Introduction to Metabolism and Glycolysis 8

thePoint® For additional ancillary materials related to this chapter, please visit thePoint.

I. METABOLISM OVERVIEW

In Chapter 5, individual enzymic reactions were analyzed in an effort to explain the mechanisms of catalysis. However, in cells, these reactions rarely occur in isolation. Instead, they are organized into multistep sequences called pathways, such as that of glycolysis (Fig. 8.1). In a pathway, the product of one reaction serves as the substrate of the subsequent reaction. Most pathways can be classified as either catabolic (degradative) or anabolic (synthetic). Catabolic pathways break down complex molecules, such as proteins, polysaccharides, and lipids, to a few simple molecules (for example, carbon dioxide, ammonia, and water). Anabolic pathways form complex end products from simple precursors, for example, the synthesis of the polysaccharide glycogen from glucose. [Note: Pathways that regenerate a component are called cycles.] Different pathways can intersect, forming an integrated and purposeful network of chemical reactions. Metabolism is the sum of all the chemical changes occurring in a cell, a tissue, or the body. The next several chapters focus on the central metabolic pathways that are involved in synthesizing and degrading carbohydrates, lipids, and amino acids.

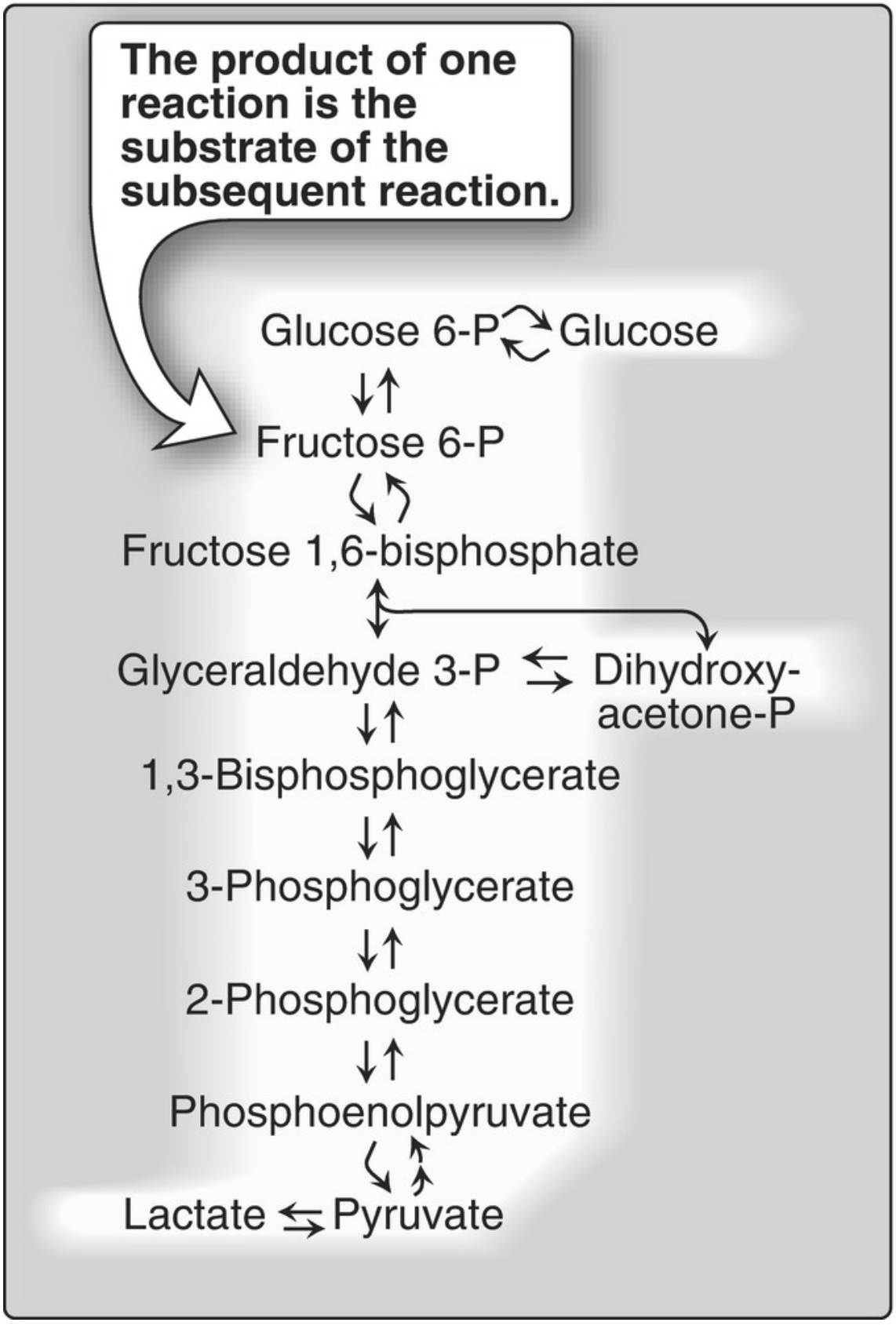
The product of one reaction is the substrate of the subsequent reaction.
Glucose 6-P
Glucose
Fructose 6-P
Fructose 1,6-bisphosphate
Glyceraldehyde 3-P
Dihydroxy-acetone-P
1,3-Bisphosphoglycerate
3-Phosphoglycerate
2-Phosphoglycerate
Phosphoenolpyruvate
Lactate
Pyruvate

Figure 8.1 Glycolysis, an example of a metabolic pathway. [Note: Pyruvate to phosphoenolpyruvate requires two reactions.] Curved reaction arrows () indicate forward and reverse reactions that are catalyzed by different enzymes. P = phosphate.

A. Metabolic map

Metabolism is best understood by examining its component pathways. Each pathway is composed of multienzyme sequences, and each enzyme, in turn, may exhibit important catalytic or regulatory features. A metabolic map containing the important central pathways of energy metabolism is presented in Figure 8.2. This “big picture” view of metabolism is useful in tracing connections between pathways, visualizing the purposeful movement of metabolic intermediates (metabolites), and depicting the effect on the flow of intermediates if a pathway is blocked (for example, by a drug or an inherited deficiency of an enzyme). [Note: The metabolome is the full complement of metabolites in an organism.] Throughout the next three units of this book, each pathway under discussion will be repeatedly featured as part of the major metabolic map shown in Figure 8.2.

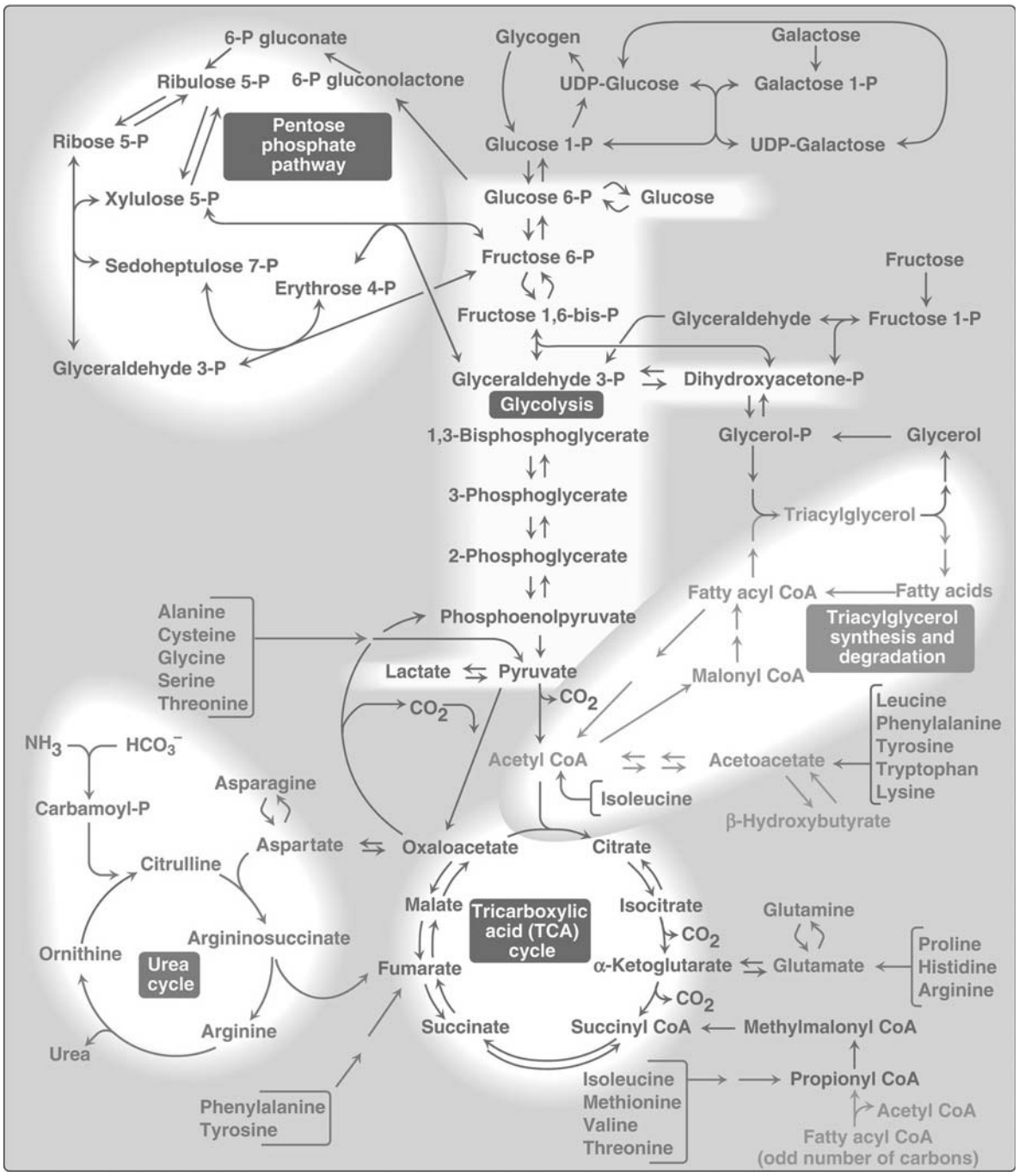

Figure 8.2 Important reactions of intermediary metabolism. Several important pathways to be discussed in later chapters are highlighted. Curved reaction arrows () indicate forward and reverse reactions that are catalyzed by different enzymes. The straight arrows () indicate forward and reverse reactions that are catalyzed by the same enzyme. Blue text = intermediates of carbohydrate metabolism; brown text = intermediates of lipid metabolism; green text =

intermediates of protein metabolism. UDP = uridine diphosphate; P = phosphate; CoA = coenzyme A; CO_2 = carbon dioxide; HCO_3^- = bicarbonate; NH_3 = ammonia.

B. Catabolic pathways

Catabolic reactions serve to capture chemical energy in the form of ATP from the degradation of energy-rich fuel molecules. ATP generation by degradation of complex molecules occurs in three stages, as shown in Figure 8.3. [Note: Catabolic pathways are typically oxidative and require oxidized coenzymes such as nicotinamide adenine dinucleotide (NAD^+).] Catabolism also allows molecules in the diet (or nutrient molecules stored in cells) to be converted into basic building blocks needed for the synthesis of complex molecules. Catabolism, then, is a convergent process (that is, a wide variety of molecules are transformed into a few common end products).

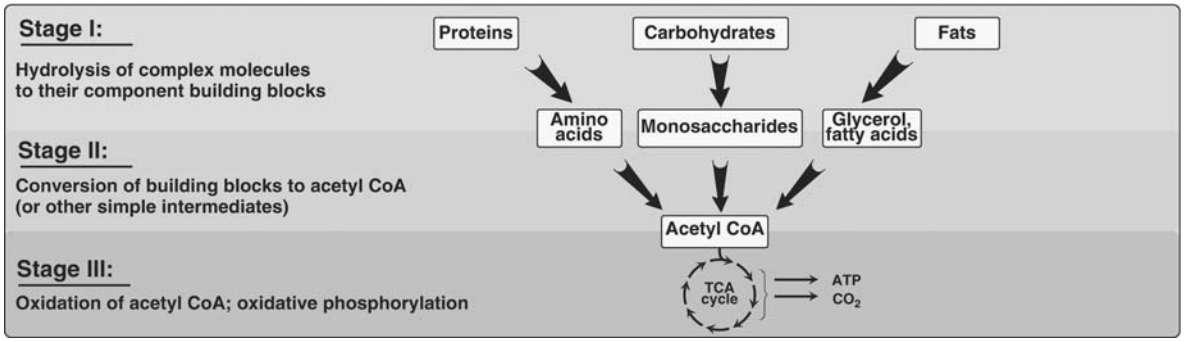

Figure 8.3 Three stages of catabolism. CoA = coenzyme A; TCA = tricarboxylic acid; CO_2 = carbon dioxide.

1. Hydrolysis of complex molecules: In the first stage, complex molecules are broken down into their component building blocks. For example, proteins are degraded to amino acids, polysaccharides to monosaccharides, and fats (triacylglycerols) to free fatty acids and glycerol.
2. Conversion of building blocks to simple intermediates: In the second stage, these diverse building blocks are further degraded to acetyl coenzyme A (CoA) and a few other simple molecules. Some energy is

captured as ATP, but the amount is small compared with the energy produced during the third stage of catabolism.

3. Oxidation of acetyl coenzyme A: The tricarboxylic acid (TCA) cycle (see p. 109) is the final common pathway in the oxidation of fuel molecules that produce acetyl CoA. Oxidation of acetyl CoA generates large amounts of ATP via oxidative phosphorylation as electrons flow from NADH and flavin adenine dinucleotide ($FADH_2$) to oxygen ([O_2] see p. 73).

C. Anabolic pathways

In contrast to catabolism, anabolism is a divergent process in which a few biosynthetic precursors (such as amino acids) form a wide variety of polymeric, or complex, products (such as proteins [Fig. 8.4]). Anabolic reactions require energy (are endergonic), which is generally provided by the hydrolysis of ATP to adenosine diphosphate (ADP) and inorganic phosphate (P_i). [Note: Catabolic reactions generate energy (are exergonic).] Anabolic reactions often involve chemical reductions in which the reducing power is most frequently provided by the electron donor NADPH (phosphorylated NADH, see p. 147).

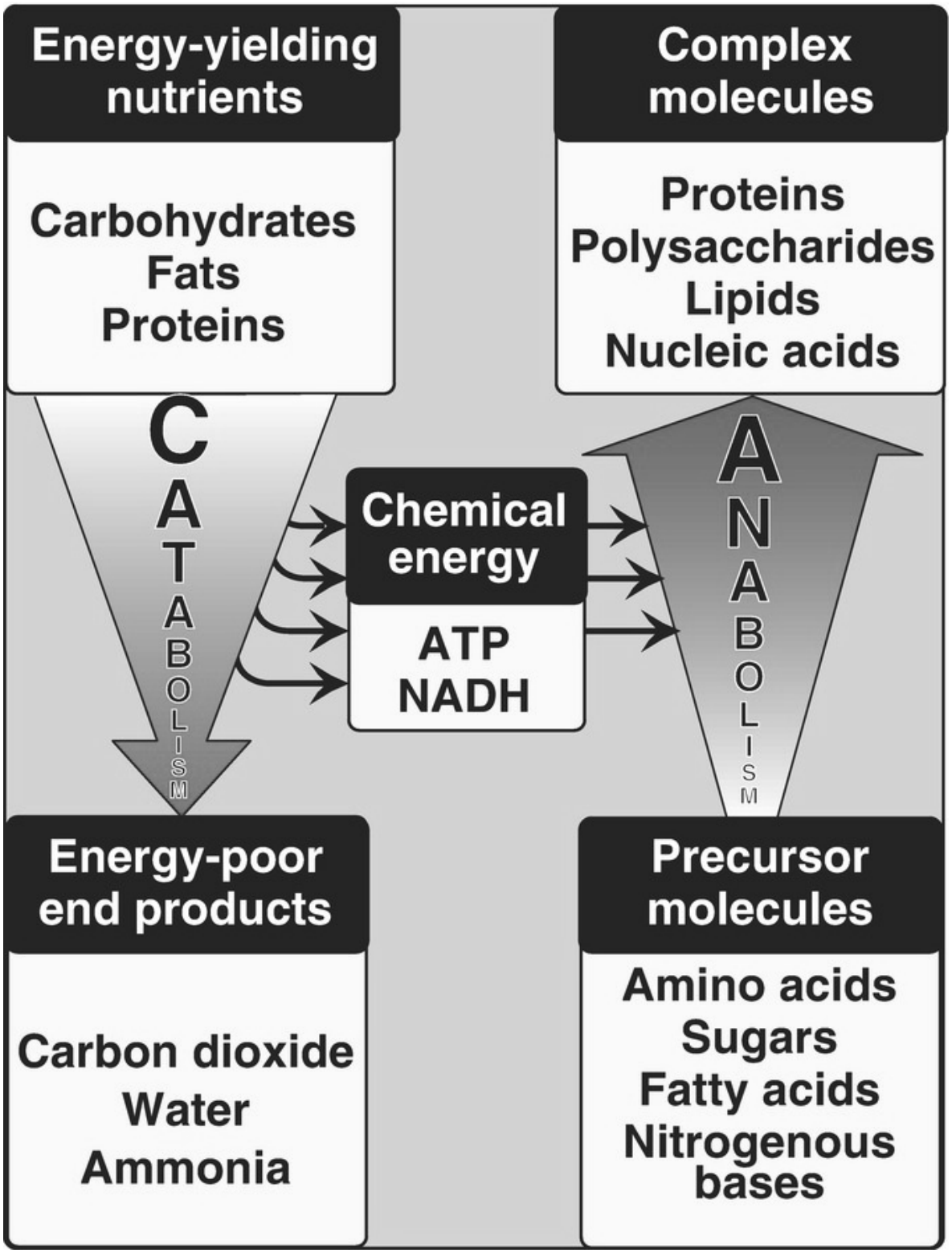

Figure 8.4 Comparison of catabolic and anabolic pathways. NADH = nicotinamide adenine dinucleotide.

II. METABOLISM REGULATION

The pathways of metabolism must be coordinated so that the production of energy or the synthesis of end products meets the needs of the cell. Furthermore, individual cells function as part of a community of interacting tissues, not in isolation. Thus, a sophisticated communication system has evolved to coordinate the functions of the body. Regulatory signals that inform an individual cell of the metabolic state of the body as a whole include hormones, neurotransmitters, and the availability of nutrients. These, in turn, influence signals generated within the cell (Fig. 8.5).

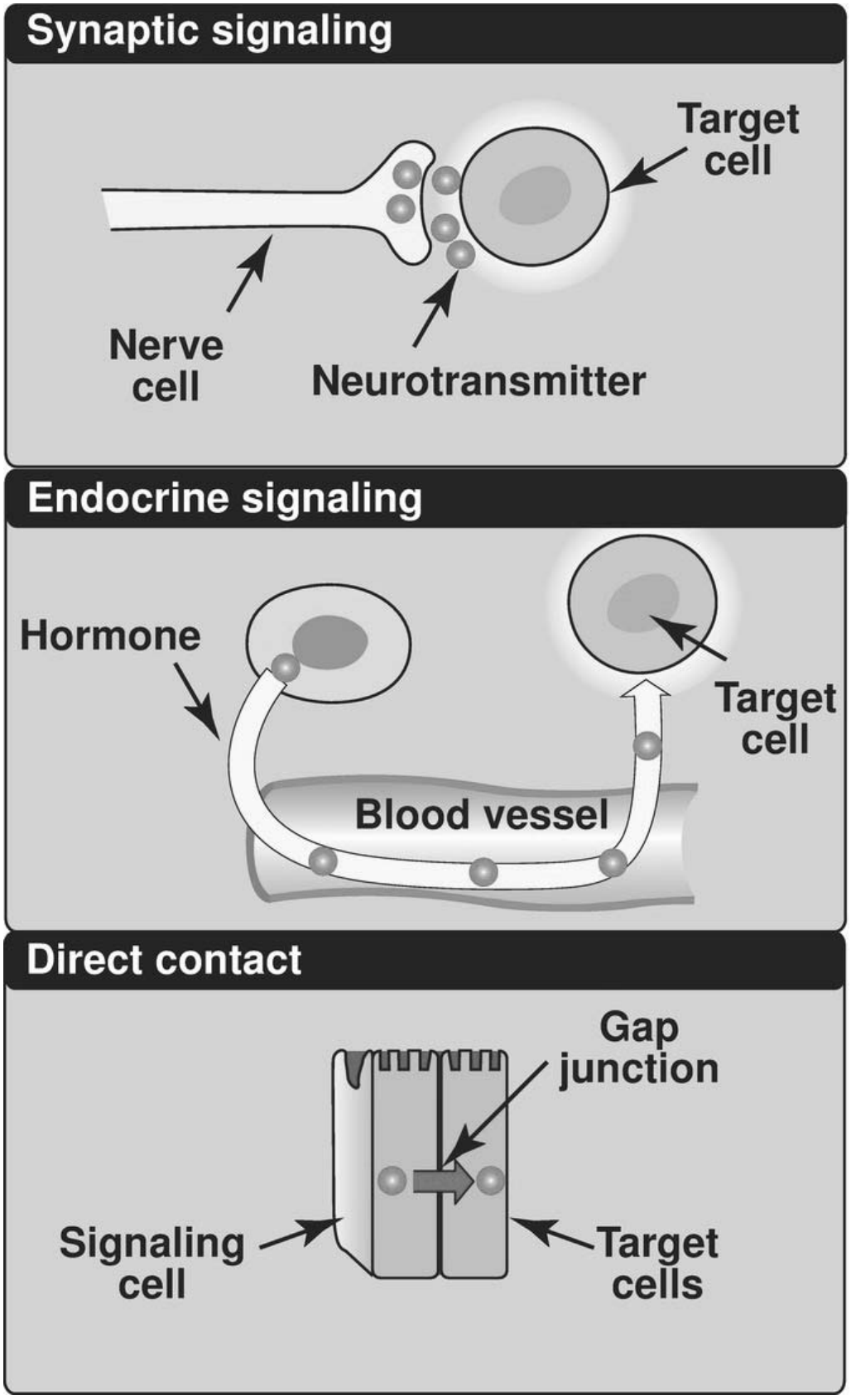
Synaptic signaling
Target cell
Nerve cell
Neurotransmitter
Endocrine signaling
Hormone
Target cell
Blood vessel
Direct contact
Gap junction
Signaling cell
Target cells

Figure 8.5 Some commonly used mechanisms for transmission of regulatory signals between cells.

A. Intracellular communication

The rate of a metabolic pathway can respond to regulatory signals that arise from within the cell. For example, the rate may be influenced by the availability of substrates, product inhibition, or alterations in the levels of allosteric activators or inhibitors. These intracellular signals typically elicit rapid responses and are important for the moment-to-moment regulation of metabolism.

B. Intercellular communication

The ability to respond to intercellular signals is essential for the development and survival of organisms. Signaling between cells provides for long-range integration of metabolism and usually results in a response, such as a change in gene expression, that is slower than is seen with intracellular signals. Communication between cells can be mediated, for example, by surface-to-surface contact and, in some tissues, by formation of gap junctions, allowing direct communication between the cytoplasms of adjacent cells. However, for energy metabolism, the most important route of communication is chemical signaling between cells by blood-borne hormones or by neurotransmitters.

C. Second messenger systems

Hormones and neurotransmitters can be thought of as signals and their receptors as signal detectors. Receptors respond to a bound ligand by initiating a series of reactions that ultimately result in specific intracellular responses. Second messenger molecules, so named because they intervene between the original extracellular messenger (the neurotransmitter or hormone) and the ultimate intracellular effect, are part of the cascade of events that converts (transduces) ligand binding into a response. Two of the most widely recognized second messenger systems are the

calcium/phosphatidylinositol system (see p. 205) and the ***adenylyl cyclase (adenylate cyclase)*** system, which is particularly important in regulating the pathways of intermediary metabolism. Both involve the binding of ligands, such as epinephrine or glucagon, to specific G protein–coupled receptors (GPCR) on the cell (plasma) membrane. GPCR are characterized by an extracellular ligand-binding domain, seven transmembrane α helices, and an intracellular domain that interacts with trimeric G proteins (Fig. 8.6). [Note: Insulin, another key regulator of metabolism, binds a membrane tyrosine kinase receptor (see p. 311) and not a GPCR.]

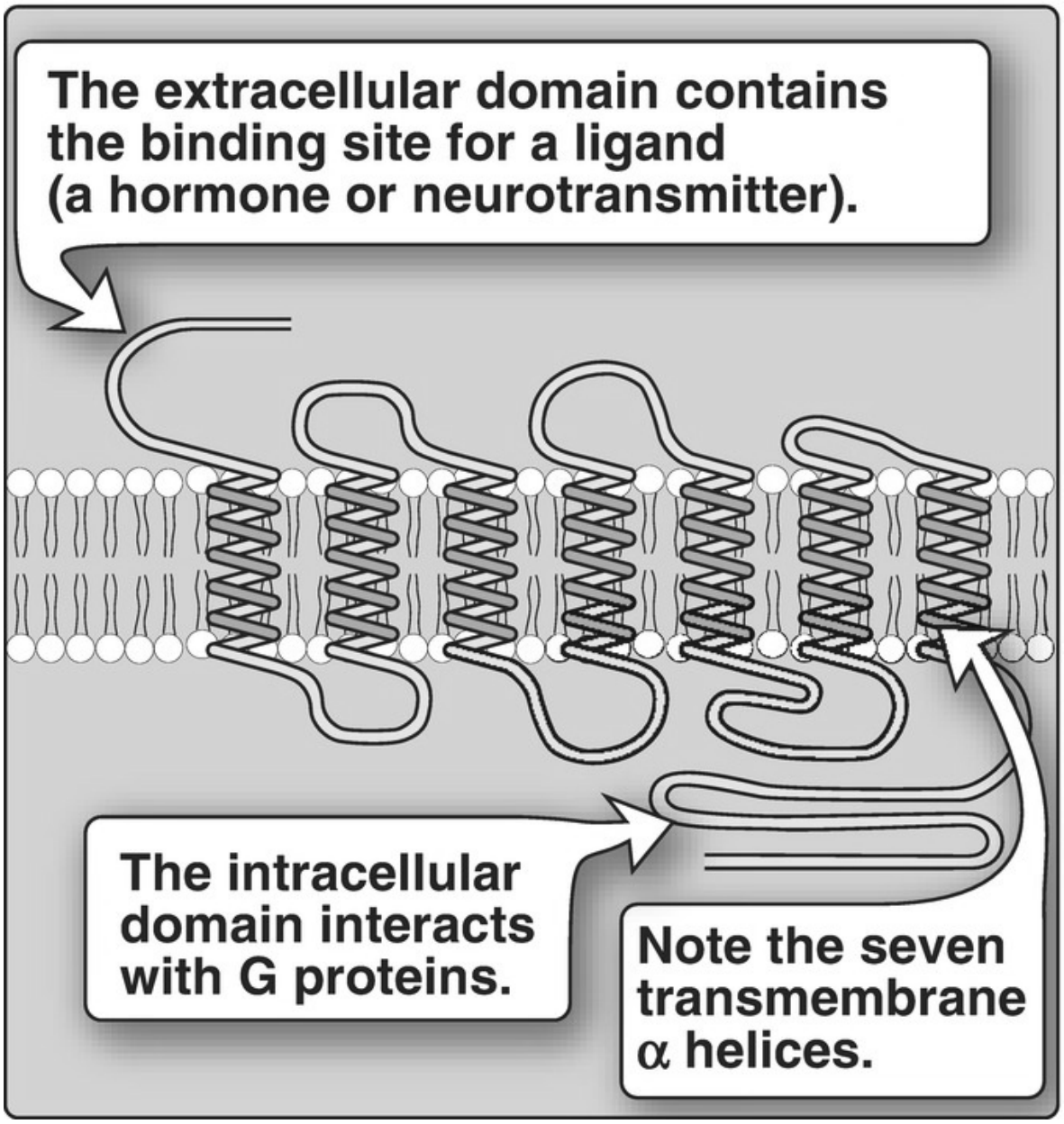

Figure 8.6 Structure of a typical G protein–coupled receptor of the plasma membrane.

D. Adenylyl cyclase

The recognition of a chemical signal by some GPCR, such as the β- and α_2-adrenergic receptors, triggers either an increase or a decrease in the activity of ***adenylyl cyclase (AC)***. This is a membrane-bound enzyme that converts

ATP to 3′,5′-adenosine monophosphate (cyclic AMP, or cAMP). The chemical signals are most often hormones or neurotransmitters, each of which binds to a unique type of GPCR. Therefore, tissues that respond to more than one signal must have several different GPCR, each of which can be linked to ***AC***.

1. Guanosine triphosphate–dependent regulatory proteins: The effect of the activated, occupied GPCR on second messenger formation is indirect, mediated by specialized trimeric proteins (α, β, and γ subunits) of the cell membrane. These proteins, referred to as G proteins because the α subunit binds guanosine di- or triphosphates (GDP or GTP), form a link in the chain of communication between the receptor and ***AC***. In the inactive form of a G protein, the α subunit is bound to GDP (Fig. 8.7). Ligand binding causes a conformational change in the receptor, triggering replacement of this GDP with GTP. The GTP-bound form of the α subunit dissociates from the βγ subunits and moves to ***AC***, affecting enzyme activity. Many molecules of active Gα protein are formed by one activated receptor. [Note: The ability of a hormone or neurotransmitter to stimulate or inhibit ***AC*** depends on the type of Gα protein that is linked to the receptor. One type, designated G_s, stimulates ***AC*** (see Fig. 8.7), whereas another type, designated G_i, inhibits the enzyme (not shown).] The actions of the Gα–GTP complex are short-lived because Gα has an inherent ***GTPase*** activity, resulting in the rapid hydrolysis of GTP to GDP. This causes inactivation of Gα, its dissociation from ***AC***, and its reassociation with the βγ dimer.

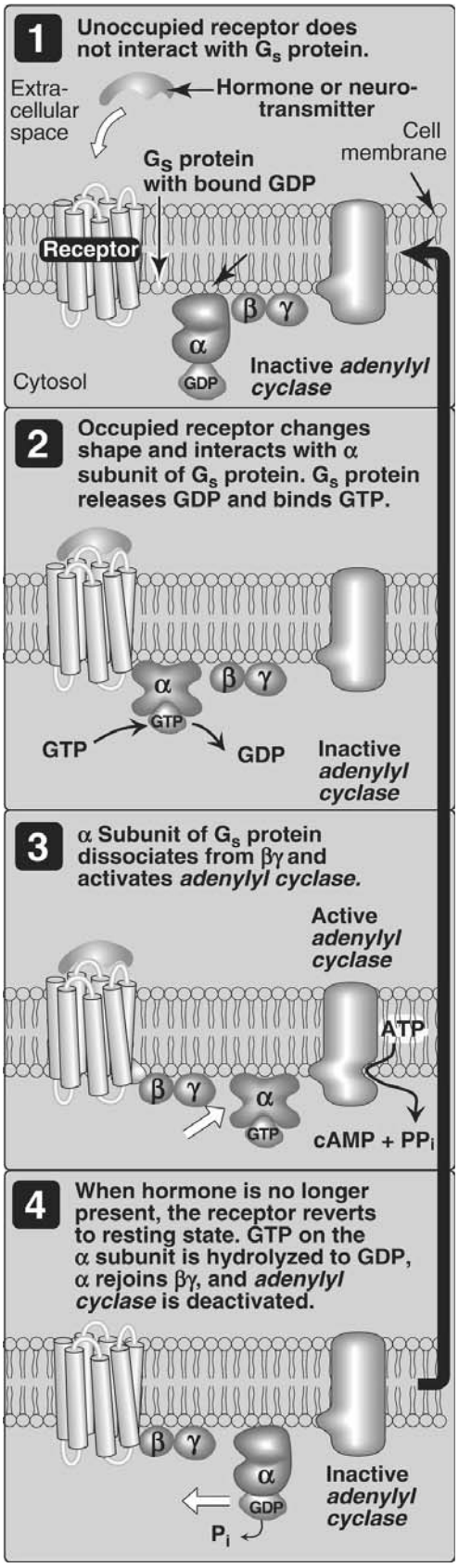
1
Unoccupied receptor does not interact with G_s protein.
Extra-cellular space
Hormone or neuro-transmitter
Cell membrane
G_S protein with bound GDP
Receptor
β γ
α
GDP
Cytosol
Inactive *adenylyl cyclase*
2
Occupied receptor changes shape and interacts with α subunit of G_s protein. G_s protein releases GDP and binds GTP.
α
β γ
GTP
GTP
GDP
Inactive *adenylyl cyclase*
3
α Subunit of G_s protein dissociates from βγ and activates *adenylyl cyclase.*
Active *adenylyl cyclase*
ATP
β γ
α
GTP
cAMP + PP_i
4
When hormone is no longer present, the receptor reverts to resting state. GTP on the α subunit is hydrolyzed to GDP, α rejoins βγ, and *adenylyl cyclase* is deactivated.
β γ
α
GDP
P_i
Inactive *adenylyl cyclase*

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***. GDP and GTP = guanosine di- and triphosphates; cAMP = cyclic adenosine monophosphate.

Toxins from Vibrio cholerae (cholera) and Bordetella pertussis (whooping cough) cause inappropriate activation of ***AC*** through covalent modification (ADP-ribosylation) of different G proteins. With cholera, the ***GTPase*** activity of $G\alpha_s$ is inhibited in intestinal cells. With whooping cough, $G\alpha_i$ is inactivated in respiratory tract cells.

2. Protein kinases: The next step in the cAMP second messenger system is the activation of a family of enzymes called ***cAMP-dependent protein kinases*** such as ***protein kinase A (PKA)***, as shown in Figure 8.8. cAMP activates ***PKA*** by binding to its two regulatory subunits, causing the release of its two catalytically active subunits. These subunits transfer phosphate from ATP to specific serine or threonine residues of protein substrates. The phosphorylated proteins may act directly on the cell's ion channels or, if enzymes, may become activated or inhibited. [Note: Several types of ***protein kinases*** are not cAMP dependent, for example, ***protein kinase C***, described on p. 205.]

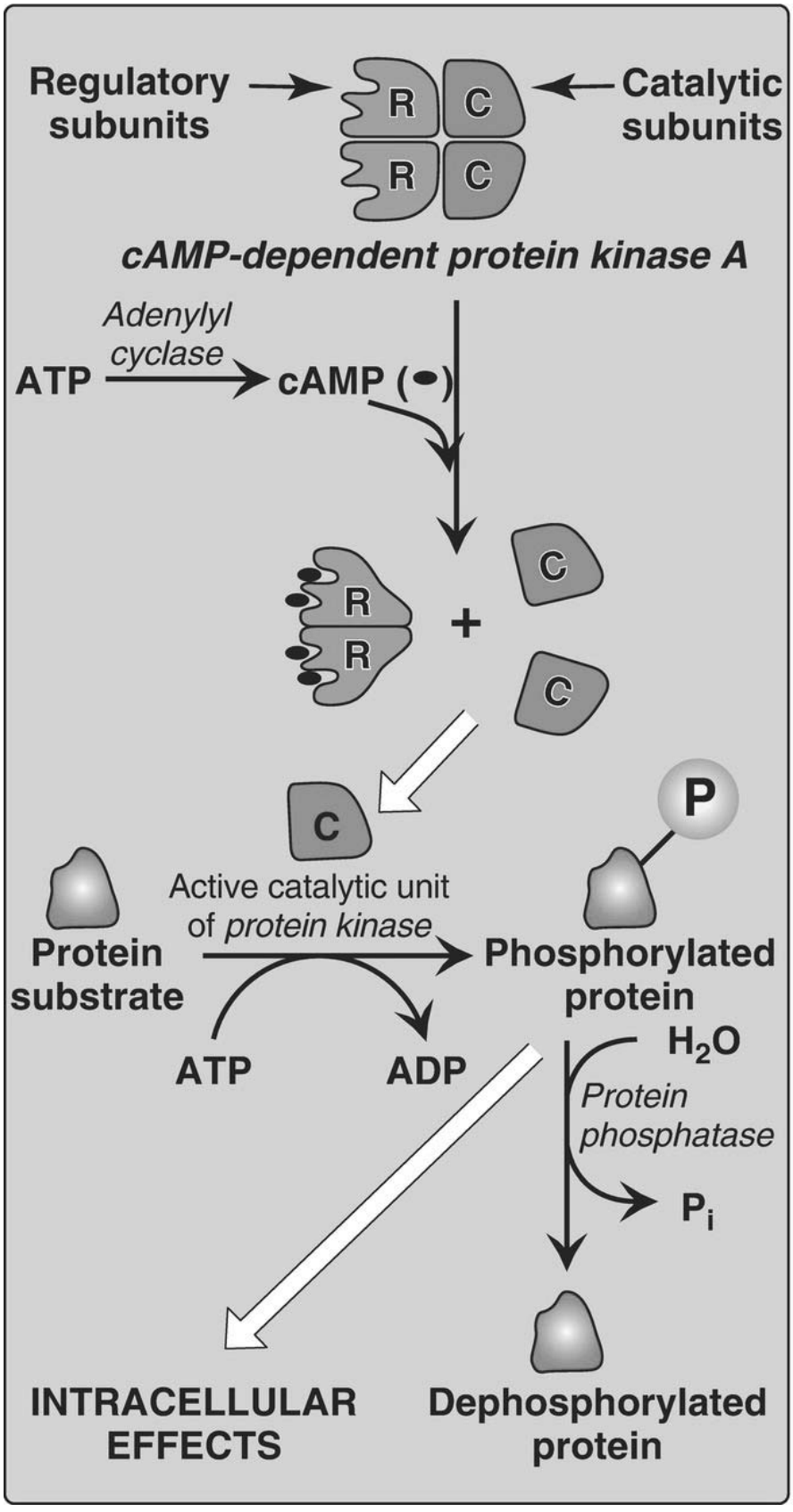
Regulatory subunits
R
C
R
C
Catalytic subunits
cAMP-dependent protein kinase A
Adenylyl cyclase
ATP
cAMP (•)
R
R
+
C
C
C
P
Active catalytic unit of protein kinase
Protein substrate
Phosphorylated protein
ATP
ADP
H2O
Protein phosphatase
Pi
INTRACELLULAR EFFECTS
Dephosphorylated protein

Figure 8.8 Actions of cyclic adenosine monophosphate (cAMP). = phosphate; ADP = adenosine diphosphate; P_i = inorganic phosphate.

3. Protein phosphatases: The phosphate groups added to proteins by ***protein kinases*** are removed by ***protein phosphatases***, enzymes that hydrolytically cleave phosphate esters (see Fig. 8.8). This insures that changes in protein activity induced by phosphorylation are not permanent.
4. cAMP hydrolysis: cAMP is rapidly hydrolyzed to 5′-AMP by ***cAMP phosphodiesterase*** that cleaves the cyclic 3′,5′-phosphodiester bond. 5′-AMP is not an intracellular signaling molecule. Therefore, the effects of neurotransmitter- or hormone-mediated increases of cAMP are rapidly terminated if the extracellular signal is removed. [Note: ***cAMP phosphodiesterase*** is inhibited by caffeine, a methylxanthine derivative.]

III. GLYCOLYSIS OVERVIEW

The glycolytic pathway is used by all tissues for the oxidation of glucose to provide energy (as 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 (Fig. 8.9A). Pyruvate is the end product of glycolysis in cells with mitochondria and an adequate supply of O_2. This series of ten reactions is called aerobic glycolysis because O_2 is required to reoxidize the NADH formed during the oxidation of glyceraldehyde 3-phosphate (Fig. 8.9B). 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^+ (Fig. 8.9C). This conversion of glucose to lactate is called anaerobic glycolysis because it can occur without the participation of O_2. Anaerobic glycolysis allows the production of ATP in tissues that lack mitochondria (for example, red blood cells [RBC] and parts of the eye) or in cells deprived of sufficient O_2 (hypoxia).

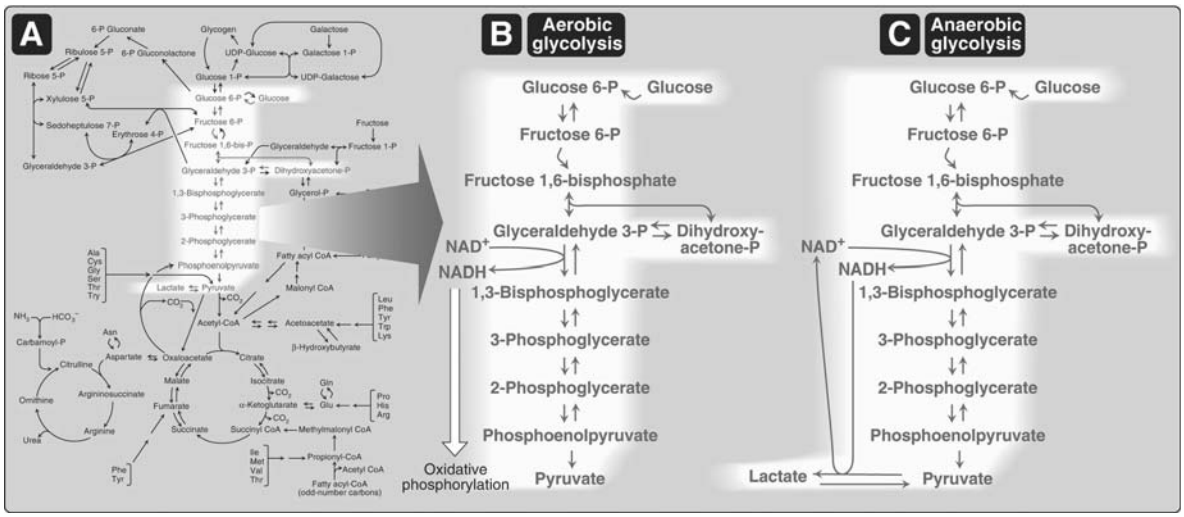

Figure 8.9 A. Glycolysis shown as one of the essential pathways of energy metabolism. B. Reactions of aerobic glycolysis. C. Reactions of anaerobic glycolysis. NAD(H) = nicotinamide adenine dinucleotide; P = phosphate.

IV. GLUCOSE TRANSPORT INTO CELLS

Glucose cannot diffuse directly into cells but enters by one of two transport systems: a sodium (Na^+)- and ATP-independent transport system or a Na^+- and ATP-dependent cotransport system.

A. Sodium- and ATP-independent transport system

This passive system is mediated by a family of 14 glucose transporter (GLUT) isoforms found in cell membranes. They are designated GLUT-1 to GLUT-14. These monomeric protein transporters exist in the membrane in two conformational states (Fig. 8.10). Extracellular glucose binds to the transporter, which then alters its conformation, transporting glucose across the cell membrane via facilitated diffusion. Because GLUT transport one molecule at a time, they are uniporters.

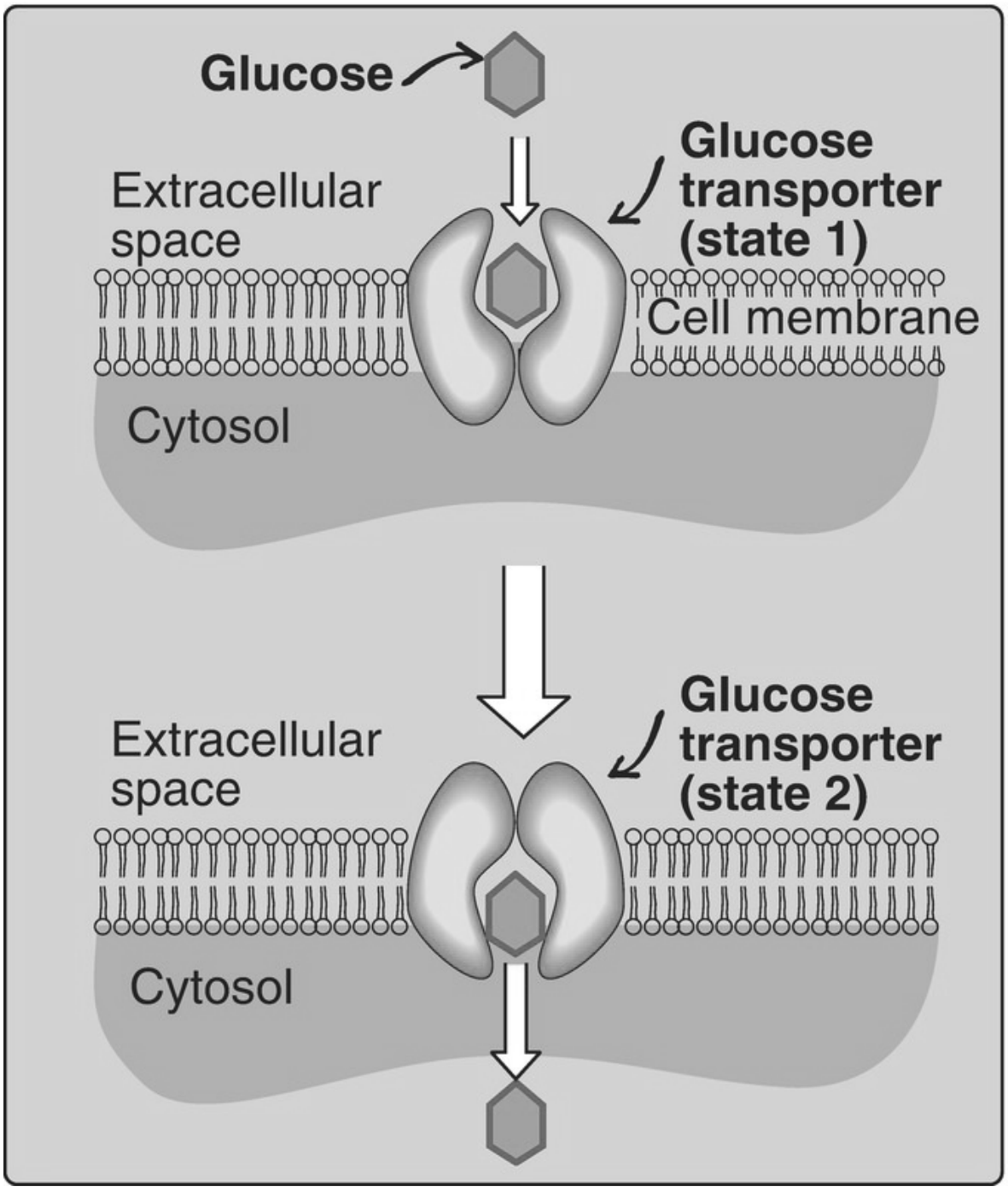

Figure 8.10 Schematic representation of the facilitated transport of glucose through a cell membrane. [Note: Glucose transporter proteins are monomeric and contain 12 transmembrane α helices.]

1. Tissue specificity: GLUT display a tissue-specific pattern of expression.

For example, GLUT-3 is the primary isoform in neurons. GLUT-1 is abundant in RBC 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. (See p. 311 for a discussion of insulin and glucose transport.)] GLUT-2 is abundant in the liver, kidneys, and pancreatic β cells. The other GLUT isoforms also have tissue-specific distributions.

2. Specialized functions: In facilitated diffusion, transporter-mediated glucose movement is down a concentration gradient (that is, from a high concentration to a lower one, therefore requiring no 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 kidneys, 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 (see p. 87).

B. Sodium- and ATP-dependent cotransport system

This energy-requiring process transports glucose against (up) its concentration gradient (that is, from low extracellular concentrations to higher intracellular concentrations) as Na^+ is transported down its electrochemical gradient. [Note: The gradient is created by the ***Na^+-potassium (K^+) ATPase*** (see Fig. 7.10, p. 87).] Because this secondary active transport process requires the concurrent uptake (symport) of Na^+, the transporter is a sodium-dependent glucose cotransporter (SGLT). This type of cotransport occurs in the epithelial cells of the intestine (see p. 87), renal tubules, and choroid plexus. [Note: The choroid plexus, part of the blood–brain barrier, also contains GLUT-1.]

V. GLYCOLYSIS REACTIONS

The conversion of glucose to pyruvate occurs in two stages (Fig. 8.11). 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 (see p. 102) per glucose molecule metabolized.

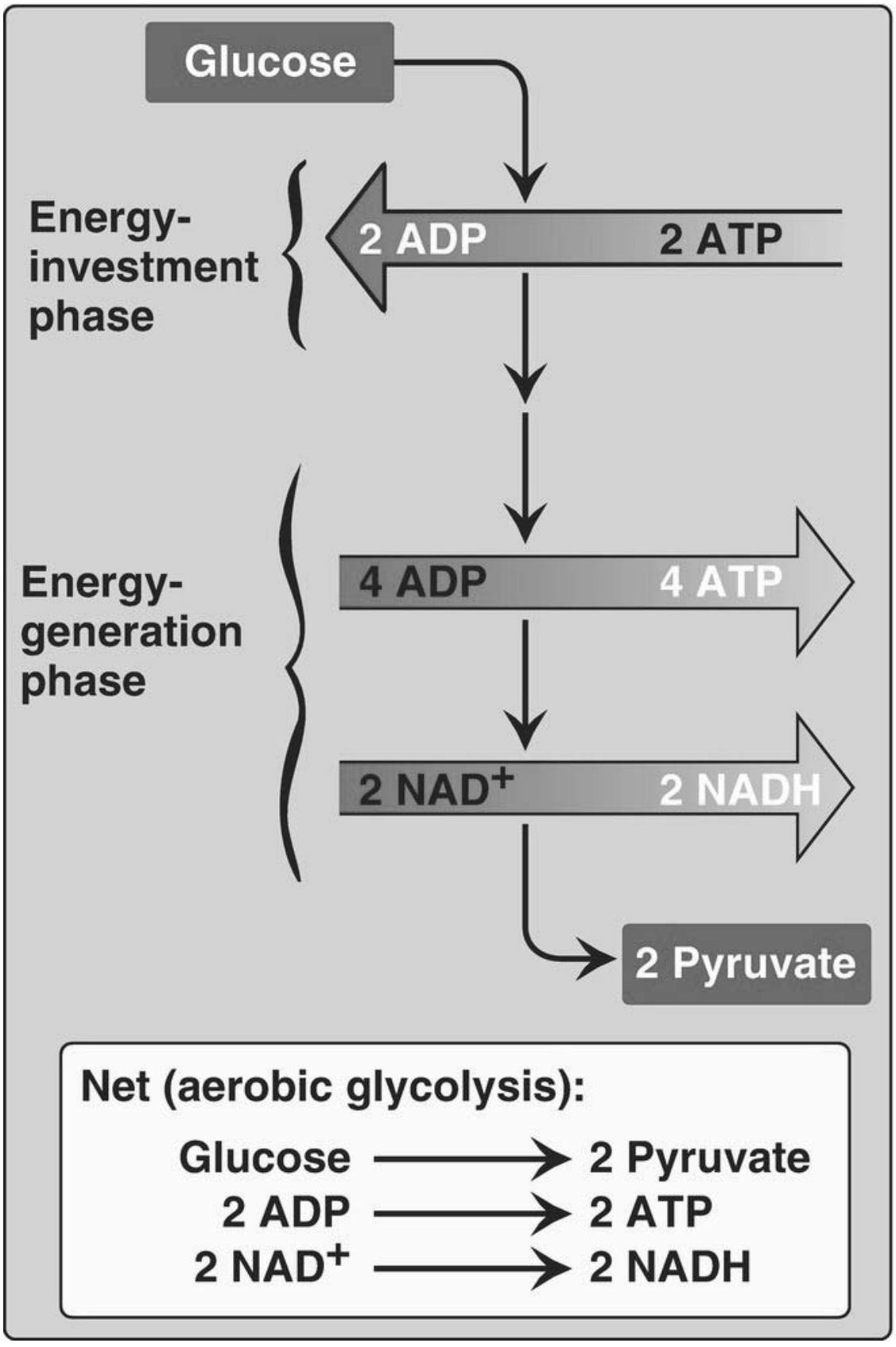
Glucose
Energy-investment phase
2 ADP
2 ATP
Energy-generation phase
4 ADP
4 ATP
2 NAD+
2 NADH
2 Pyruvate
Net (aerobic glycolysis):
Glucose → 2 Pyruvate
2 ADP → 2 ATP
2 NAD+ → 2 NADH

Figure 8.11 Two phases of aerobic glycolysis. NAD(H) = nicotinamide adenine dinucleotide; ADP = adenosine diphosphate.

A. Glucose phosphorylation

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. Therefore, the irreversible phosphorylation of glucose (Fig. 8.12) effectively traps the sugar as cytosolic glucose 6-phosphate and commits it to further metabolism in the cell. Mammals have four isozymes (I–IV) of the enzyme ***hexokinase*** that catalyze the phosphorylation of glucose to glucose 6-phosphate.

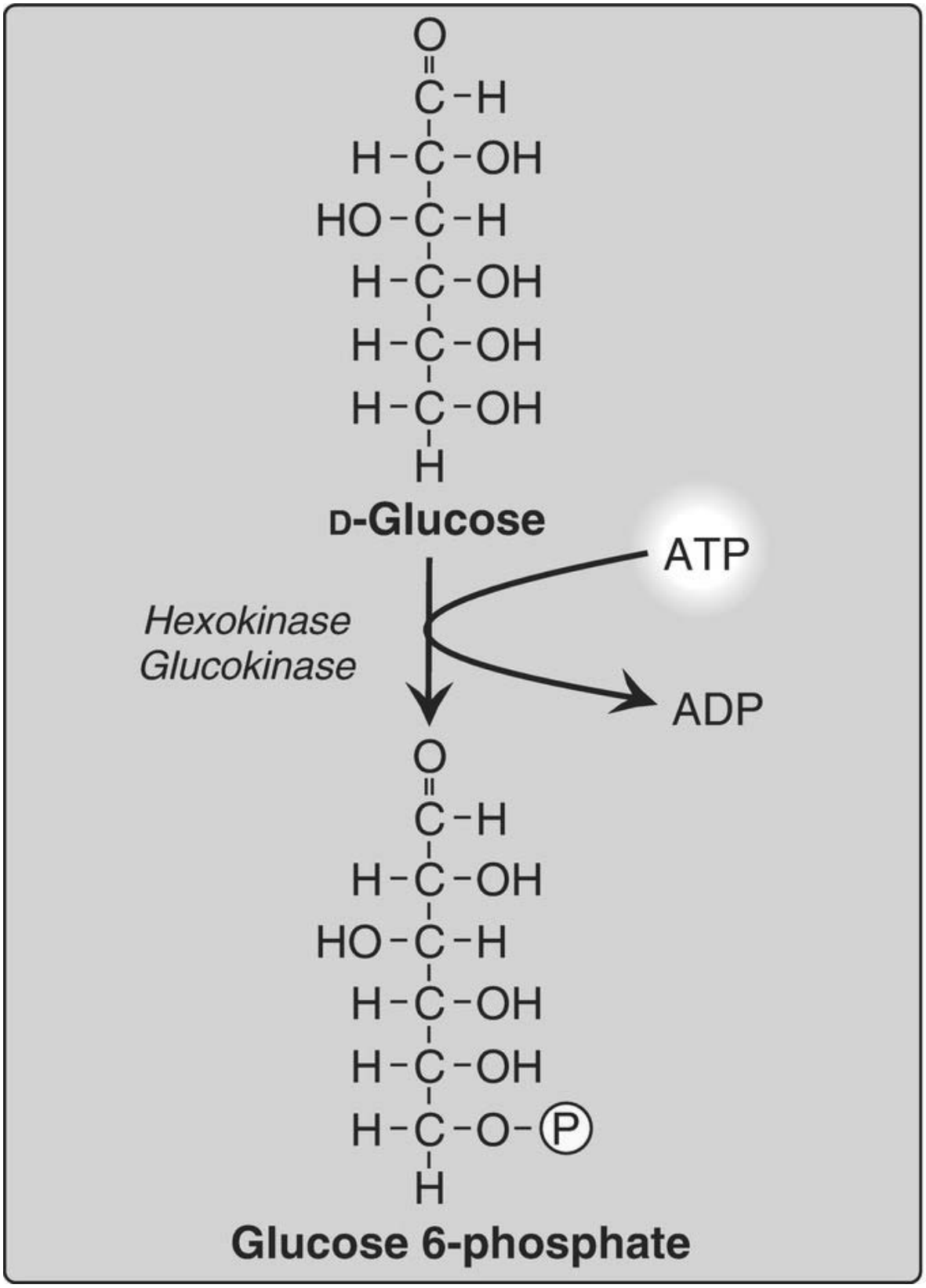
O
C-H
H-C-OH
HO-C-H
H-C-OH
H-C-OH
H-C-OH
H
D-Glucose
ATP
Hexokinase
Glucokinase
ADP
O
C-H
H-C-OH
HO-C-H
H-C-OH
H-C-OH
H-C-O-P
H
Glucose 6-phosphate

Figure 8.12 Energy-investment phase: phosphorylation of glucose. [Note: ***Kinases*** utilize ATP complexed with a divalent metal ion, most typically magnesium.] ADP = adenosine diphosphate; P = phosphate.

1. Hexokinases I–III: In most tissues, glucose phosphorylation is catalyzed by one of these isozymes of ***hexokinase***, which is one of three regulatory enzymes of glycolysis (along with ***phosphofructokinase*** and ***pyruvate kinase***). 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 (K_m) and, therefore, a high affinity (see p. 59) for glucose. This permits the efficient phosphorylation and subsequent metabolism of glucose even when tissue concentrations of glucose are low (Fig. 8.13). However, because these isozymes have a low maximal velocity ([V_{max}] see p. 57) for glucose, they do not sequester (trap) cellular phosphate in the form of phosphorylated glucose or phosphorylate more glucose than the cell can use. [Note: These isozymes have broad substrate specificity and are able to phosphorylate several hexoses in addition to glucose.]

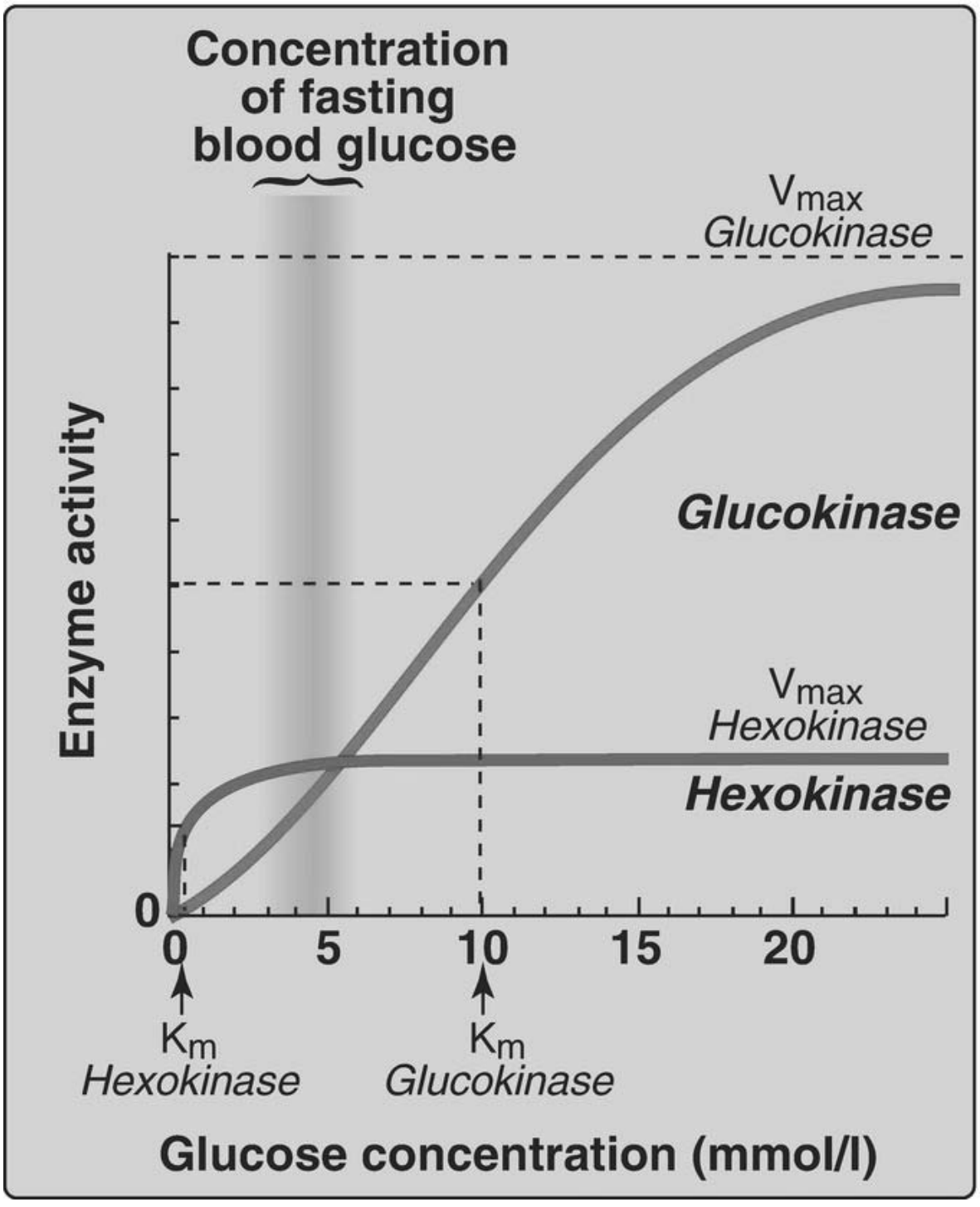

Figure 8.13 Effect of glucose concentration on the rate of phosphorylation catalyzed by ***hexokinase*** and ***glucokinase***. K_m = Michaelis constant; V_{max} = maximal velocity.

2. Hexokinase IV: In liver parenchymal cells and pancreatic β cells, ***glucokinase*** (the ***hexokinase IV*** isozyme) is the predominant enzyme responsible for glucose phosphorylation. In β cells, ***glucokinase*** functions as a glucose sensor, determining the threshold for insulin secretion (see p. 309). [Note: ***Hexokinase IV*** also serves as a glucose sensor in hypothalamic neurons, playing a key role in the adrenergic response to hypoglycemia (see p. 315).] 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 Fig. 8.13). 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 hepatocyte membrane.]
   b. Regulation: ***Glucokinase*** activity is not directly inhibited by glucose 6-phosphate as are the other ***hexokinases***. Instead, it 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***). Regulation is achieved by reversible binding to the hepatic protein glucokinase regulatory protein (GKRP). In the presence of fructose 6-phosphate, ***glucokinase*** binds tightly to GKRP and is translocated to the nucleus, thereby rendering the enzyme inactive (Fig. 8.14). When glucose levels in the blood (and also in the hepatocyte, as a result of GLUT-2) increase, ***glucokinase*** is released from GKRP, and the enzyme reenters the cytosol where it phosphorylates glucose to glucose 6-phosphate. [Note: GKRP is a competitive inhibitor of glucose use by ***glucokinase***.]

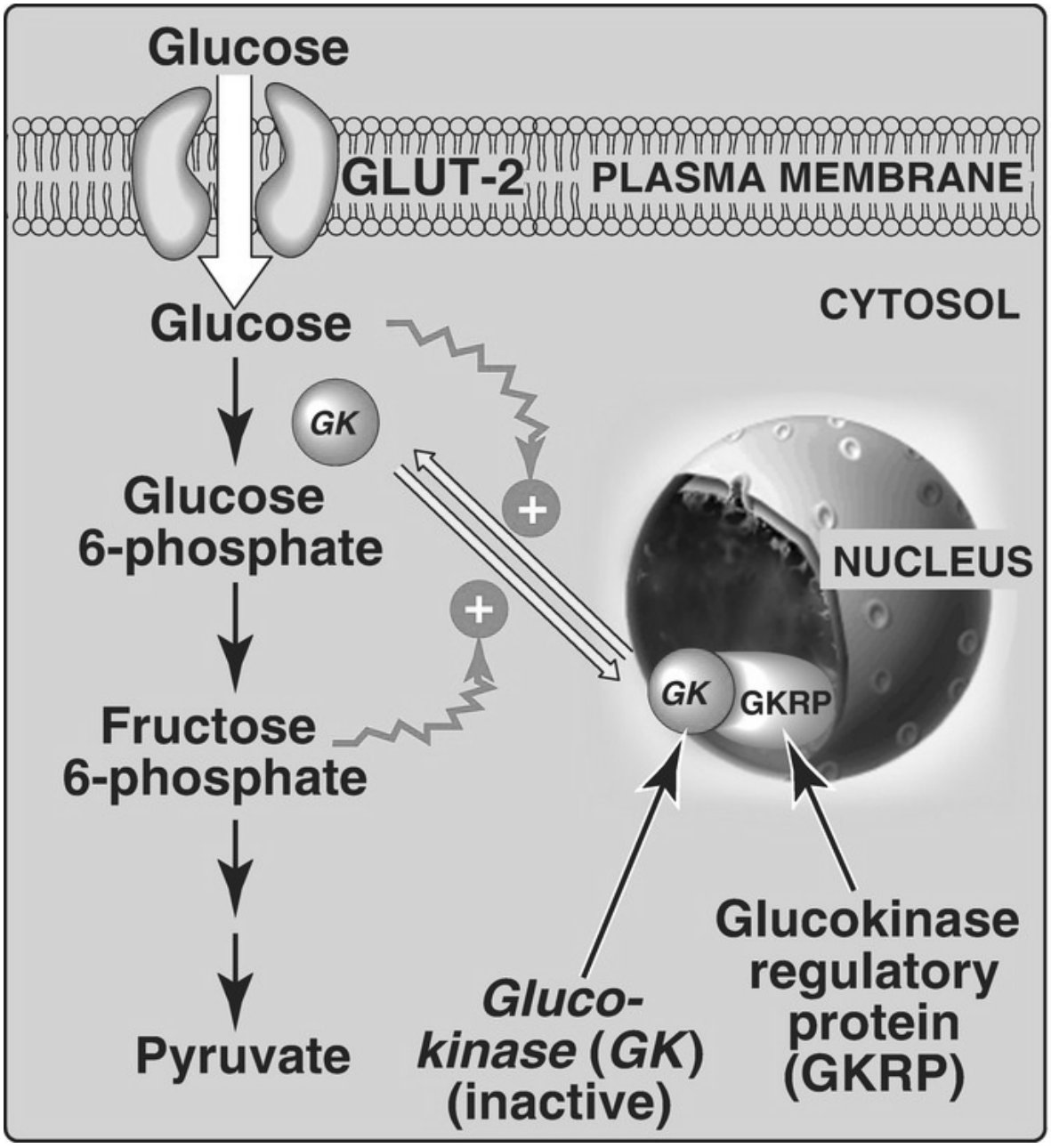

Figure 8.14 Regulation of ***glucokinase*** activity by glucokinase regulatory protein. GLUT = glucose transporter.

Glucokinase functions as a glucose sensor in 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 and hyperglycemia.

B. Glucose 6-phosphate isomerization

The isomerization of glucose 6-phosphate to fructose 6-phosphate is catalyzed by ***phosphoglucose isomerase*** (Fig. 8.15). The reaction is readily reversible and is not a rate-limiting or regulated step.

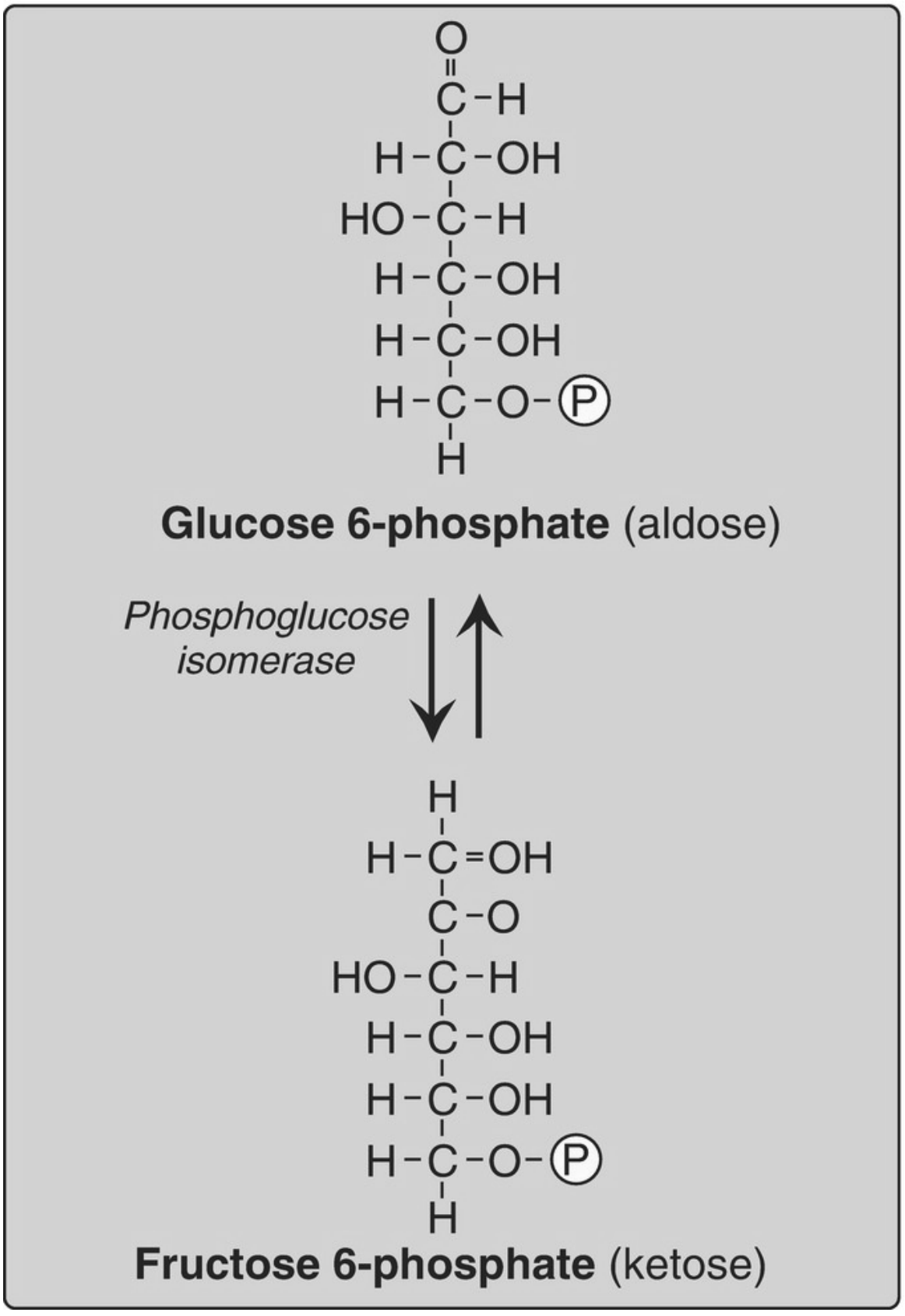
O
C-H
H-C-OH
HO-C-H
H-C-OH
H-C-OH
H-C-O-Ⓟ
H
Glucose 6-phosphate (aldose)
Phosphoglucose isomerase
H
H-C=OH
C-O
HO-C-H
H-C-OH
H-C-OH
H-C-O-Ⓟ
H
Fructose 6-phosphate (ketose)

Figure 8.15 Aldose-ketose isomerization of glucose 6-phosphate to fructose 6-phosphate. P = phosphate.

C. Fructose 6-phosphate phosphorylation

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 (Fig. 8.16). ***PFK-1*** is controlled by the available concentrations of the substrates ATP and fructose 6-phosphate as well as by other regulatory molecules.

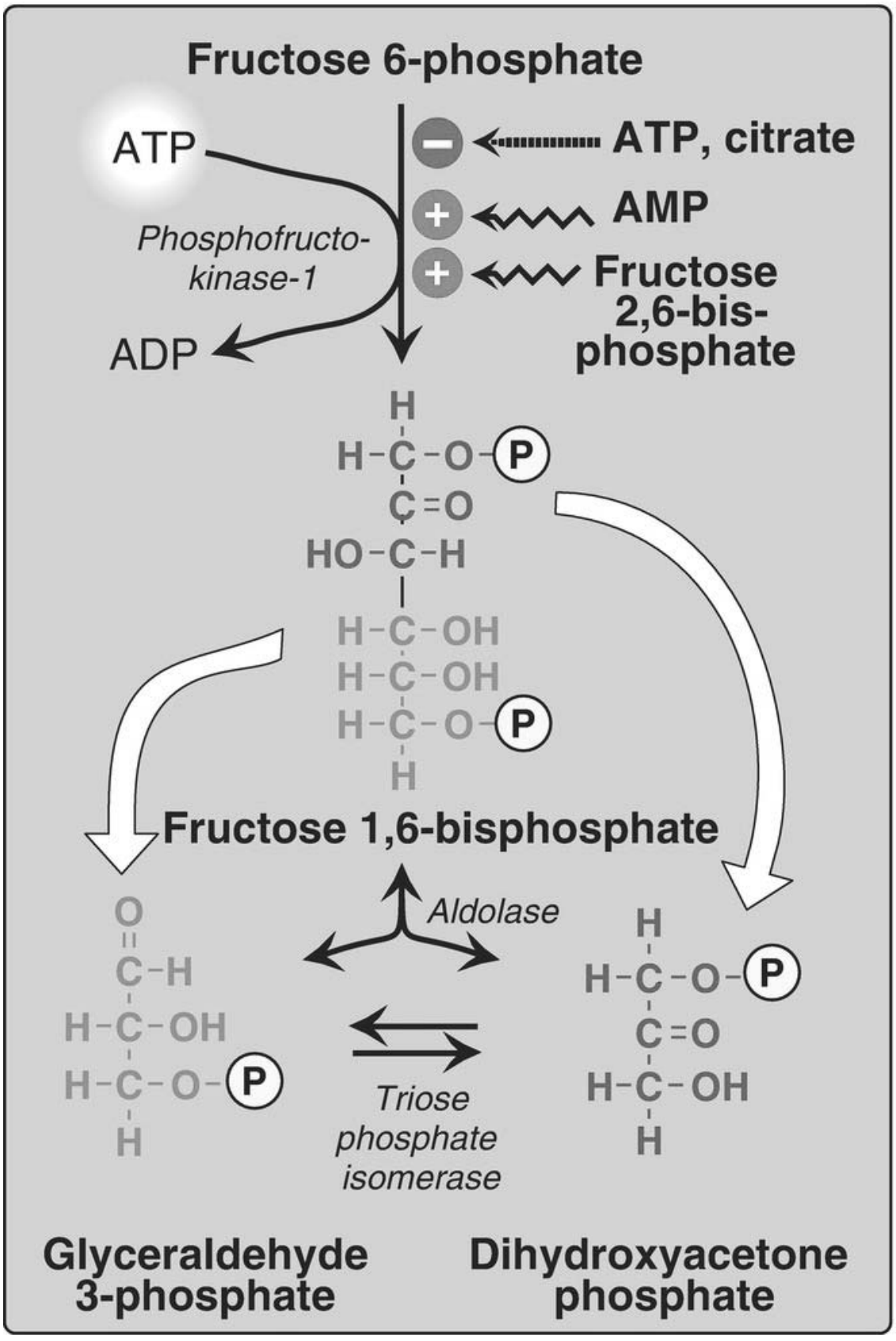
Fructose 6-phosphate
ATP
ATP, citrate
AMP
Fructose 2,6-bis-phosphate
Phosphofructo-kinase-1
ADP
Fructose 1,6-bisphosphate
Aldolase
Triose phosphate isomerase
Glyceraldehyde 3-phosphate
Dihydroxyacetone phosphate

Figure 8.16 Energy-investment phase (continued): conversion of fructose 6-phosphate to triose phosphates. P = phosphate; AMP and ADP = adenosine mono- and diphosphates.

1. Regulation by intracellular energy levels: ***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 (see p. 111), also inhibit ***PFK-1***. [Note: Inhibition by citrate favors the use of glucose for glycogen synthesis (see p. 126).] 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 Fig. 8.16) and is able to activate the enzyme even when ATP levels are high. It is formed from fructose 6-phosphate by ***phosphofructokinase-2 (PFK-2)***. Unlike ***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 to fructose 6-phosphate. In the liver isozyme, phosphorylation of ***PFK-2*** inactivates the ***kinase*** domain and activates the ***phosphatase*** domain (Fig. 8.17). The opposite is seen in the cardiac isozyme. Skeletal ***PFK-2*** is not covalently regulated. [Note: Fructose 2,6-bisphosphate is an inhibitor of ***fructose 1,6-bisphosphatase***, an enzyme of gluconeogenesis (see p. 121). The reciprocal actions of fructose 2,6-bisphosphate on glycolysis (activation) and gluconeogenesis (inhibition) insure that both pathways are not fully active at the same time, preventing a futile cycle of glucose oxidation to pyruvate followed by glucose resynthesis from pyruvate.]

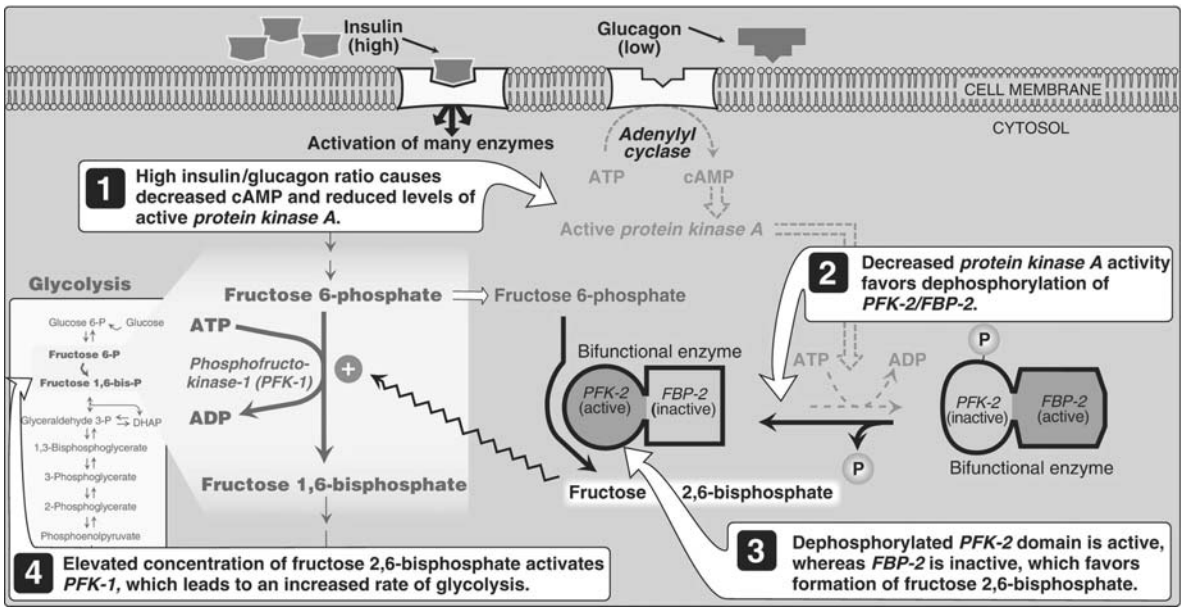

Figure 8.17 Effect of elevated insulin concentration on the intracellular concentration of fructose 2,6-bisphosphate in the liver. ***PFK-2*** = ***phosphofructokinase-2***; ***FBP-2*** = ***fructose 2,6-bisphosphatase***; AMP and ADP = adenosine mono- and diphosphates; cAMP = cyclic AMP; = phosphate.

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 hepatic fructose 2,6-bisphos- phate (***PFK-2*** is dephosphorylated) and, thus, in the rate of glycolysis (see Fig. 8.17). Therefore, fructose 2,6-bisphosphate acts as an intracellular signal of glucose abundance.

b. During fasting: By contrast, the elevated levels of glucagon and low levels of insulin that occur during fasting (see p. 327) cause a decrease in hepatic fructose 2,6-bisphosphate (***PFK-2*** is phosphorylated). This results in inhibition of glycolysis and activation of gluconeogenesis.

D. Fructose 1,6-bisphosphate cleavage

Aldolase cleaves fructose 1,6-bisphosphate to dihydroxyacetone phosphate (DHAP) and glyceraldehyde 3-phosphate (see Fig. 8.16). The reaction is reversible and not regulated. [Note: ***Aldolase B***, the hepatic isoform, also cleaves fructose 1-phosphate and functions in dietary fructose metabolism (see p. 138).]

E. Dihydroxyacetone phosphate isomerization

Triose phosphate isomerase interconverts DHAP and glyceraldehyde 3-phosphate (see Fig. 8.16). 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 (see p. 188).]

F. Glyceraldehyde 3-phosphate oxidation

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 (Fig. 8.18). [Note: Because there is a limited amount of NAD^+ in the cell, the NADH formed by the ***dehydrogenase*** reaction must be oxidized for glycolysis to continue. Two major mechanisms for oxidizing NADH to NAD^+ are the reduction of pyruvate to lactate by ***lactate dehydrogenase (LDH)*** (anaerobic, see p. 96) and the electron transport chain ([ETC] aerobic, see p. 74). Because NADH cannot cross the inner mitochondrial membrane, the ETC requires the malate-aspartate and glycerol 3-phosphate substrate shuttles to move NADH reducing equivalents into the mitochondrial matrix (see p. 79).]

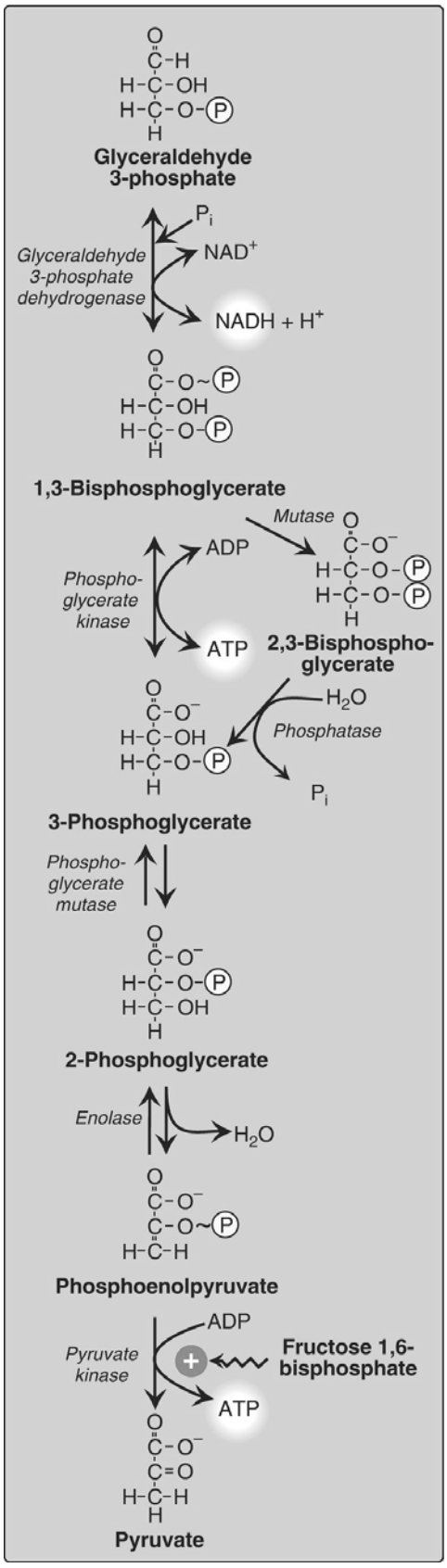
Glyceraldehyde 3-phosphate
Pi
NAD+
Glyceraldehyde 3-phosphate dehydrogenase
NADH + H+
1,3-Bisphosphoglycerate
Mutase
ADP
Phospho-glycerate kinase
ATP
2,3-Bisphospho-glycerate
H2O
Phosphatase
Pi
3-Phosphoglycerate
Phospho-glycerate mutase
2-Phosphoglycerate
Enolase
H2O
Phosphoenolpyruvate
ADP
Pyruvate kinase
Fructose 1,6-bisphosphate
ATP
Pyruvate

Figure 8.18 Energy-generating phase: conversion of glyceraldehyde 3-phosphate to pyruvate. NAD(H) = nicotinamide adenine dinucleotide; P = phosphate; P_i = inorganic phosphate; ~ = high-energy bond; ADP = adenosine diphosphate.

1. 1,3-Bisphosphoglycerate synthesis: 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. This phosphate group, linked to carbon 1 of the 1,3-BPG product by a high-energy bond (see p. 73), conserves much of the free energy (see p. 69) produced by the oxidation of glyceraldehyde 3-phosphate. This high-energy phosphate drives ATP synthesis in the next reaction of glycolysis.
2. Arsenic poisoning: The toxicity of arsenic is due primarily to the inhibition by trivalent arsenic (arsenite) of enzymes such as the ***pyruvate dehydrogenase complex (PDHC)***, which require lipoic acid as a coenzyme (see p. 110). 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 (see Fig. 8.18). 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 binding to the F_1 domain of ***ATP synthase*** (see p. 78), resulting in formation of ADP-arsenate that is rapidly hydrolyzed.]
3. 2,3-Bisphosphoglycerate synthesis in RBC: Some of the 1,3-BPG is converted to 2,3-BPG by the action of ***bisphosphoglycerate mutase*** (see Fig. 8.18). 2,3-BPG, which is found in only trace amounts in most cells, is present at high concentration in RBC and serves to increase O_2 delivery (see p. 31). 2,3-BPG is hydrolyzed by a ***phosphatase*** to 3-phosphoglycerate, which is also an intermediate in glycolysis (see Fig. 8.18). In the RBC, glycolysis is modified by inclusion of these shunt reactions.

G. 3-Phosphoglycerate synthesis and ATP production

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 Fig. 8.18). 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 reaction 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 ETC (see J. below and p. 113 for other examples).]

H. Phosphate group shift

The shift of the phosphate group from carbon 3 to carbon 2 of phosphoglycerate by ***phosphoglycerate mutase*** is freely reversible.

I. 2-Phosphoglycerate dehydration

The dehydration of 2-phosphoglycerate by ***enolase*** redistributes the energy within the substrate, forming phosphoenolpyruvate (PEP), which contains a high-energy enol phosphate (see Fig. 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 (see p. 405).]

J. Pyruvate synthesis and ATP production

The conversion of PEP to pyruvate, catalyzed by ***pyruvate kinase*** (***PK***), is 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 Fig. 8.18).

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

2. Covalent regulation in the liver: Phosphorylation by ***cAMP-dependent PKA*** leads to inactivation of the hepatic isozyme of ***PK*** (Fig. 8.19). 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 partly 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.

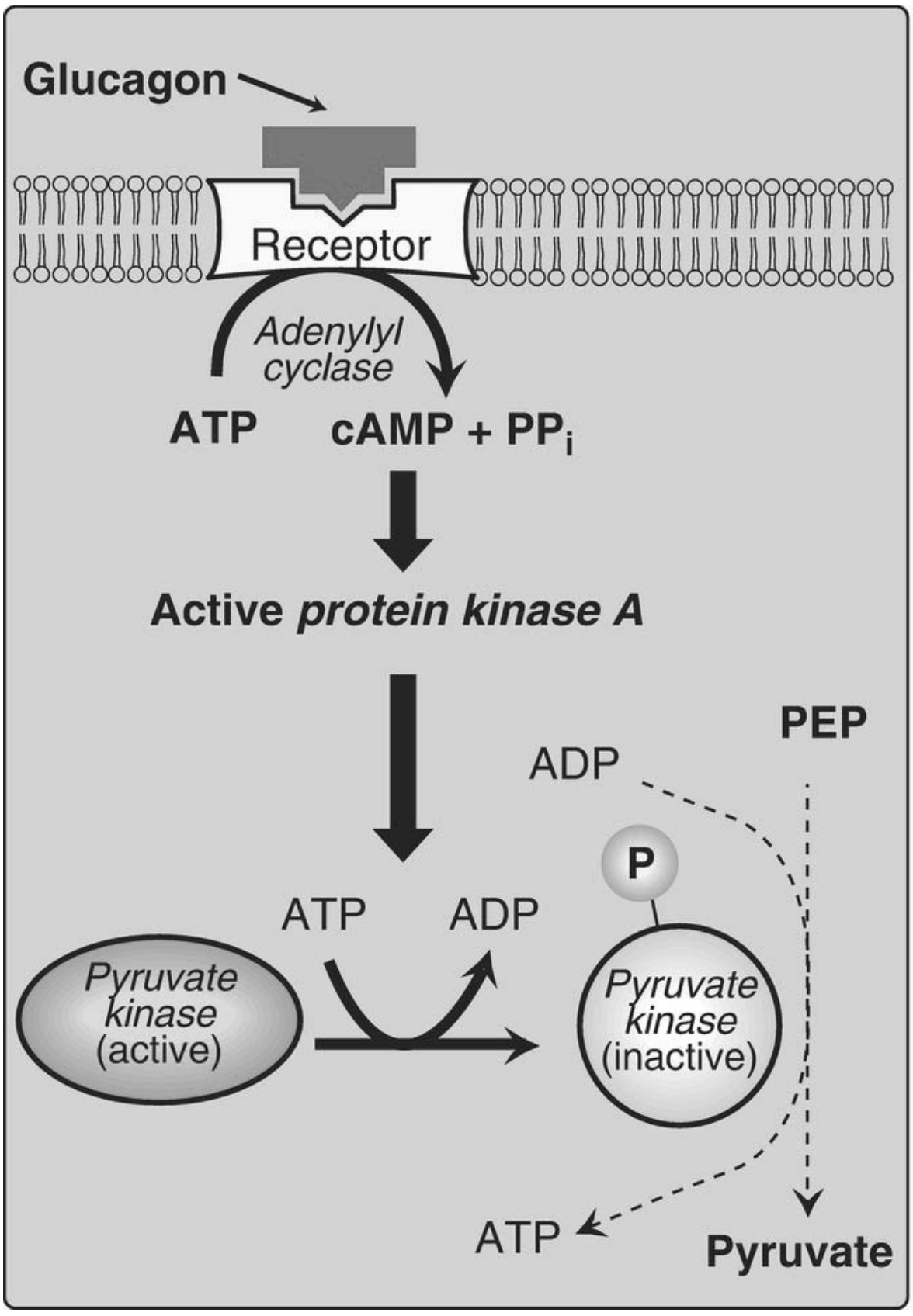
Glucagon
Receptor
Adenylyl cyclase
ATP
cAMP + PPi
Active protein kinase A
PEP
ADP
P
ATP
ADP
Pyruvate kinase (active)
Pyruvate kinase (inactive)
ATP
Pyruvate

Figure 8.19 Covalent modification of hepatic ***pyruvate kinase*** results in inactivation of the enzyme. cAMP = cyclic adenosine monophosphate; PEP = phosphoenolpyruvate; = phosphate; PP_i = pyrophosphate; ADP = adenosine diphosphate.

3. Pyruvate kinase deficiency: Because mature RBC lack mitochondria, they are completely dependent on glycolysis for ATP production. ATP is required to meet the metabolic needs of RBC 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 by substrate-level phosphorylation. The resulting alterations in the RBC membrane lead to changes in cell shape and, ultimately, to phagocytosis by cells of the mononuclear phagocyte system, particularly splenic macrophages. The premature death and lysis of RBC result in mild-to-severe nonspherocytic hemolytic anemia, with the severe form requiring regular transfusions. Among patients with rare genetic defects of glycolytic enzymes, the majority has a deficiency in ***PK***. [Note: Liver ***PK*** is encoded by the same gene as the RBC isozyme. However, liver cells show no effect because they can synthesize more ***PK*** and can also 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 RBC compensate by synthesizing increased levels of 2,3-BPG (see p. 31). Almost all individuals with ***PK*** deficiency have a mutant enzyme that shows altered kinetics or decreased stability (Fig. 8.20). Individuals heterozygous for ***PK*** deficiency have resistance to the most severe forms of malaria.

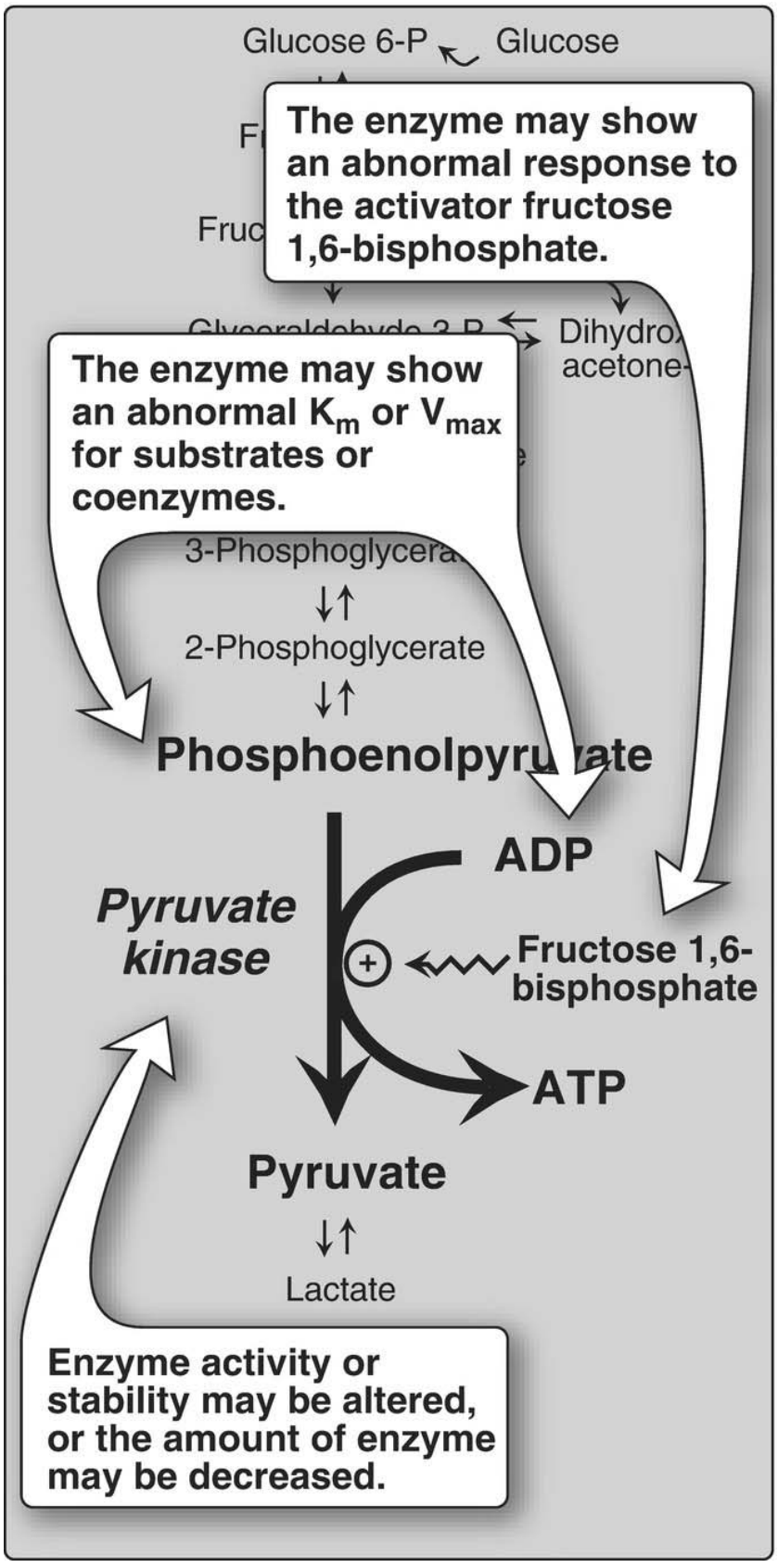
Glucose 6-P
Glucose
The enzyme may show an abnormal response to the activator fructose 1,6-bisphosphate.
Fruc
Dihydro
acetone-
The enzyme may show an abnormal K_m or V_{max} for substrates or coenzymes.
3-Phosphoglycera
2-Phosphoglycerate
Phosphoenolpyru ate
ADP
Pyruvate kinase
Fructose 1,6-bisphosphate
ATP
Pyruvate
Lactate
Enzyme activity or stability may be altered, or the amount of enzyme may be decreased.

Figure 8.20 Alterations observed with various mutant forms of ***pyruvate kinase***. K_m = Michaelis constant; V_{max} = maximal velocity; ADP = adenosine diphosphate.

The tissue-specific expression of ***PK*** in RBC and the liver results from the use of different start sites in transcription (see p. 473) of the gene that encodes the enzyme.

K. Pyruvate reduction to lactate

Lactate, formed from pyruvate by ***LDH***, is the final product of anaerobic glycolysis in eukaryotic cells (Fig. 8.21). Reduction to lactate is the major fate for pyruvate in tissues that are poorly vascularized (for example, the lens and cornea of the eye and the kidney medulla) or in RBC that lack mitochondria.

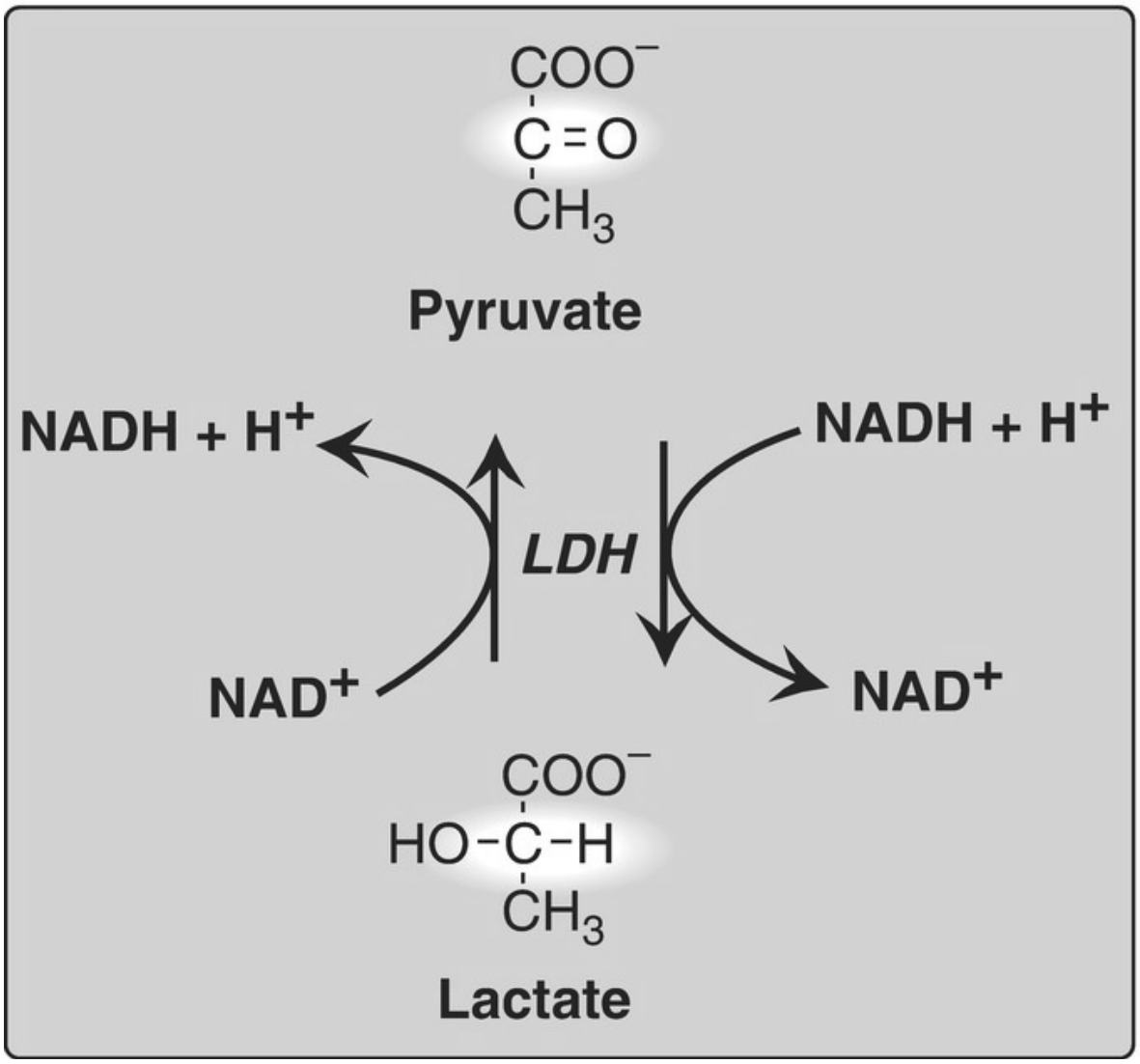

Figure 8.21 Interconversion of pyruvate and lactate by ***lactate dehydrogenase*** (***LDH***). NAD(H) = nicotinamide adenine dinucleotide.

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, see p. 113) exceeds the oxidative capacity of the ETC. This results in an elevated $NADH/NAD^+$ ratio, favoring reduction of pyruvate to lactate by ***LDH***. 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 (see p. 118).

2. Lactate utilization: The direction of the ***LDH*** reaction depends on the relative intracellular concentrations of pyruvate and lactate and on the ratio of NADH/NAD^+. For example, in the liver and heart, this ratio is lower than in exercising muscle. Consequently, the liver and heart oxidize lactate (obtained from the blood) to pyruvate. In the liver, pyruvate is either converted to glucose by gluconeogenesis or converted to acetyl CoA that is oxidized in the TCA cycle. Heart muscle exclusively oxidizes lactate to carbon dioxide and water 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 with myocardial infarction, pulmonary embolism, and uncontrolled hemorrhage, or when an individual is in shock. The failure to bring adequate amounts of O_2 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 lifesaving during the period required to reestablish adequate blood flow to the tissues.] The additional O_2 required to recover from a period when O_2 availability has been inadequate is termed the O_2 debt. [Note: The O_2 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 O_2 debt in patients and the monitoring of their recovery.]

L. Energy yield from glycolysis

Despite the production of some ATP by substrate-level phosphorylation during glycolysis, the end product, pyruvate or lactate, still contains most of the energy originally contained in glucose. The TCA cycle is required to release that energy completely (see p. 109).

1. Anaerobic glycolysis: A net of two molecules of ATP are generated for each molecule of glucose converted to two molecules of lactate (Fig. 8.22). There is no net production or consumption of NADH.

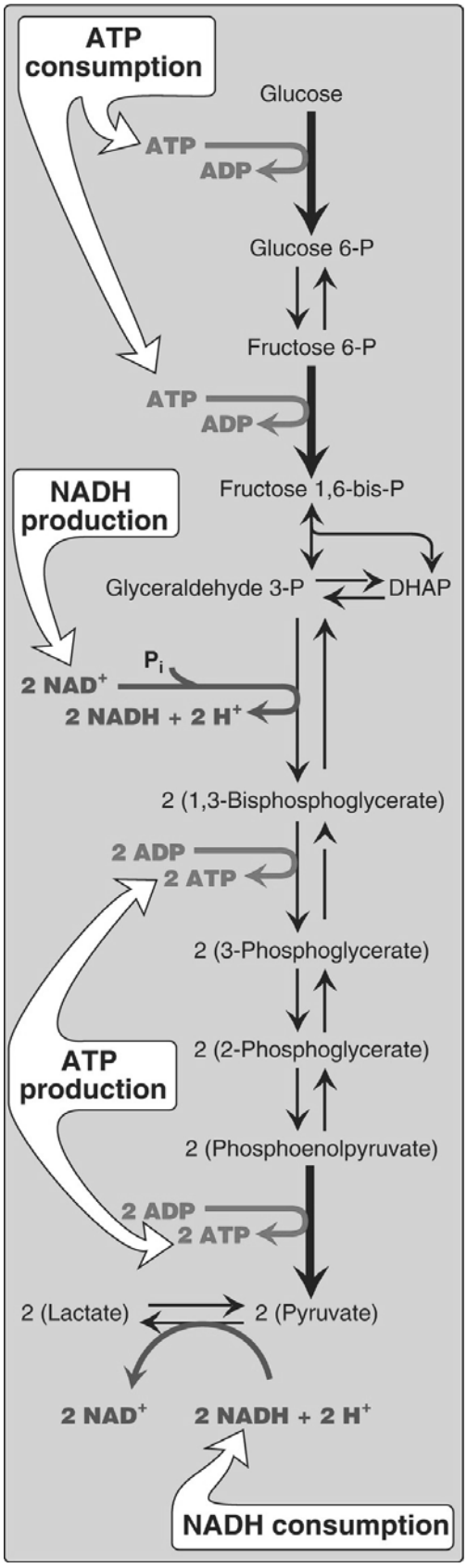
ATP consumption
Glucose
ATP
ADP
Glucose 6-P
Fructose 6-P
ATP
ADP
NADH production
Fructose 1,6-bis-P
Glyceraldehyde 3-P
DHAP
Pi
2 NAD+
2 NADH + 2 H+
2 (1,3-Bisphosphoglycerate)
2 ADP
2 ATP
2 (3-Phosphoglycerate)
2 (2-Phosphoglycerate)
ATP production
2 (Phosphoenolpyruvate)
2 ADP
2 ATP
2 (Lactate)
2 (Pyruvate)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

Figure 8.22 Summary of anaerobic glycolysis. Reactions involving the production or consumption of ATP or nicotinamide adenine dinucleotide (NADH) are indicated. The three irreversible reactions of glycolysis are shown with thick arrows. DHAP = dihydroxyacetone phosphate; ADP = adenosine diphosphate; P = phosphate.

2. Aerobic glycolysis: The generation 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 ETC, producing three ATP for each NADH molecule entering the chain (see p. 77). [Note: NADH cannot cross the inner mitochondrial membrane, and substrate shuttles are required (see p. 79).]

VI. HORMONAL REGULATION

Regulation of the activity of the irreversible glycolytic enzymes by allosteric activation/inhibition or covalent phosphorylation/dephosphorylation is short term (that is, the effects occur over minutes or hours). Superimposed on these effects on the activity of preexisting enzyme molecules are the long-term hormonal effects on the number of new enzyme molecules. These hormonal effects can result in 10- to 20-fold increases in enzyme synthesis that typically occur over hours to days. Regular consumption of meals rich in carbohydrate or administration of insulin initiates an increase in the amount of ***glucokinas***e, ***PFK-1***, and ***PK*** in the liver (Fig. 8.23). The change reflects an increase in gene transcription, resulting in increased enzyme synthesis. Increased availability of these three enzymes favors the conversion of glucose to pyruvate, a characteristic of the absorptive state (see p. 321). [Note: The transcriptional effects of insulin and carbohydrate (specifically glucose) are mediated by the transcription factors sterol regulatory element–binding protein-1c and carbohydrate response element–binding protein, respectively. These factors also regulate transcription of genes involved in fatty acid synthesis (see p. 184).] Conversely, gene expression of the three enzymes is decreased when plasma glucagon is high and insulin is low (for example, as seen in fasting or diabetes).

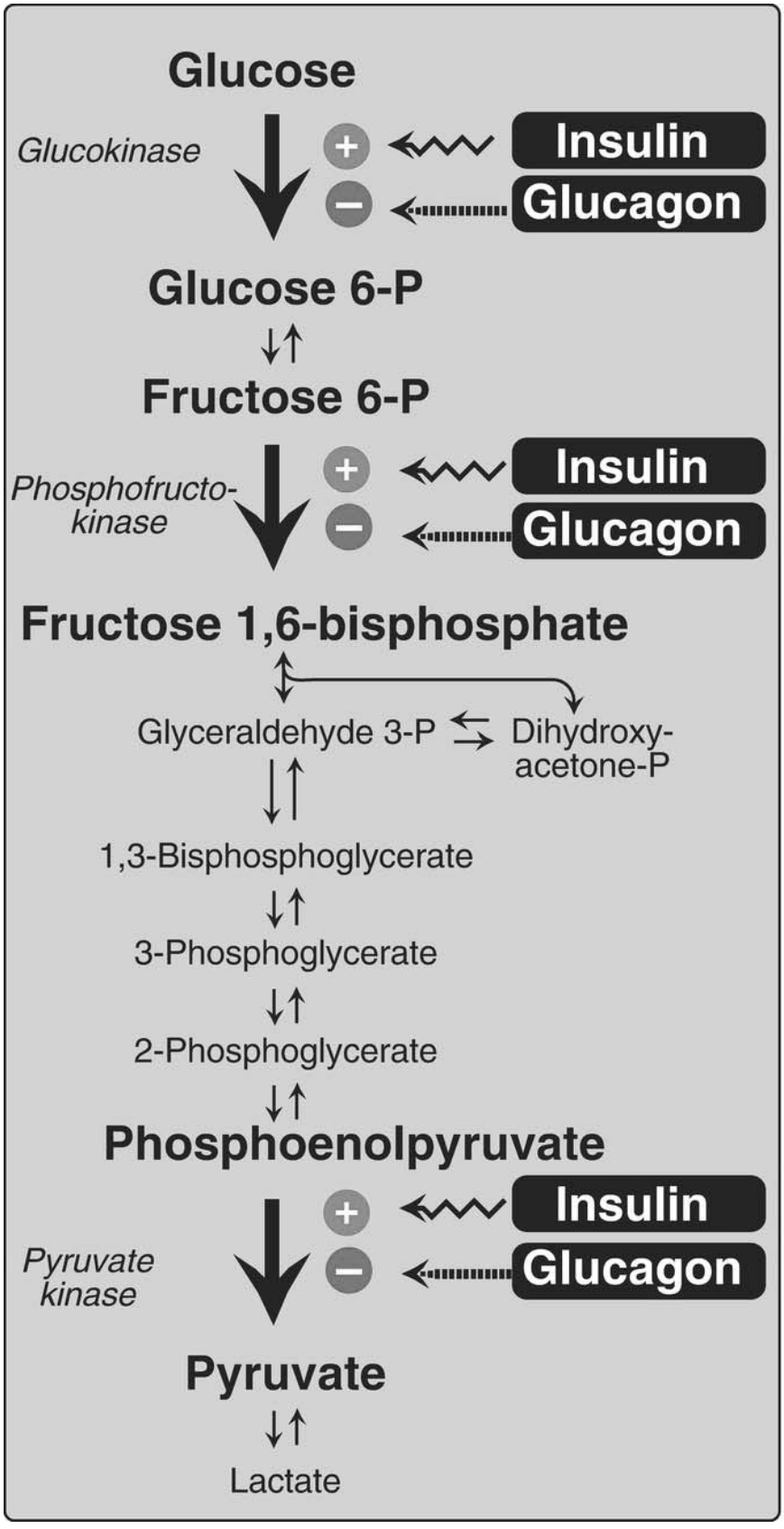
Glucose
Glucokinase
+
−
Insulin
Glucagon
Glucose 6-P
Fructose 6-P
Phosphofructo-kinase
+
−
Insulin
Glucagon
Fructose 1,6-bisphosphate
Glyceraldehyde 3-P
Dihydroxy-acetone-P
1,3-Bisphosphoglycerate
3-Phosphoglycerate
2-Phosphoglycerate
Phosphoenolpyruvate
Pyruvate kinase
+
−
Insulin
Glucagon
Pyruvate
Lactate

Figure 8.23 Effect of insulin and glucagon on the expression of key enzymes of glycolysis in the liver. P = phosphate.

VII. ALTERNATE FATES OF PYRUVATE

Pyruvate can be metabolized to products other than lactate.

A. Oxidative decarboxylation to acetyl CoA

Oxidative decarboxylation of pyruvate by the ***PDHC*** is an important pathway in tissues with a high oxidative capacity such as cardiac muscle (Fig. 8.24). ***PDHC*** irreversibly converts pyruvate, the end product of aerobic glycolysis, into acetyl CoA, a TCA cycle substrate (see p. 109) and the carbon source for fatty acid synthesis (see p. 183).

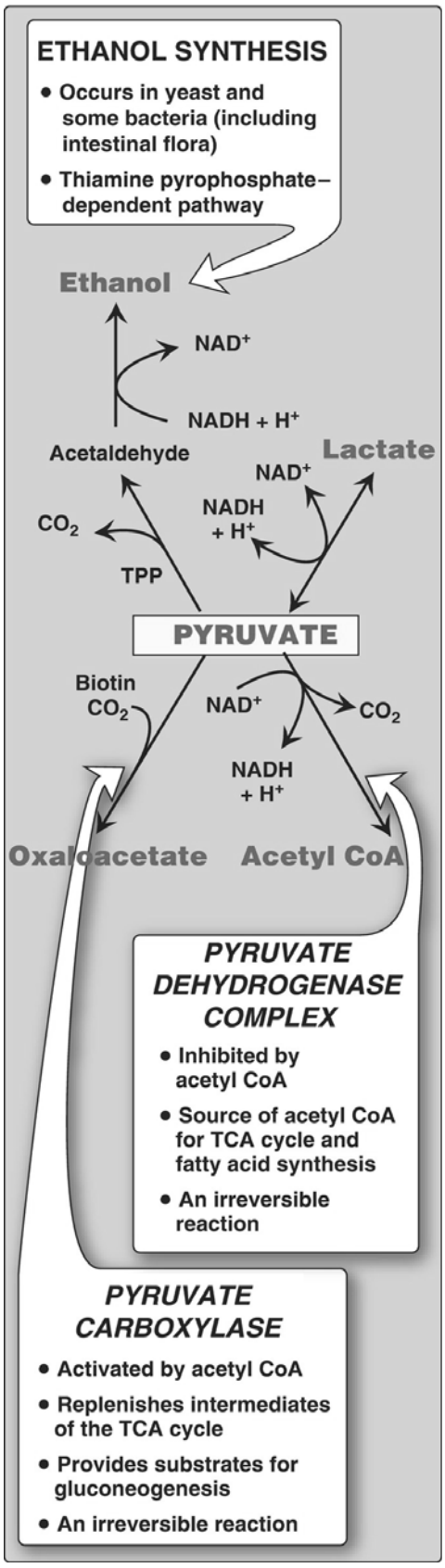
ETHANOL SYNTHESIS
• Occurs in yeast and some bacteria (including intestinal flora)
• Thiamine pyrophosphate–dependent pathway
Ethanol
NAD+
NADH + H+
Acetaldehyde
Lactate
NAD+
NADH + H+
CO2
TPP
PYRUVATE
Biotin
CO2
NAD+
CO2
NADH + H+
Oxaloacetate
Acetyl CoA
PYRUVATE DEHYDROGENASE COMPLEX
• Inhibited by acetyl CoA
• Source of acetyl CoA for TCA cycle 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

Figure 8.24 Summary of the metabolic fates of pyruvate. TPP = thiamine pyrophosphate. TCA = tricarboxylic acid; NAD(H) = nicotinamide adenine dinucleotide; CoA = coenzyme A; CO_2 = carbon dioxide.

B. Carboxylation to oxaloacetate

Carboxylation of pyruvate to oxaloacetate by ***pyruvate carboxylase*** is a biotin-dependent reaction (see Fig. 8.24). This irreversible reaction is important because it replenishes the TCA cycle intermediate and provides substrate for gluconeogenesis (see p. 118).

C. Reduction to ethanol (microorganisms)

The reduction of pyruvate to ethanol occurs by the two reactions summarized in Figure 8.24. The decarboxylation of pyruvate to acetaldehyde by thiamine-requiring ***pyruvate decarboxylase*** occurs in yeast and certain other microorganisms but not in humans.