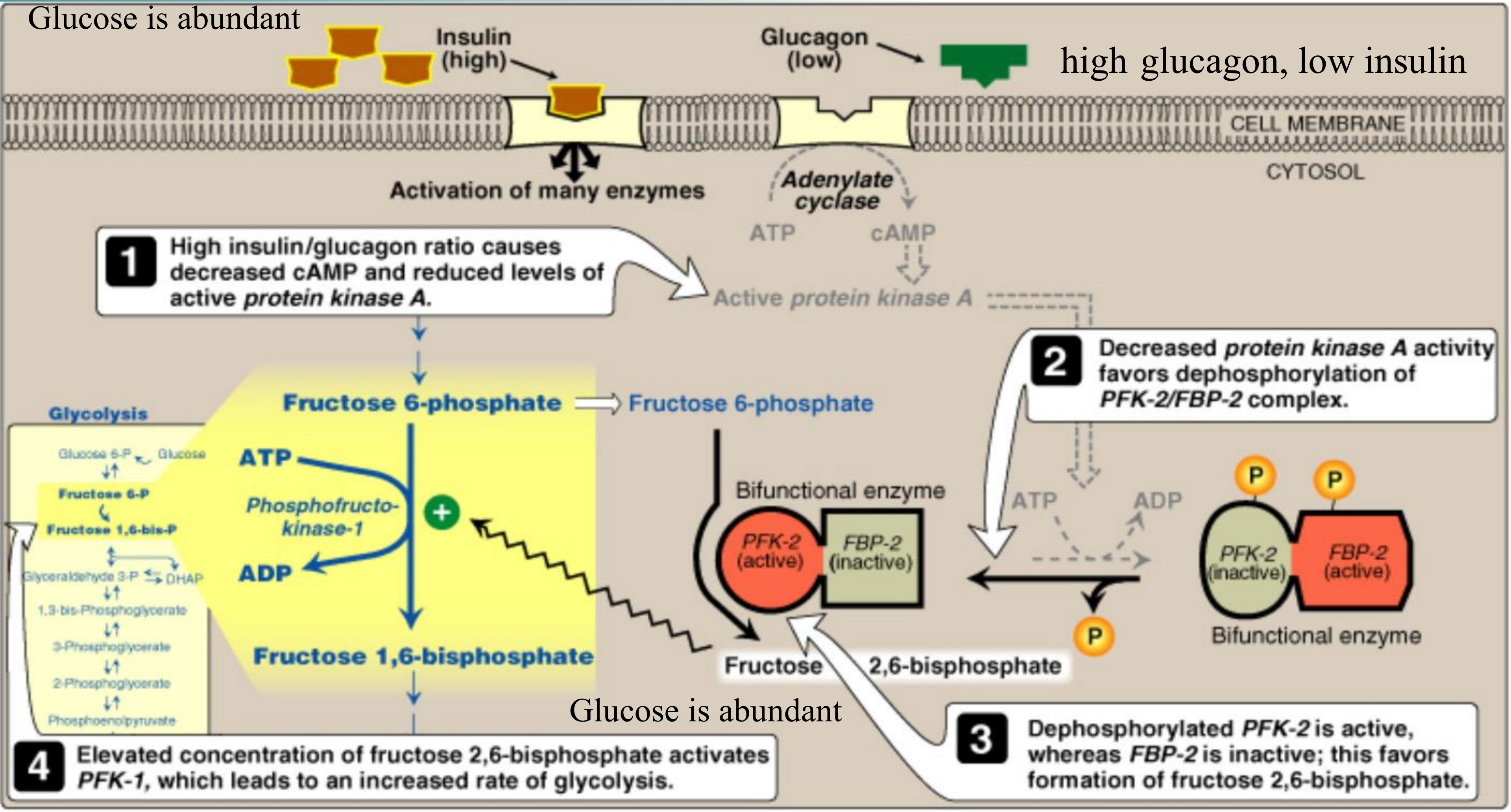
Well fed state

During starvation,

Glucose is abundant

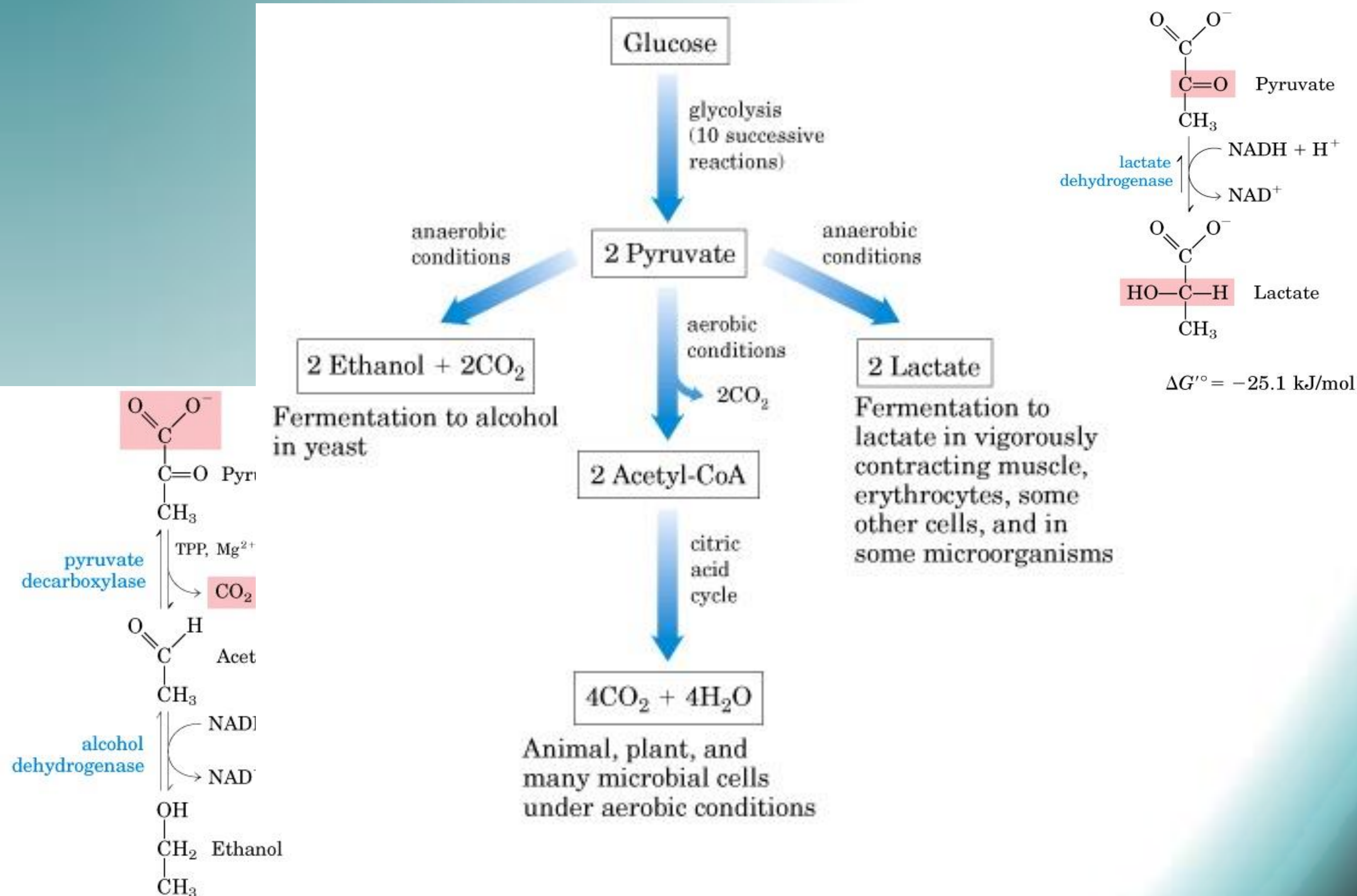
high glucagon, low insulin

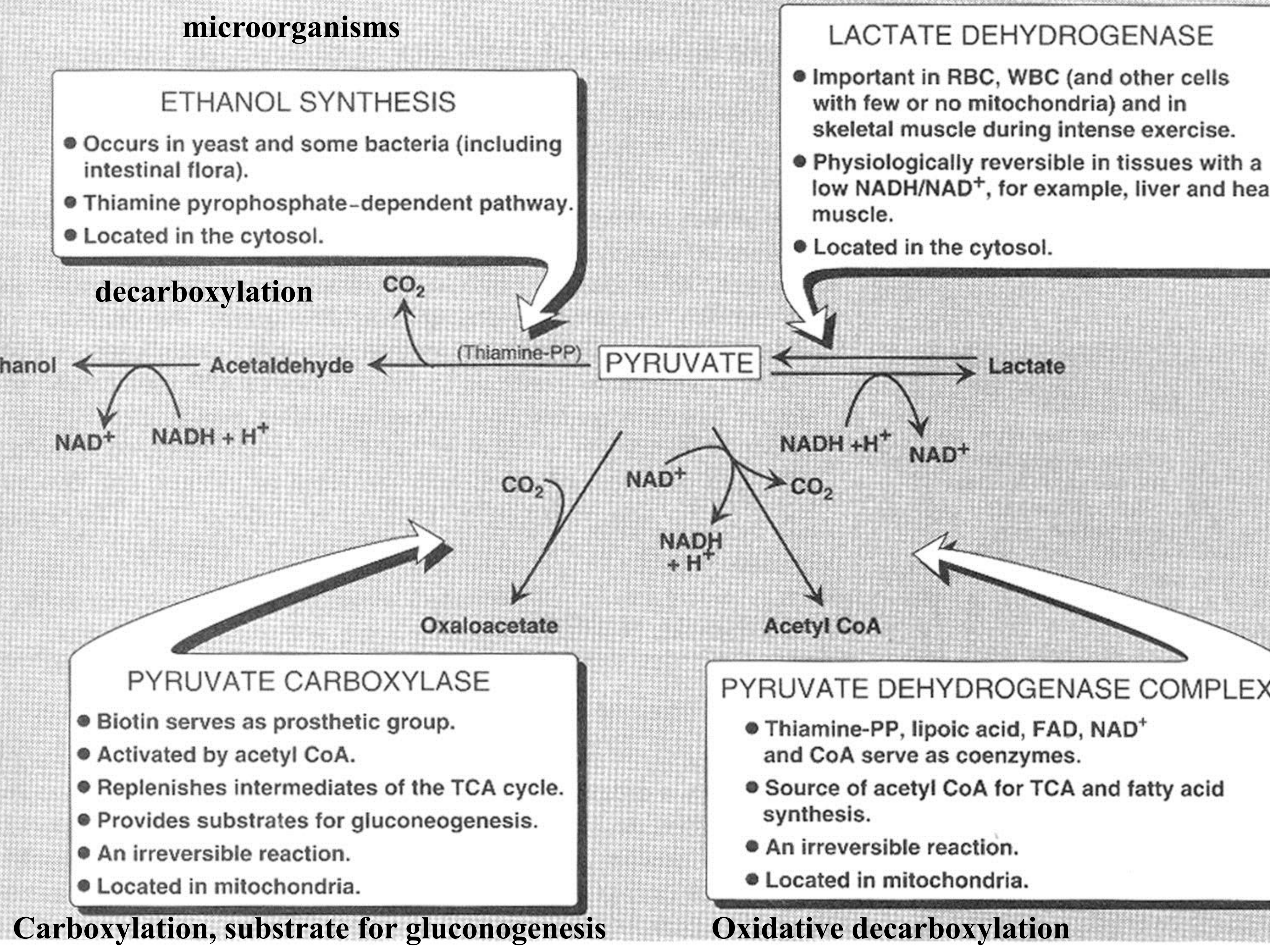


Glucose is abundant

Fructose 2,6-biphosphate is converted back to fructose 6-P by fructose biphosphatase 2 (FBP-2) while fructose 2,6-biphosphate is formed by phosphofructokinase 2 (PFK-2). The kinase and phosphatase activities are on different domains. Insulin and glucagon actions on the kinases.

Three possible catabolic fates of pyruvate from glycolysis




microorganisms
ETHANOL SYNTHESIS
• Occurs in yeast and some bacteria (including intestinal flora).
• Thiamine pyrophosphate-dependent pathway.
• Located in the cytosol.
LACTATE DEHYDROGENASE
• Important in RBC, WBC (and other cells with few or no mitochondria) and in skeletal muscle during intense exercise.
• Physiologically reversible in tissues with a low NADH/NAD+, for example, liver and hea
muscle.
• Located in the cytosol.
decarboxylation
CO2
(Thiamine-PP)
hanol
Acetaldehyde
PYRUVATE
Lactate
NAD+
NADH + H+
NADH +H+
NAD+
CO2
NAD+
CO2
NADH + H+
Oxaloacetate
Acetyl CoA
PYRUVATE CARBOXYLASE
• Biotin serves as prosthetic group.
• Activated by acetyl CoA.
• Replenishes intermediates of the TCA cycle.
• Provides substrates for gluconeogenesis.
• An irreversible reaction.
• Located in mitochondria.
PYRUVATE DEHYDROGENASE COMPLEX
• Thiamine-PP, lipoic acid, FAD, NAD+ and CoA serve as coenzymes.
• Source of acetyl CoA for TCA and fatty acid synthesis.
• An irreversible reaction.
• Located in mitochondria.
Carboxylation, substrate for gluconogenesis
Oxidative decarboxylation

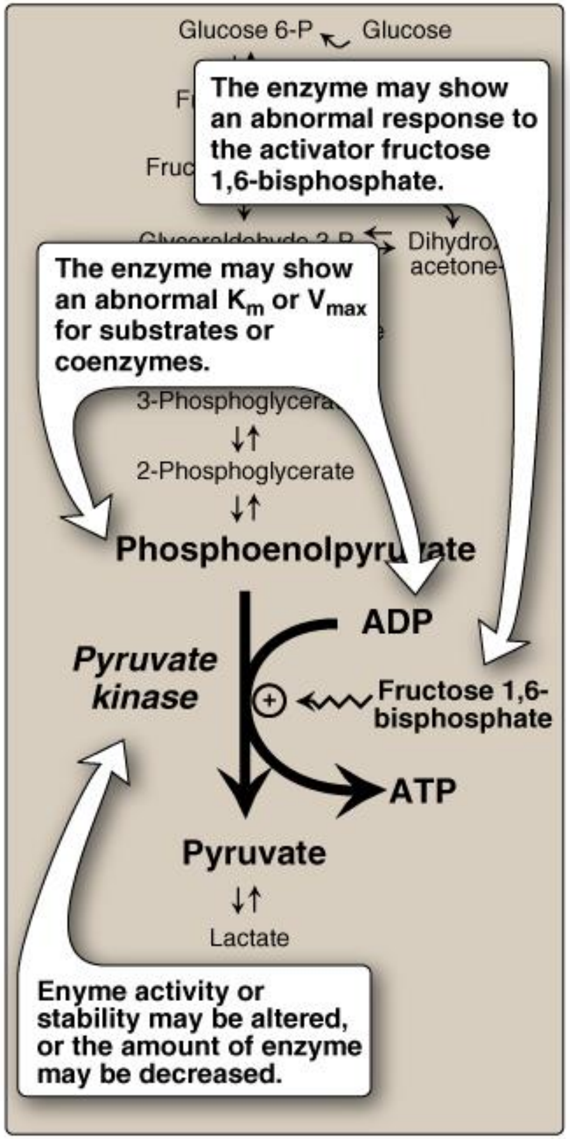


Figure 8.20

Alterations observed with various mutant forms of pyruvate kinase.

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How many ATP's are formed from anaerobic and aerobic glycolysis? Each NADH=3ATP

Patients with defects in glycolitic enzymes 95% show a defect in pyruvate kinase (PK) and about 4% in glucose phosphate isomerase. They exibit hemolytic anemia due to erythrocyte destruction.

Meal rich in carbohydrates or administration of insulin

Starvation or diabetes

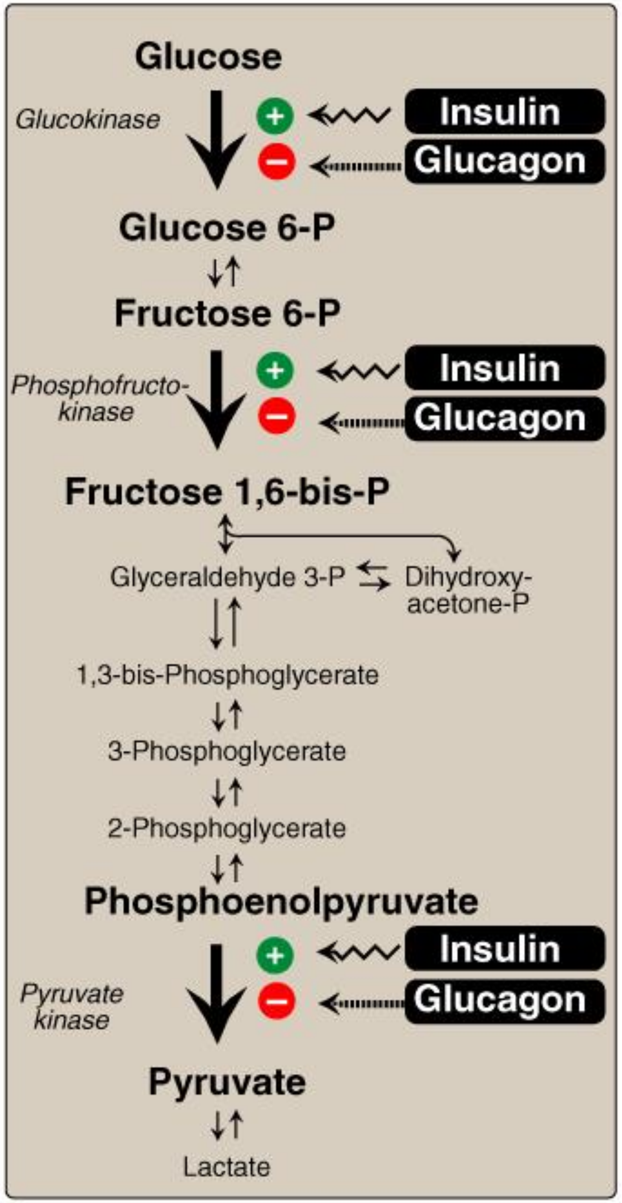

Figure 8.23
Effect of insulin and glucagon on the synthesis of key enzymes of glycolysis in liver.

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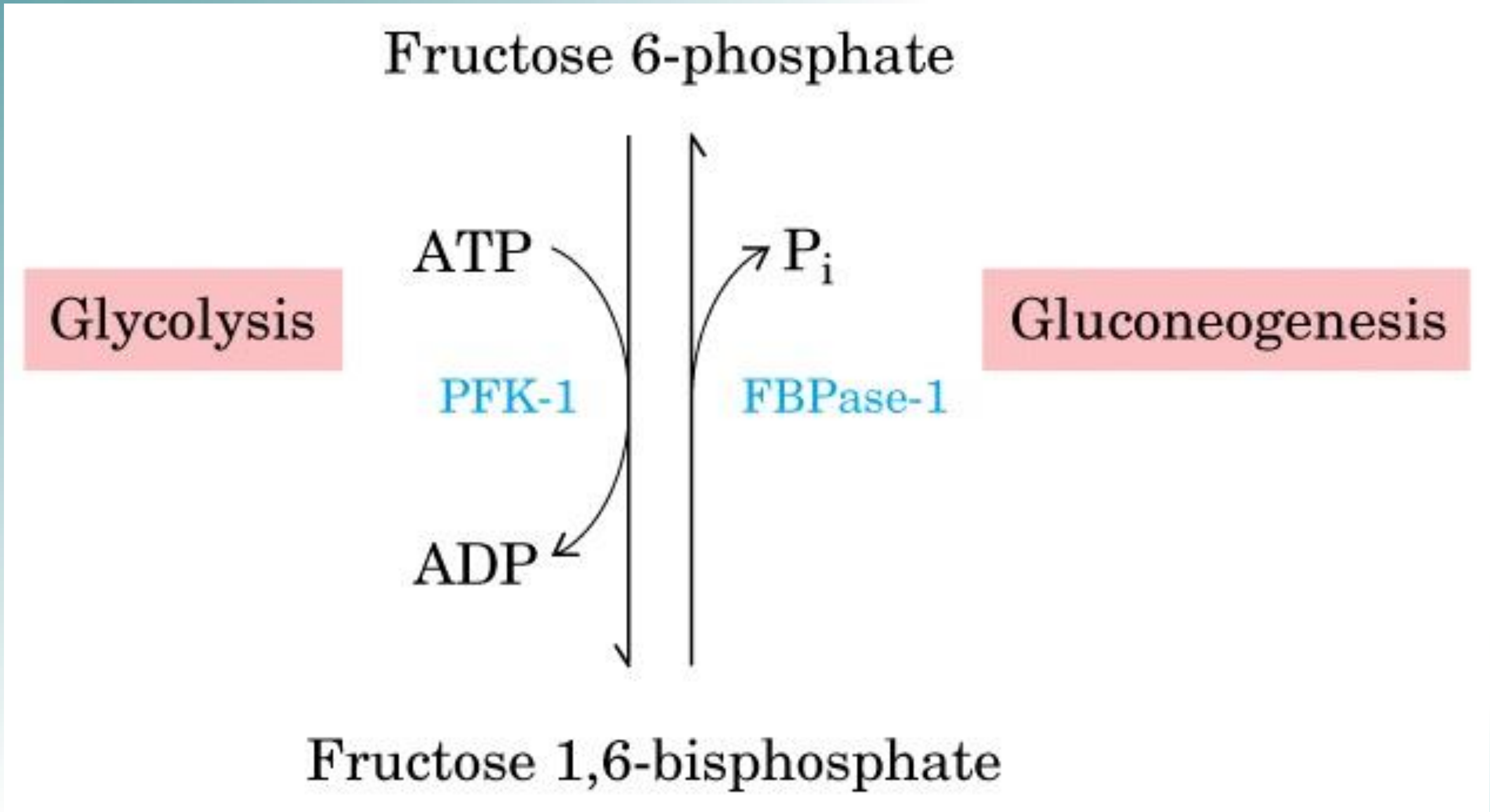
These effects are due to increase or decrease in transcription of specific genes. These effects can result in 10-20 fold increases in enzyme activity

Rate limiting step enzymes in gluconeogenesis, glucagon activates transcription of PEP carboxykinase, fructose 6-phosphatase, glucose 6-phosphatase (old Lipp page 102)


Fructose 6-phosphate
ATP
ADP
PFK-1
P_i
FBPase-1
Glycolysis
Gluconeogenesis
Fructose 1,6-bisphosphate

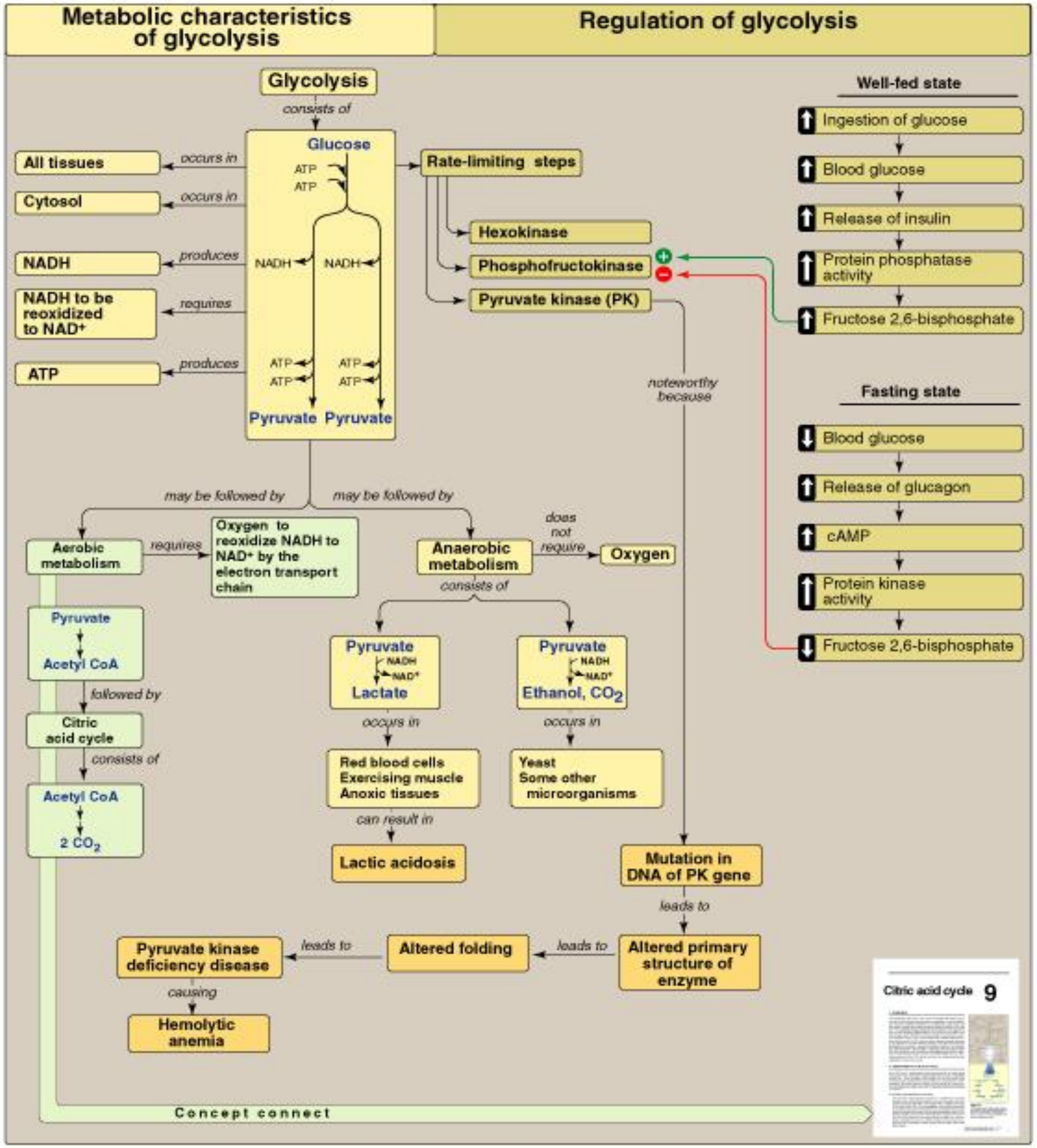


Figure 8.25

Key concept map for glycolysis.

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table 15–2

Cytosolic Concentrations of Enzymes and Intermediates of the Glycolytic Pathway in Skeletal Muscle

Enzyme	Concentration (μM)	Intermediate	Concentration (μM)
Aldolase	810	Glucose 6-phosphate	3,900
Triose phosphate isomerase	220	Fructose 6-phosphate	1,500
Glyceraldehyde 3-phosphate		Fructose 1,6-bisphosphate	80
dehydrogenase	1,400	Dihydroxyacetone phosphate	160
Phosphoglycerate kinase	130	Glyceraldehyde 3-phosphate	80
Phosphoglycerate mutase	240	1,3-Bisphosphoglycerate	50
Enolase	540	3-Phosphoglycerate	200
Pyruvate kinase	170	2-Phosphoglycerate	20
Lactate dehydrogenase	300	Phosphenolpyruvate	65
Phosphoglucomutase	32	Pyruvate	380
		Lactate	3,700
		ATP	8,000
		ADP	600
		P_i	8,000
		NAD^+	540
		NADH	50

Source: From Srivastava, D.K. & Bernhard, S.A. (1987) Biophysical chemistry of metabolic reaction sequences in concentrated solution and in the cell. *Annu. Rev. Biophys. Biophys. Chem.* **16,** 175–204.

How well have you learned?

When a muscle is stimulated to contract aerobically, less lactic acid is formed than when it contracts anaerobically because:

a. glycolysis does not occur to a significant extent under aerobic conditions.

b. muscle is metabolically less active under aerobic than anaerobic conditions.

c. the lactic acid generated is rapidly incorporated into lipids under aerobic conditions.

d. under aerobic conditions in muscle, the major energy-yielding pathway is the pentose phosphate pathway which does not produce lactate.

e. under aerobic conditions most of the pyruvate generated as a result of glycolysis is oxidized by the citric acid cycle rather than reduced to lactate.

The steps of glycolysis between glyceraldehyde 3-phosphate and 3 phosphoglycerate involve all of the following except:

a. ATP synthesis.
b. catalysis by phosphoglycerate kinase
c. oxidation of NADH to NAD^+
d. the formation of 1,3-bisphosphoglycerate
e. utilization of Pi.

Glycolysis in the erythrocyte produces pyruvate that is further metabolized to:

a. CO_2
b. Ethanol
c. Glucose
d. Hemoglobin
e. lactate