

Introduction to Carbohydrates

7

I. OVERVIEW

Carbohydrates are the most abundant organic molecules in nature. They have a wide range of functions, including providing a significant fraction of the dietary calories for most organisms, acting as a storage form of energy in the body, and serving as cell membrane components that mediate some forms of intercellular communication. Carbohydrates also serve as a structural component of many organisms, including the cell walls of bacteria, the exoskeleton of many insects, and the fibrous cellulose of plants. The empiric formula for many of the simpler carbohydrates is $(\text{CH}_2\text{O})_n$, hence the name “hydrate of carbon.”

II. CLASSIFICATION AND STRUCTURE OF CARBOHYDRATES

Monosaccharides (simple sugars) can be classified according to the number of carbon atoms they contain. Examples of some monosaccharides commonly found in humans are listed in Figure 7.1. Carbohydrates with an aldehyde as their most oxidized functional group are called aldoses, whereas those with a keto as their most oxidized functional group are called ketoses (Figure 7.2). For example, glyceraldehyde is an aldose, whereas dihydroxyacetone is a ketose. Carbohydrates that have a free carbonyl group have the suffix *-ose*. [Note: Ketoses (with some exceptions, for example, fructose) have an additional two letters in their suffix: *-ulose*, for example, xylulose.] Monosaccharides can be linked by glycosidic bonds to create larger structures (Figure 7.3). Disaccharides contain two monosaccharide units, oligosaccharides contain from three to about ten monosaccharide units, whereas polysaccharides contain more than ten monosaccharide units, and can be hundreds of sugar units in length.

A. Isomers and epimers

Compounds that have the same chemical formula but have different structures are called isomers. For example, fructose, glucose, mannose, and galactose are all isomers of each other, having the same chemical formula, $\text{C}_6\text{H}_{12}\text{O}_6$. Carbohydrate isomers that differ in configuration around only one specific carbon atom (with the exception of the carbonyl carbon, see “anomers” below) are defined as epimers of each other. For example, glucose and galactose are C-4

Generic names	Examples
3 Carbons: trioses	Glyceraldehyde
4 Carbons: tetroses	Erythrose
5 Carbons: pentoses	Ribose
6 Carbons: hexoses	Glucose
7 Carbons: heptoses	Sedoheptulose
9 Carbons: nonoses	Neuraminic acid

Figure 7.1

Examples of monosaccharides found in humans, classified according to the number of carbons they contain.

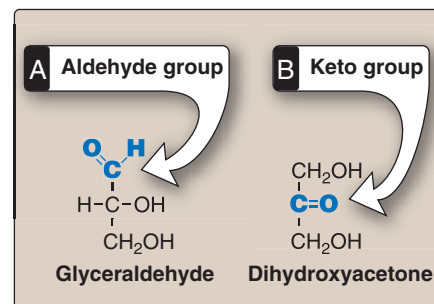
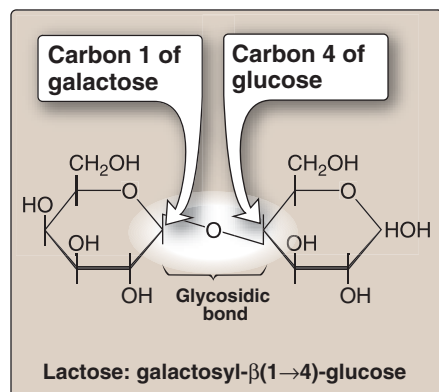
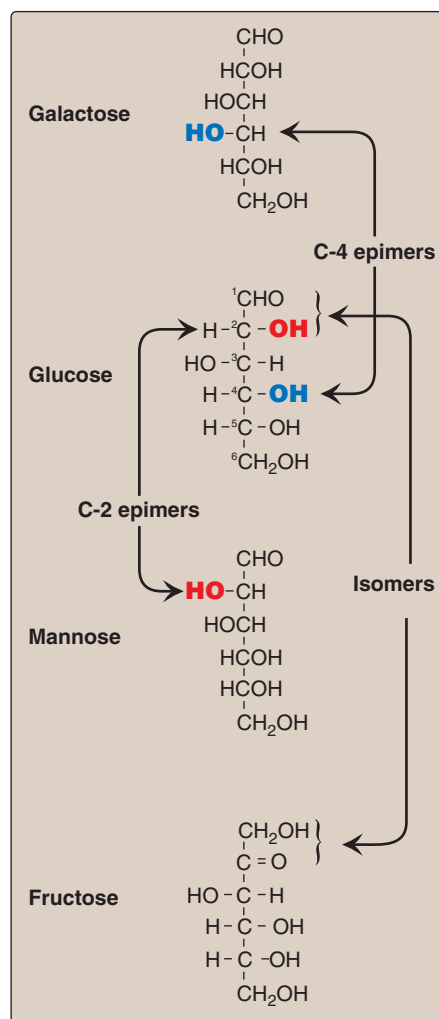


Figure 7.2

Examples of an aldose (A) and a ketose (B) sugar.

**Figure 7.3**

A glycosidic bond between two hexoses producing a disaccharide.

**Figure 7.4**

C-2 and C-4 epimers and an isomer of glucose.

epimers—their structures differ only in the position of the -OH group at carbon 4. [Note: The carbons in sugars are numbered beginning at the end that contains the carbonyl carbon—that is, the aldehyde or keto group (Figure 7.4).] Glucose and mannose are C-2 epimers. However, galactose and mannose are NOT epimers—they differ in the position of -OH groups at two carbons (2 and 4) and are, therefore, defined only as isomers (see Figure 7.4).

B. Enantiomers

A special type of isomerism is found in the pairs of structures that are mirror images of each other. These mirror images are called enantiomers, and the two members of the pair are designated as a D- and an L-sugar (Figure 7.5). The vast majority of the sugars in humans are D-sugars. In the D isomeric form, the -OH group on the asymmetric carbon (a carbon linked to four different atoms or groups) farthest from the carbonyl carbon is on the right, whereas in the L-isomer it is on the left. Enzymes known as *racemases* are able to interconvert D- and L-isomers.

C. Cyclization of monosaccharides

Less than 1% of each of the monosaccharides with five or more carbons exists in the open-chain (acyclic) form. Rather, they are predominantly found in a ring (cyclic) form, in which the aldehyde (or keto) group has reacted with an alcohol group on the same sugar, making the carbonyl carbon (carbon 1 for an aldose or carbon 2 for a ketose) asymmetric. [Note: Pyranose refers to a six-membered ring consisting of five carbons and one oxygen, for example, glucopyranose (Figure 7.6), whereas furanose denotes a five-membered ring with four carbons and one oxygen.]

1. Anomeric carbon: Cyclization creates an anomeric carbon (the former carbonyl carbon), generating the α and β configurations of the sugar, for example, α -D-glucopyranose and β -D-glucopyranose (see Figure 7.6). These two sugars are both glucose but are anomers of each other. [Note: In the α configuration, the OH on the anomeric C projects to the same side as the ring in a modified Fischer projection formula (Figure 7.6A), and is trans to the CH_2OH group in a Haworth projection formula (Figure 7.6B). Because the α and β forms are not mirror images, they are referred to as diastereomers.] Enzymes are able to distinguish between these two structures and use one or the other preferentially. For example, glycogen is synthesized from α -D-glucopyranose, whereas cellulose is synthesized from β -D-glucopyranose. The cyclic α and β anomers of a sugar in solution are in equilibrium with each other, and can be spontaneously interconverted (a process called mutarotation, see Figure 7.6).

2. Reducing sugars: If the hydroxyl group on the anomeric carbon of a cyclized sugar is not linked to another compound by a glycosidic bond, the ring can open. The sugar can act as a reducing agent, and is termed a reducing sugar. Such sugars can react with chromogenic agents (for example, Benedict's reagent or Fehling's solution) causing the reagent to be reduced and colored, with the aldehyde group of the acyclic sugar becoming oxidized. [Note:

Only the state of the oxygen in the aldehyde group determines if the sugar is reducing or nonreducing.]

A colorimetric test can detect a reducing sugar in urine. A positive result is indicative of an underlying pathology because sugars are not normally present in urine, and can be followed up by more specific tests to identify the reducing sugar.

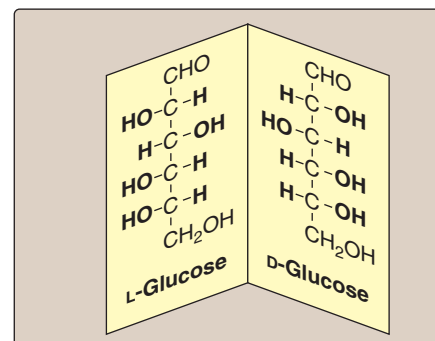


Figure 7.5

Enantiomers (mirror images) of glucose.

D. Joining of monosaccharides

Monosaccharides can be joined to form disaccharides, oligosaccharides, and polysaccharides. Important disaccharides include lactose (galactose + glucose), sucrose (glucose + fructose), and maltose (glucose + glucose). Important polysaccharides include branched glycogen (from animal sources) and starch (plant sources) and unbranched cellulose (plant sources); each is a polymer of glucose. The bonds that link sugars are called glycosidic bonds. These are formed by enzymes known as *glycosyltransferases* that use nucleotide sugars such as UDP-glucose as substrates.

- 1. Naming glycosidic bonds:** Glycosidic bonds between sugars are named according to the numbers of the connected carbons, and with regard to the position of the anomeric hydroxyl group of the sugar involved in the bond. If this anomeric hydroxyl is in the α configuration, the linkage is an α -bond. If it is in the β configuration, the linkage is a β -bond. Lactose, for example, is synthesized by forming a glycosidic bond between carbon 1 of β -galactose and carbon 4 of glucose. The linkage is, therefore, a $\beta(1\rightarrow4)$ glycosidic bond (see Figure 7.3). [Note: Because the anomeric end of the glucose residue is not involved in the glycosidic linkage it (and, therefore, lactose) remains a reducing sugar.]

E. Complex carbohydrates

Carbohydrates can be attached by glycosidic bonds to non-carbohydrate structures, including purine and pyrimidine bases (found in

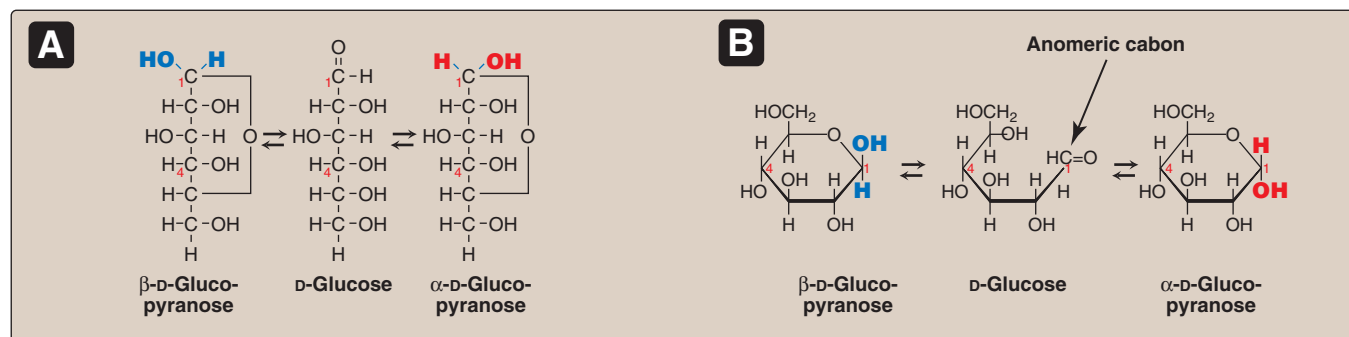
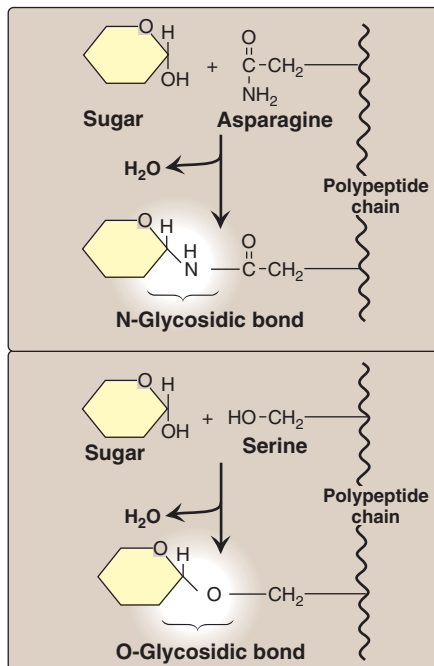
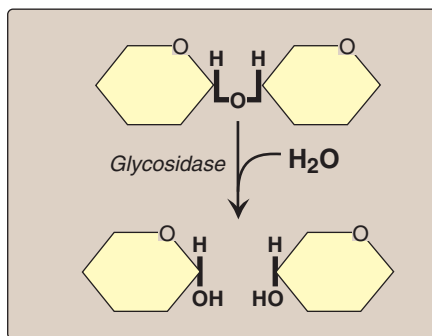


Figure 7.6

A The interconversion (mutarotation) of the α and β anomeric forms of glucose shown as modified Fischer projection formulas. B. The interconversion shown as Haworth projection formulas. Carbon 1 is the anomeric carbon. [Note: Glucose is a reducing sugar.]

**Figure 7.7**

Glycosides: examples of N- and O-glycosidic bonds.

**Figure 7.8**

Hydrolysis of a glycosidic bond.

nucleic acids), aromatic rings (such as those found in steroids and bilirubin), proteins (found in glycoproteins and proteoglycans), and lipids (found in glycolipids).

- 1. N- and O-glycosides:** If the group on the non-carbohydrate molecule to which the sugar is attached is an -NH_2 group, the structure is an N-glycoside and the bond is called an N-glycosidic link. If the group is an -OH , the structure is an O-glycoside, and the bond is an O-glycosidic link (Figure 7.7). [Note: All sugar–sugar glycosidic bonds are O-type linkages.]

III. DIGESTION OF DIETARY CARBOHYDRATES

The principal sites of dietary carbohydrate digestion are the mouth and intestinal lumen. This digestion is rapid and is catalyzed by enzymes known as *glycoside hydrolases* (*glycosidases*) that hydrolyze glycosidic bonds. Because there is little monosaccharide present in diets of mixed animal and plant origin, the enzymes are primarily *endoglycosidases* that hydrolyze polysaccharides and oligosaccharides, and *disaccharidases* that hydrolyze tri- and disaccharides into their reducing sugar components (Figure 7.8). *Glycosidases* are usually specific for the structure and configuration of the glycosyl residue to be removed, as well as for the type of bond to be broken. The final products of carbohydrate digestion are the monosaccharides, glucose, galactose and fructose, which are absorbed by cells of the small intestine.

A. Digestion of carbohydrates begins in the mouth

The major dietary polysaccharides are of plant (starch, composed of amylose and amylopectin) and animal (glycogen) origin. During mastication, salivary α -*amylase* acts briefly on dietary starch and glycogen, hydrolyzing random $\alpha(1\rightarrow4)$ bonds. [Note: There are both $\alpha(1\rightarrow4)$ - and $\beta(1\rightarrow4)$ -*endoglucosidases* in nature, but humans do not produce the latter. Therefore, we are unable to digest cellulose—a carbohydrate of plant origin containing $\beta(1\rightarrow4)$ glycosidic bonds between glucose residues.] Because branched amylopectin and glycogen also contain $\alpha(1\rightarrow6)$ bonds, which α -*amylase* cannot hydrolyze, the digest resulting from its action contains a mixture of short, branched and unbranched oligosaccharides known as dextrins (Figure 7.9) [Note: Disaccharides are also present as they, too, are resistant to *amylase*.] Carbohydrate digestion halts temporarily in the stomach, because the high acidity inactivates salivary α -*amylase*.

B. Further digestion of carbohydrates by pancreatic enzymes occurs in the small intestine

When the acidic stomach contents reach the small intestine, they are neutralized by bicarbonate secreted by the pancreas, and pancreatic α -*amylase* continues the process of starch digestion.

C. Final carbohydrate digestion by enzymes synthesized by the intestinal mucosal cells

The final digestive processes occur primarily at the mucosal lining of the upper jejunum, and include the action of several *disacchari-*

dases (Figure 7.10). For example, *isomaltase* cleaves the $\alpha(1\rightarrow6)$ bond in isomaltose and *maltase* cleaves maltose and maltotriose, each producing glucose, *sucrase* cleaves sucrose producing glucose and fructose, and *lactase* (β -galactosidase) cleaves lactose producing galactose and glucose. Trehalose, an $\alpha(1\rightarrow1)$ disaccharide of glucose found in mushrooms and other fungi, is cleaved by *trehalase*. These enzymes are secreted through, and remain associated with, the luminal side of the brush border membranes of the intestinal mucosal cells. [Note: The substrates for *isomaltase* are broader than its name suggests, as it hydrolyzes the majority of maltose.]

Sucrase and *isomaltase* are enzymic activities of a single protein which is cleaved into two functional subunits that remain associated in the cell membrane, forming the *sucrase-isomaltase* complex. *Maltase* forms a similar complex with an *exoglucosidase* (*glucoamylase*) that cleaves $\alpha(1\rightarrow4)$ glycosidic bonds in dextrins.

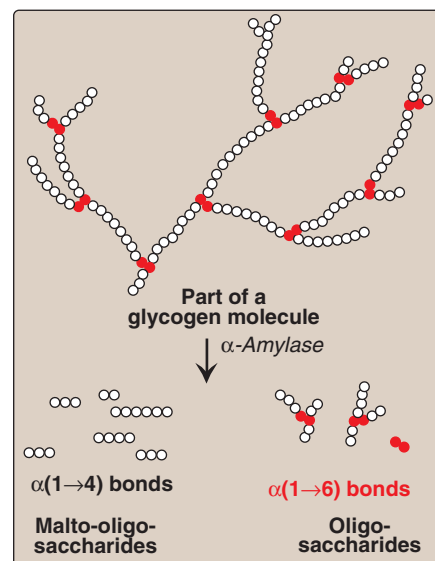


Figure 7.9

Degradation of dietary glycogen by salivary or pancreatic α -amylase.

D. Absorption of monosaccharides by intestinal mucosal cells

The duodenum and upper jejunum absorb the bulk of the dietary sugars. However, different sugars have different mechanisms of absorption. For example, galactose and glucose are transported into the mucosal cells by an active, energy-requiring process that requires a concurrent uptake of sodium ions; the transport protein is the sodium-dependent glucose cotransporter 1 (SGLT-1). Fructose uptake requires a sodium-independent monosaccharide transporter (GLUT-5) for its absorption. All three monosaccharides are transported from the intestinal mucosal cell into the portal circulation by yet another transporter, GLUT-2. (See p. 97 for a discussion of these transporters.)

E. Abnormal degradation of disaccharides

The overall process of carbohydrate digestion and absorption is so efficient in healthy individuals that ordinarily all digestible dietary carbohydrate is absorbed by the time the ingested material reaches the lower jejunum. However, because it is monosaccharides that are absorbed, any defect in a specific *disaccharidase* activity of the intestinal mucosa causes the passage of undigested carbohydrate into the large intestine. As a consequence of the presence of this osmotically active material, water is drawn from the mucosa into the large intestine, causing osmotic diarrhea. This is reinforced by the bacterial fermentation of the remaining carbohydrate to two- and three-carbon compounds (which are also osmotically active) plus large volumes of CO_2 and H_2 gas, causing abdominal cramps, diarrhea, and flatulence.

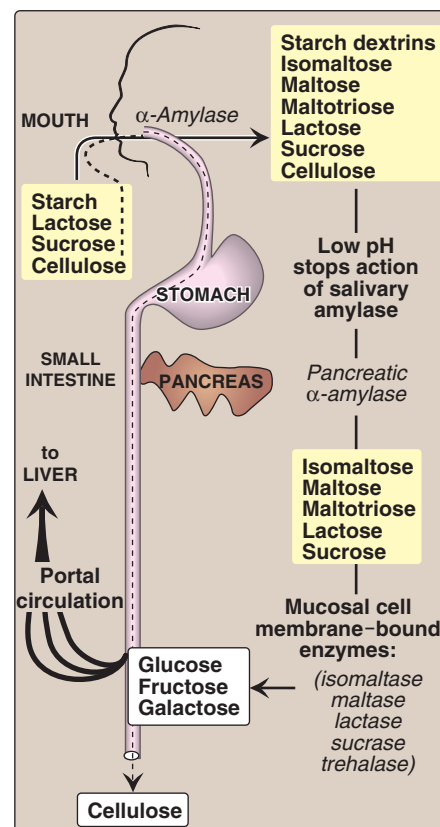


Figure 7.10

Digestion of carbohydrates. [Note: Indigestible cellulose enters the colon and is excreted.]

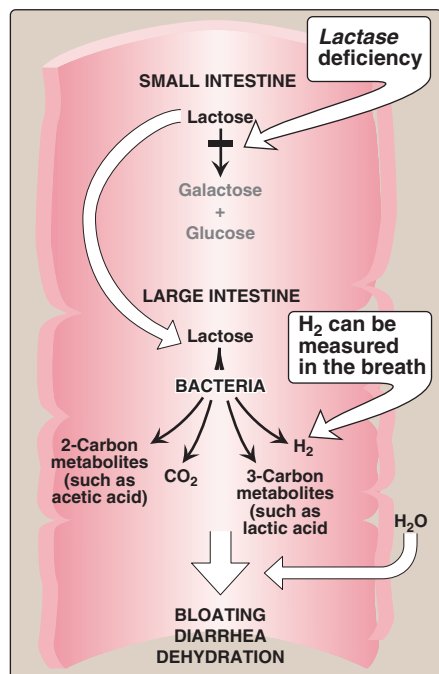


Figure 7.11

Abnormal lactose metabolism.

1. **Digestive enzyme deficiencies:** Genetic deficiencies of the individual *disaccharidases* result in disaccharide intolerance. Alterations in disaccharide degradation can also be caused by a variety of intestinal diseases, malnutrition, or drugs that injure the mucosa of the small intestine. For example, brush border enzymes are rapidly lost in normal individuals with severe diarrhea, causing a temporary, acquired enzyme deficiency. Thus, patients suffering or recovering from such a disorder cannot drink or eat significant amounts of dairy products or sucrose without exacerbating the diarrhea.
2. **Lactose intolerance:** More than three quarters of the world's adults are lactose intolerant (Figure 7.11). This is particularly manifested in certain populations. For example, up to 90% of adults of African or Asian descent are *lactase*-deficient and, therefore, are less able to metabolize lactose than individuals of Northern European origin. The age-dependent loss of *lactase* activity represents a reduction in the amount of enzyme rather than a modified inactive enzyme. It is thought to be caused by small variations in the DNA sequence of a region on chromosome 2 that controls expression of the gene for *lactase*, also on chromosome 2. Treatment for this disorder is to reduce consumption of milk while eating yogurts and cheeses, as well as green vegetables such as broccoli, to ensure adequate calcium intake; to use *lactase*-treated products; or to take *lactase* in pill form prior to eating. [Note: Because the loss of *lactase* is the norm for most of the world's adults, use of the term "adult hypolactasia" for lactose intolerance is becoming more common.]
3. **Sucrase-isomaltase complex deficiency:** This deficiency results in an intolerance of ingested sucrose. The disorder is found in about 10% of the Inuit people of Greenland and Canada, whereas 2% of North Americans are heterozygous for the deficiency. Treatment includes the dietary restriction of sucrose, and enzyme replacement therapy.
4. **Diagnosis:** Identification of a specific enzyme deficiency can be obtained by performing oral tolerance tests with the individual disaccharides. Measurement of hydrogen gas in the breath is a reliable test for determining the amount of ingested carbohydrate not absorbed by the body, but which is metabolized instead by the intestinal flora (see Figure 7.11).

IV. CHAPTER SUMMARY

Monosaccharides (simple sugars, Figure 7.12) containing an aldehyde group are called **aldoses** and those with a keto group are called **ketoses**. **Disaccharides**, **oligosaccharides**, and **polysaccharides** consist of monosaccharides linked by **glycosidic bonds**. Compounds with the same chemical formula are called **isomers**. If two monosaccharide isomers differ in configuration around one specific carbon atom (with the exception of the carbonyl carbon), they are defined as **epimers** of each other. If a pair of sugars are mirror images (**enantiomers**), the two members of the pair are designated as **D-** and **L-sugars**. When a

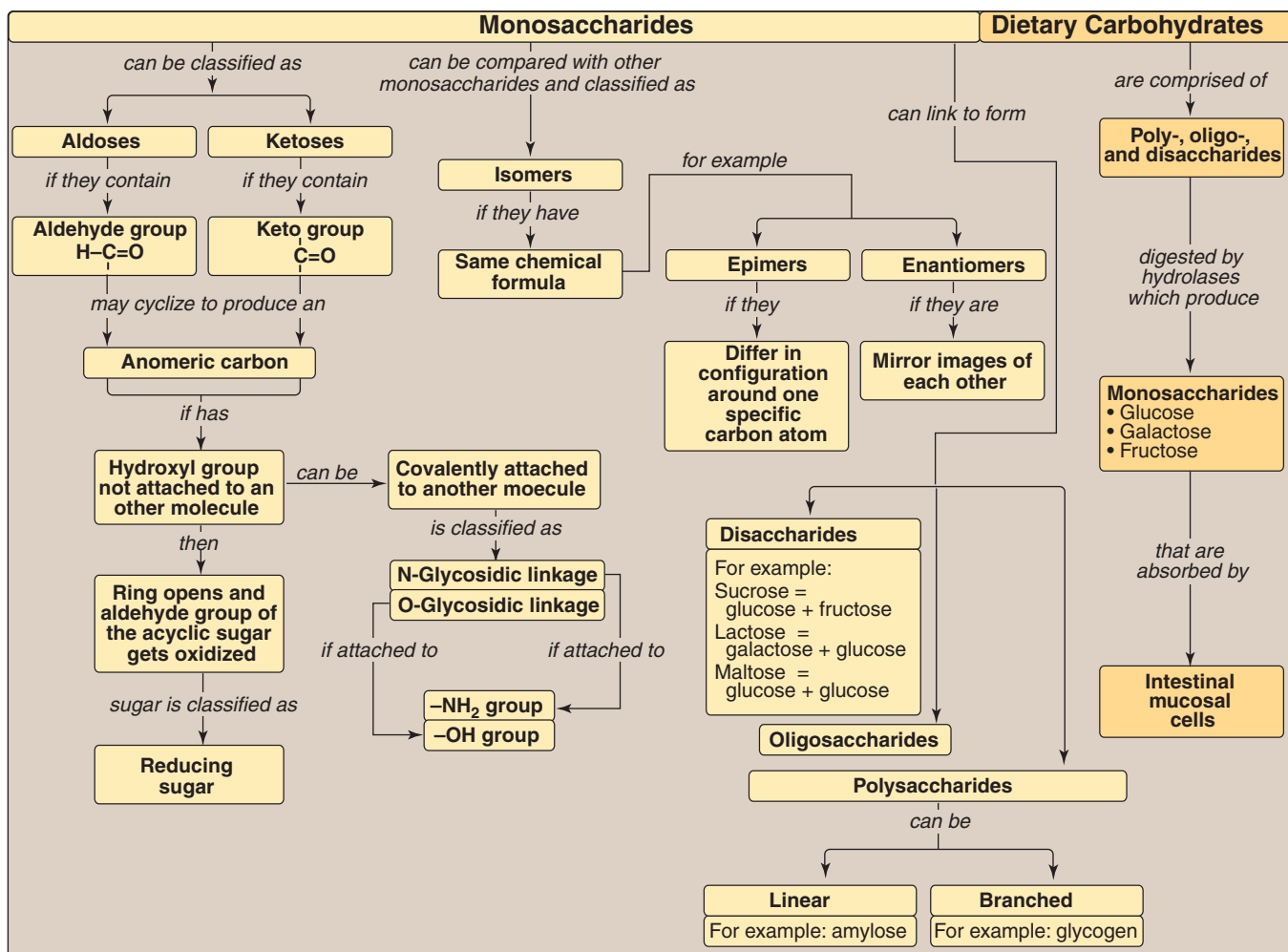


Figure 7.12
Key concept map for structure of monosaccharides.

sugar cyclizes, an **anomeric carbon** is created from the aldehyde group of an aldose or keto group of a ketose. This carbon can have two configurations, α or β . If the aldehyde group on an acyclic sugar gets oxidized as a chromogenic agent gets reduced, that sugar is a **reducing sugar**. A sugar with its anomeric carbon linked to another structure is called a **glycosyl residue**. Sugars can be attached either to an $-\text{NH}_2$ or an $-\text{OH}$ group, producing **N-** and **O-glycosides**. **Salivary α -amylase** acts on **dietary polysaccharides** (glycogen, amylose, amylopectin), producing **oligosaccharides**. **Pancreatic α -amylase** continues the process of polysaccharide digestion. The final digestive processes occur at the **mucosal lining** of the **small intestine**. Several **disaccharidases** [for example, **lactase** (β -galactosidase), **sucrase**, **maltase**, and **isomaltase**] produce monosaccharides (glucose, galactose, and fructose). These enzymes are secreted by and remain associated with the luminal side of the **brush border membranes** of **intestinal mucosal cells**. Absorption of the monosaccharides requires specific transporters. If carbohydrate degradation is deficient (as a result of heredity, intestinal disease, malnutrition, or drugs that injure the mucosa of the small intestine), undigested carbohydrate will pass into the large intestine, where it can cause **osmotic diarrhea**. Bacterial fermentation of the compounds produces large volumes of CO_2 and H_2 gas, causing abdominal cramps, diarrhea, and flatulence. **Lactose intolerance**, caused by a lack of **lactase**, is by far the most common of these deficiencies.

Study Question

Choose the ONE correct answer.

7.1 Which of the following statements best describes glucose?

- A. It is a ketose and usually exists as a furanose ring in solution.
- B. It is a C-4 epimer of galactose.
- C. It is utilized in biological systems only in the L-isomeric form.
- D. It is produced from dietary starch by the action of α -amylase.
- E. Homopolysaccharides of glucose, formed by the action of glycosyltransferases, are always branched molecules that contain only β -glycosidic linkages.

Correct answer = B. Glucose and galactose differ only in configuration around carbon 4, and so are C-4 epimers that are interconvertible by the action of an epimerase. Glucose is an aldose sugar that typically exists as a pyranose ring in solution; fructose, however, is a ketose with a furanose ring. The D-isomeric form of carbohydrates is most typically the form found in biologic systems, in contrast to amino acids. Salivary amylase does not produce monosaccharides. Homopolysaccharides of glucose include branched glycogen in which the glycosidic linkages are the α form, as well as unbranched cellulose that has β linkages.

7.2 A young black man entered his physician's office complaining of bloating and diarrhea. His eyes were sunken and the physician noted additional signs of dehydration. The patient's temperature was normal. He explained that the episode had occurred following a birthday party at which he had participated in an ice cream eating contest. The patient reported prior episodes of a similar nature following ingestion of a significant amount of dairy products. This clinical picture is most probably due to a deficiency in:

- A. salivary α -amylase.
- B. isomaltase.
- C. pancreatic α -amylase.
- D. sucrase.
- E. lactase.

Correct answer = E. The physical symptoms suggest a deficiency in an enzyme responsible for carbohydrate degradation. The symptoms observed following the ingestion of dairy products suggest that the patient is deficient in lactase.

7.3 Routine examination of the urine of an asymptomatic pediatric patient showed a positive reaction with Clinitest (a copper reduction method of detecting reducing sugars), but a negative reaction with the glucose oxidase test. Which one of the following sugars is least likely to be present (assuming a single elevated saccharide)?

- A. Lactose
- B. Fructose
- C. Sucrose
- D. Xylulose
- E. Galactose

Correct answer = C. Clinitest is a nonspecific test that produces a change in color if urine is positive for reducing substances, including reducing sugars (glucose, fructose, galactose, xylulose, lactose), amino acids, ascorbic acid, and certain drugs and drug metabolites. Because sucrose is not a reducing sugar, it is not detected by Clinitest. Glucose oxidase method will not detect increased levels of galactose or other sugars in urine. It is therefore important that a copper reduction method be used as a screening test. In those instances when the copper method is positive and the glucose oxidase method is negative, glucosuria is ruled out.

7.4 α -Glucosidase inhibitors such as acarbose and miglitol taken with meals are used in the treatment of diabetes. Explain. What effect should these drugs have on the digestion of lactose?

α -Glucosidase inhibitors slow the production of glucose from dietary carbohydrates, thereby reducing the post-prandial rise in blood glucose and facilitating better blood glucose control in diabetics. These drugs have no effect on lactose digestion because the disaccharide lactose contains a β -glycosidic bond, not an α .

Glycolysis

8

I. INTRODUCTION TO METABOLISM

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, but rather are organized into multistep sequences called pathways, such as that of glycolysis (Figure 8.1). In a pathway, the product of one reaction serves as the substrate of the subsequent reaction. Different pathways can also intersect, forming an integrated and purposeful network of chemical reactions. These are collectively called metabolism, which is the sum of all the chemical changes occurring in a cell, a tissue, or the body. Most pathways can be classified as either catabolic (degradative) or anabolic (synthetic). Catabolic reactions break down complex molecules, such as proteins, polysaccharides, and lipids, to a few simple molecules, for example, CO_2 , NH_3 (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.] In the following chapters, this text focuses on the central metabolic pathways that are involved in synthesizing and degrading carbohydrates, lipids, and amino acids.

A. Metabolic map

It is convenient to investigate metabolism by examining its component pathways. Each pathway is composed of multienzyme sequences, and each enzyme, in turn, may exhibit important catalytic or regulatory features. To provide the reader with the “big picture,” a metabolic map containing the important central pathways of energy metabolism is presented in Figure 8.2. This map is useful in tracing connections between pathways, visualizing the purposeful “movement” of metabolic intermediates, and picturing the effect on the flow of intermediates if a pathway is blocked, for example, by a drug or an inherited deficiency of an enzyme. 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.

B. Catabolic pathways

Catabolic reactions serve to capture chemical energy in the form of adenosine triphosphate (ATP) from the degradation of energy-rich fuel molecules. Catabolism also allows molecules in the diet (or nutrient molecules stored in cells) to be converted into building blocks needed for the synthesis of complex molecules. Energy generation by degradation of complex molecules occurs in three stages as shown in Figure 8.3. [Note: Catabolic pathways are typically oxidative, and require coenzymes such as NAD^+ .]

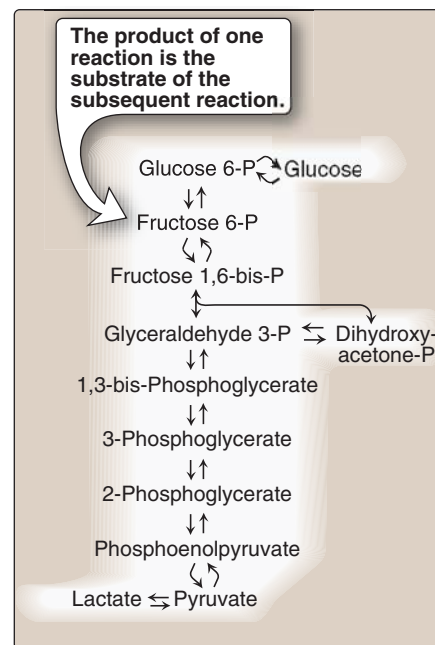
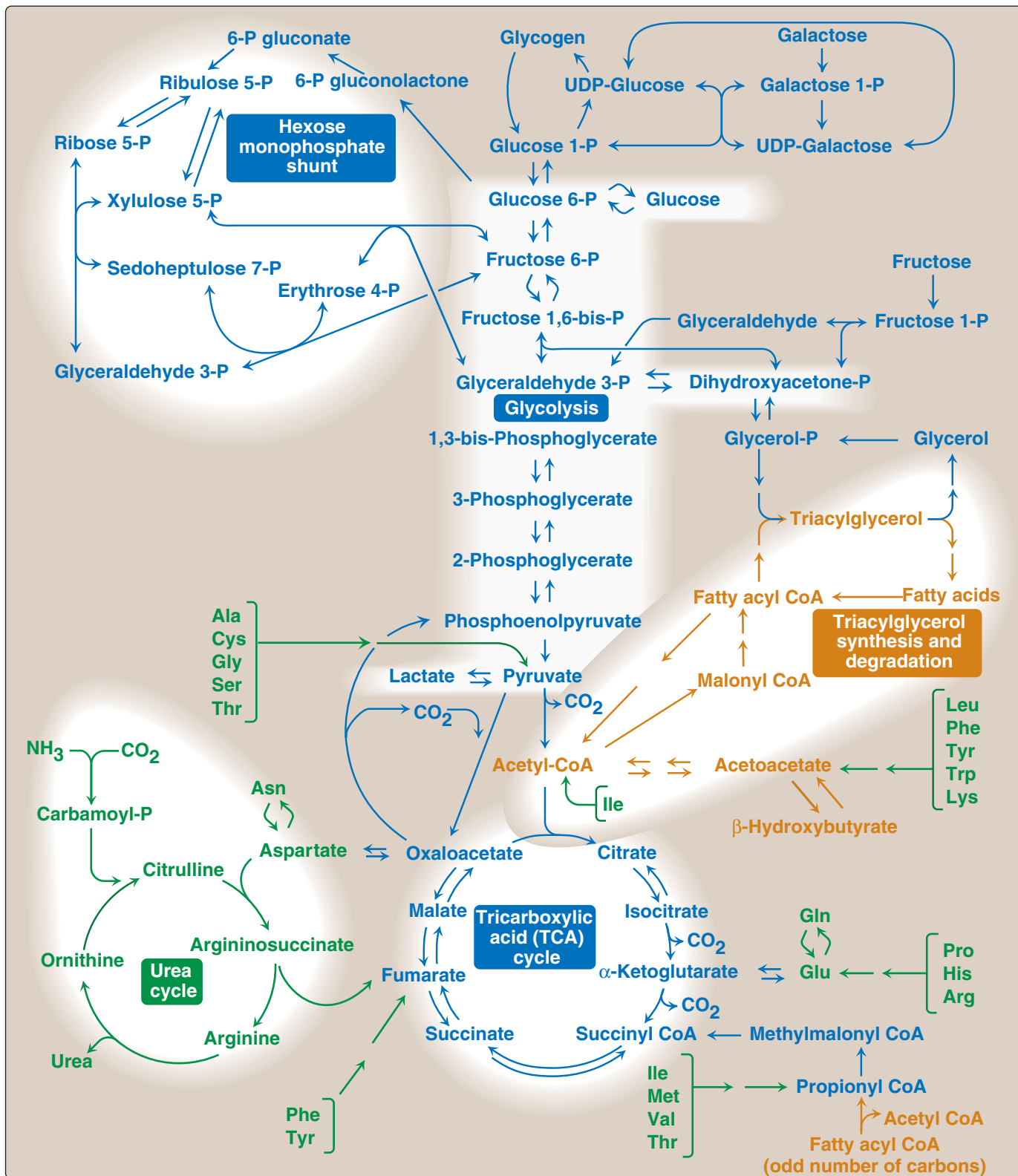
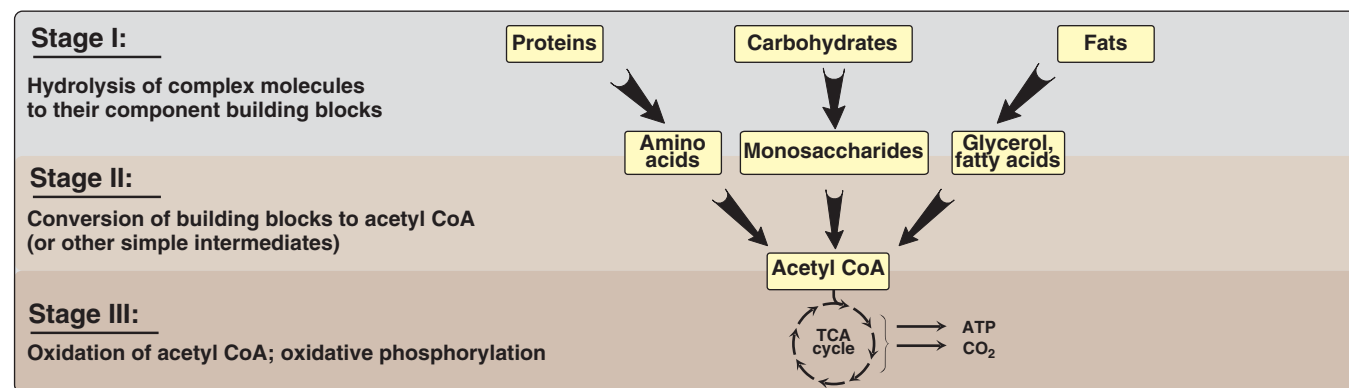


Figure 8.1

Glycolysis, an example of a metabolic pathway.

**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.

**Figure 8.3**

Three stages of catabolism.

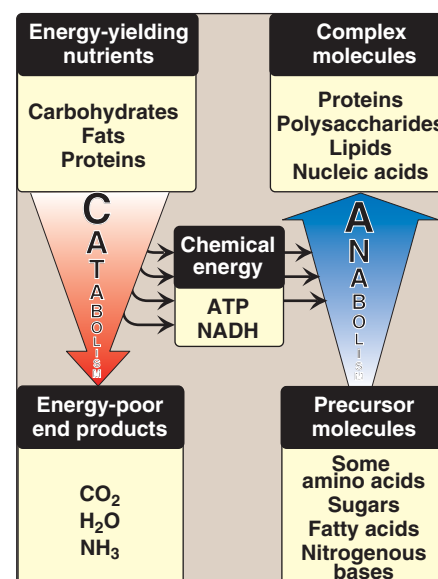
- 1. Hydrolysis of complex molecules:** In the first stage, complex molecules are broken down into their component building blocks. For example, proteins are degraded to amino acids, polysaccharides to monosaccharides, and fats (triacylglycerols) to free fatty acids and glycerol.
- 2. Conversion of building 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 CoA:** 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 FADH₂ to oxygen (see p. 73).

C. Anabolic pathways

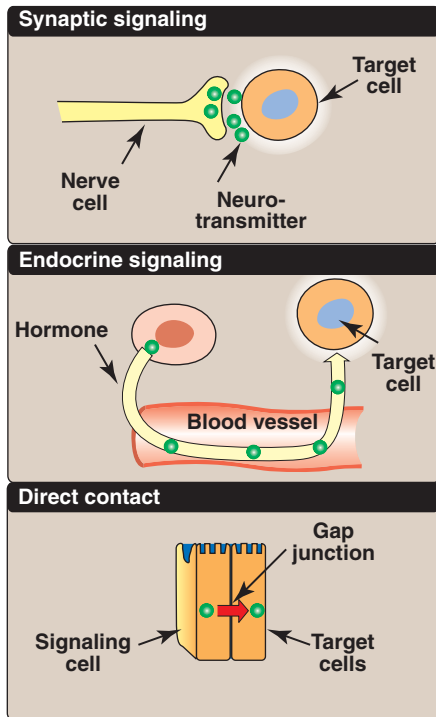
Anabolic reactions combine small molecules, such as amino acids, to form complex molecules, such as proteins (Figure 8.4). Anabolic reactions require energy (are endergonic), which is generally provided by the breakdown of ATP to adenosine diphosphate (ADP) and inorganic phosphate (P_i). Anabolic reactions often involve chemical reductions in which the reducing power is most frequently provided by the electron donor NADPH (see p. 147). Note that catabolism is a convergent process—that is, a wide variety of molecules are transformed into a few common end products. By contrast, anabolism is a divergent process in which a few biosynthetic precursors form a wide variety of polymeric or complex products.

II. REGULATION OF METABOLISM

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 do not function in isolation but, rather, are part of a community of interacting tissues. Thus, a sophisticated communication system has evolved to coordinate the functions of the

**Figure 8.4**

Comparison of catabolic and anabolic pathways.

**Figure 8.5**

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

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 (Figure 8.5).

A. Signals from within the cell (intracellular)

The rate of a metabolic pathway can respond to regulatory signals that arise from within the cell. For example, the rate of a pathway 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. Communication between cells (intercellular)

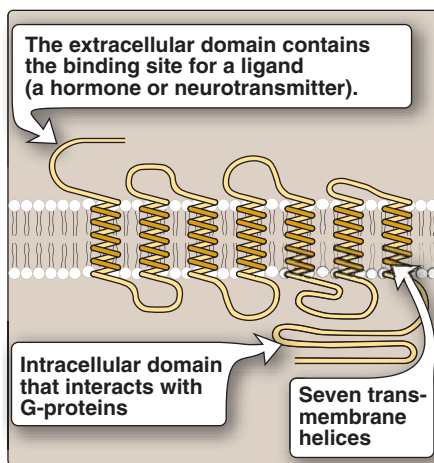
The ability to respond to extracellular signals is essential for the survival and development of all organisms. Signaling between cells provides for long-range integration of metabolism, and usually results in a response that is slower than is seen with signals that originate within the cell. Communication 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 cytoplasmic contents of adjacent cells. However, for energy metabolism, the most important route of communication is chemical signaling between cells by bloodborne hormones or by neurotransmitters.

C. Second messenger systems

Hormones or neurotransmitters can be thought of as signals, and their receptors as signal detectors. Each component serves as a link in the communication between extracellular events and chemical changes within the cell. Many receptors signal their recognition of a bound ligand by initiating a series of reactions that ultimately result in a specific intracellular response. “Second messenger” molecules—so named because they intervene between the original messenger (the neurotransmitter or hormone) and the ultimate effect on the cell—are part of the cascade of events that translates hormone or neurotransmitter binding into a cellular response. Two of the most widely recognized second messenger systems are the calcium/phosphatidylinositol system (see p. 205), and the *adenylyl cyclase* system, which is particularly important in regulating the pathways of intermediary metabolism.

D. Adenylyl cyclase

The recognition of a chemical signal by some membrane receptors, such as the β - and α_2 -adrenergic receptors, triggers either an increase or a decrease in the activity of *adenylyl cyclase* (*adenylate cyclase*). This is a membrane-bound enzyme that converts ATP to 3',5'-adenosine monophosphate (also called cyclic AMP or cAMP). The chemical signals are most often hormones or neurotransmitters, each of which binds to a unique type of membrane receptor. Therefore, tissues that respond to more than one chemical signal must have several different receptors, each of which can be linked to *adenylyl cyclase*. These receptors, known as G protein-coupled receptors (GPCR), are characterized by an extracellular ligand-binding region, seven transmembrane helices, and an intracellular domain that interacts with G proteins (Figure 8.6).

**Figure 8.6**

Structure of a typical G protein-coupled receptor (GPCR) of the plasma membrane.

1. GTP-dependent regulatory proteins: The effect of the activated, occupied GPCR on second messenger formation is not direct but, rather, is mediated by specialized trimeric proteins (α , β , γ subunits) of the cell membrane. These proteins, referred to as G proteins because they bind guanosine nucleotides (GTP and GDP), form a link in the chain of communication between the receptor and *adenylyl cyclase*. In the inactive form of a G protein, the α -subunit is bound to GDP (Figure 8.7). Binding of ligand 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 $\beta\gamma$ subunits and moves to *adenylyl cyclase*, which is thereby activated. Many molecules of active $G\alpha$ protein are formed by one activated receptor. [Note: The ability of a hormone or neurotransmitter to stimulate or inhibit *adenylyl cyclase* depends on the type of $G\alpha$ protein that is linked to the receptor. One family of G proteins, designated G_s , stimulates *adenylyl cyclase*; another family, designated G_i , inhibits the enzyme (not shown in Figure 8.7).] The actions of the $G\alpha$ -GTP complex are short-lived because $G\alpha$ has an inherent *GTPase* activity, resulting in the rapid hydrolysis of GTP to GDP. This causes inactivation of the $G\alpha$, its dissociation from *adenylyl cyclase* and reassociation with the $\beta\gamma$ dimer.

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

- 2. Protein kinases:** The next key link in the cAMP second messenger system is the activation by cAMP of a family of enzymes called cAMP-independent *protein kinases*, for example, *protein kinase A* (Figure 8.8). Cyclic AMP activates *protein kinase A* by binding to its two regulatory subunits, causing the release of active catalytic subunits. The active subunits catalyze the transfer of 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. *Protein kinase A* can also phosphorylate proteins that bind to DNA, causing changes in gene expression. [Note: Several types of *protein kinases* are not cAMP-dependent, for example, *protein kinase C* described on p. 205.]
- 3. Dephosphorylation of proteins:** The phosphate groups added to proteins by *protein kinases* are removed by *protein phosphatases*—enzymes that hydrolytically cleave phosphate esters (see Figure 8.8). This ensures that changes in protein activity induced by phosphorylation are not permanent.
- 4. Hydrolysis of cAMP:** cAMP is rapidly hydrolyzed to 5'-AMP by *cAMP phosphodiesterase*, one of a family of enzymes that cleave the cyclic 3',5'-phosphodiester bond. 5'-AMP is not an intracellular signaling molecule. Thus, the effects of neurotransmitter- or hormone-mediated

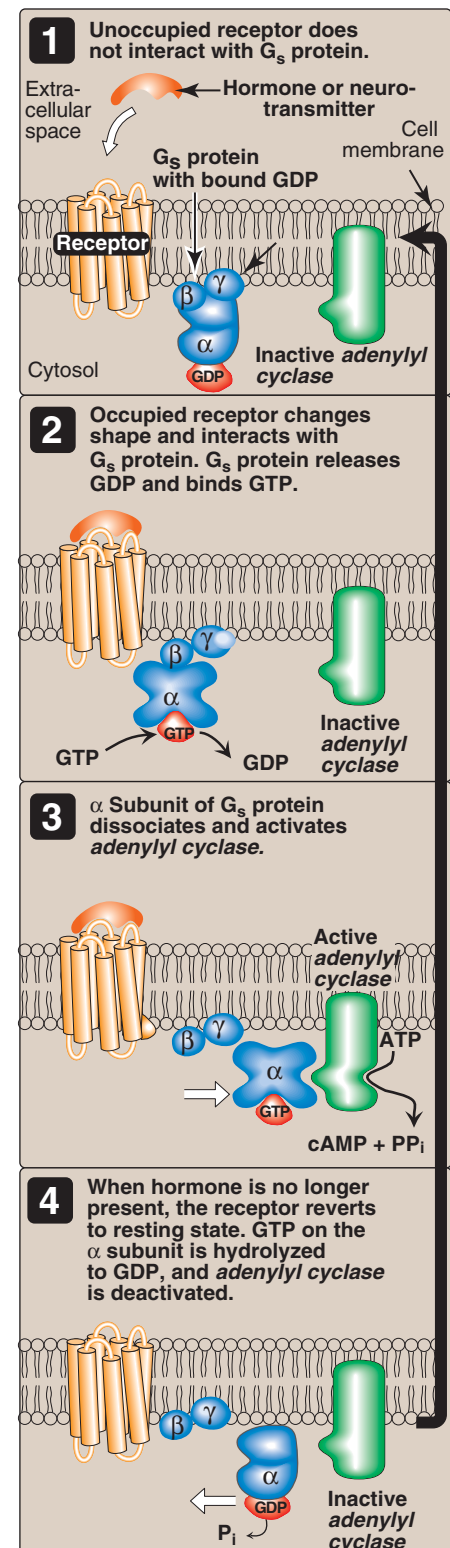


Figure 8.7

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

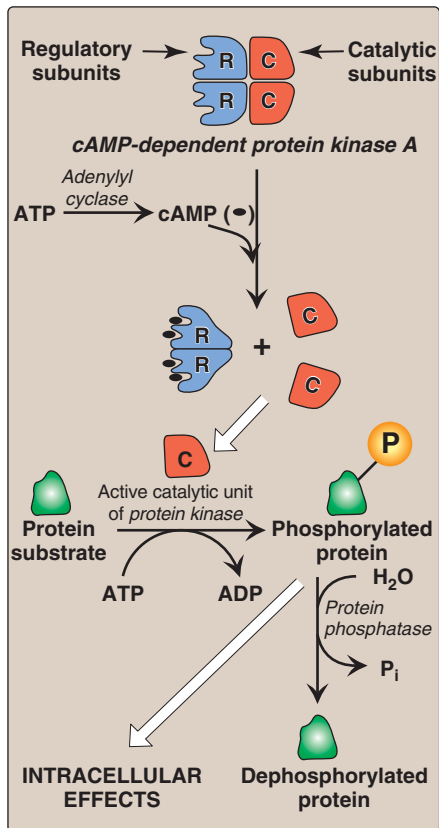


Figure 8.8
Actions of cAMP.

increases of cAMP are rapidly terminated if the extracellular signal is removed. [Note: *Phosphodiesterase* is inhibited by methylxanthine derivatives, such as theophylline and caffeine.¹]

III. OVERVIEW OF GLYCOLYSIS

The glycolytic pathway is employed by all tissues for the breakdown 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 8.9A). 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 8.9B). Aerobic glycolysis sets the stage for the oxidative decarboxylation of pyruvate to acetyl CoA, a major fuel of the TCA (or citric acid) cycle. Alternatively, pyruvate is reduced to lactate as NADH is oxidized to NAD⁺ (Figure 8.9C). This conversion 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) 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 a Na⁺-monosaccharide cotransporter system.

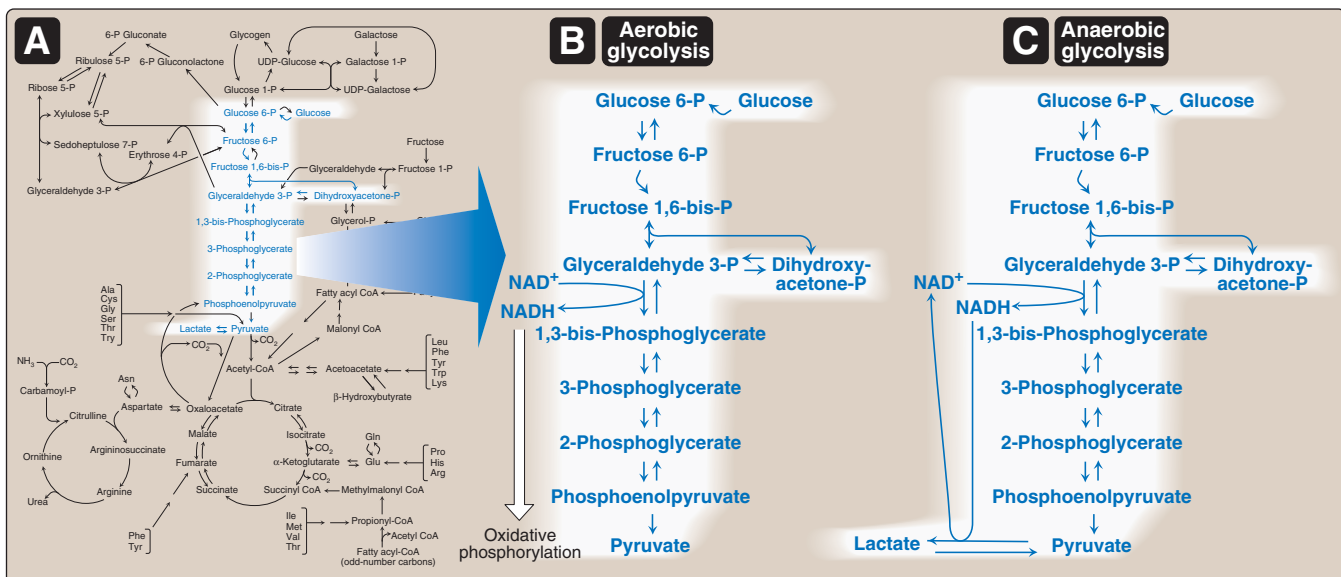


Figure 8.9

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



¹See Chapter 10 in *Lippincott's Illustrated Reviews: Pharmacology* for a discussion of the use of methylxanthine derivatives as drugs.

A. Na^+ -independent facilitated diffusion transport

This system is mediated by a family of 14 glucose transporters in cell membranes. They are designated GLUT-1 to GLUT-14 (glucose transporter isoforms 1–14). These transporters exist in the membrane in two conformational states (Figure 8.10). Extracellular glucose binds to the transporter, which then alters its conformation, transporting glucose across the cell membrane.

- 1. Tissue specificity of GLUT gene expression:** The glucose transporters 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 blood brain barrier, but is low in adult muscle, whereas GLUT-4 is abundant in adipose tissue and skeletal muscle. [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.)] The other GLUT isoforms also have tissue-specific distributions.
- 2. Specialized functions of GLUT isoforms:** In facilitated diffusion, glucose movement follows a concentration gradient, that is, from a high glucose concentration to a lower one. For example, GLUT-1, GLUT-3, and GLUT-4 are primarily involved in glucose uptake from the blood. In contrast, GLUT-2, which is found 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). [Note: GLUT-2 is also found in pancreatic β cells.] GLUT-5 is unusual in that it is the primary transporter for fructose (instead of glucose) in the small intestine and the testes.

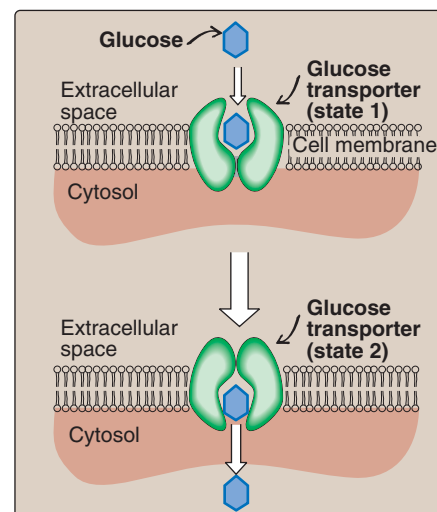


Figure 8.10

Schematic representation of the facilitated transport of glucose through a cell membrane. [Note: GLUT proteins contain 12 trans-membrane helices.]

B. Na^+ -monosaccharide cotransporter 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 carrier-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 carrier is a sodium-dependent–glucose transporter or SGLT. This type of transport 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. REACTIONS OF GLYCOLYSIS

The conversion of glucose to pyruvate occurs in two stages (Figure 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.

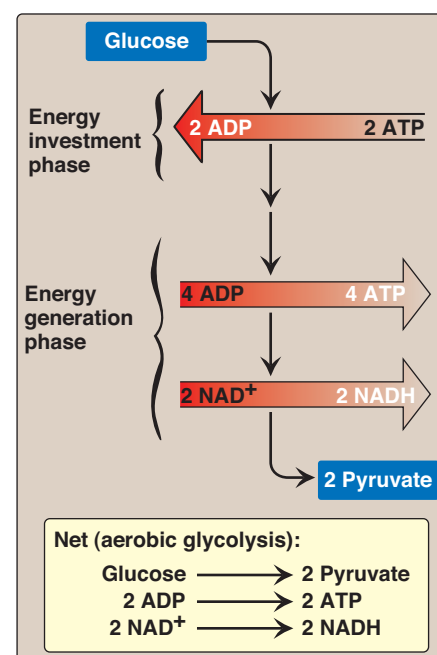
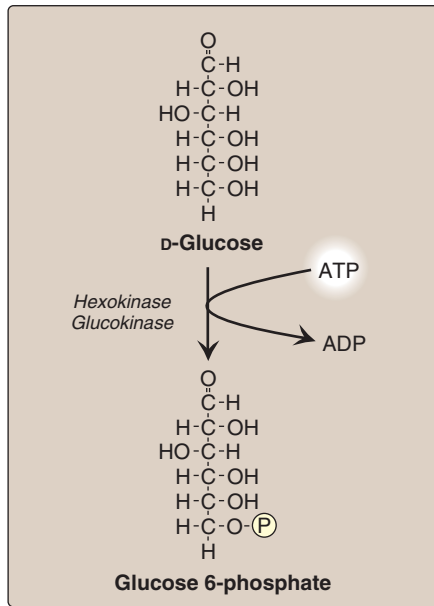


Figure 8.11

Two phases of aerobic glycolysis.

**Figure 8.12**

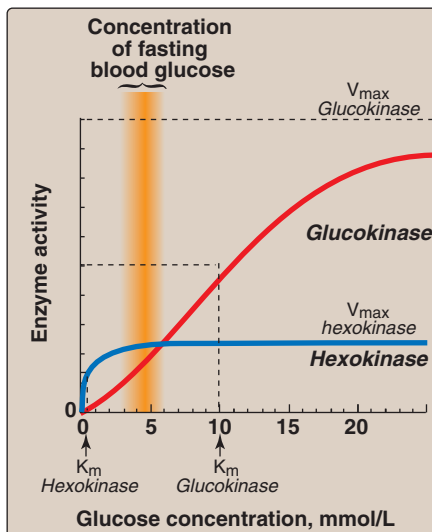
Energy investment phase:
phosphorylation of glucose.

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 8.12), therefore, effectively traps the sugar as cytosolic glucose 6-phosphate, thus committing it to further metabolism in the cell. Mammals have several isozymes of the enzyme *hexokinase* that catalyze the phosphorylation of glucose to glucose 6-phosphate.

1. Hexokinase: In most tissues, the phosphorylation of glucose is catalyzed by *hexokinase*, one of three regulatory enzymes of glycolysis (see also *phosphofructokinase* and *pyruvate kinase*). *Hexokinase* has broad substrate specificity and is able to phosphorylate several hexoses in addition to glucose. *Hexokinase* is inhibited by the reaction product, glucose 6-phosphate, which accumulates when further metabolism of this hexose phosphate is reduced. *Hexokinase* has a low 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 (Figure 8.13). *Hexokinase*, however, has a low V_{max} for glucose and, therefore, cannot sequester (trap) cellular phosphate in the form of phosphorylated hexoses, or phosphorylate more sugars than the cell can use.

2. Glucokinase: In liver parenchymal cells and β cells of the pancreas, *glucokinase* (also called *hexokinase D*, or *type IV*) is the predominant enzyme responsible for the phosphorylation of glucose. In β cells, *glucokinase* functions as the glucose sensor, determining the threshold for insulin secretion (see p. 310). In the liver, the enzyme facilitates glucose phosphorylation during hyperglycemia. [Note: *Hexokinase* also serves as a glucose sensor in neurons of the hypothalamus, playing a key role in the adrenergic response to hypoglycemia (see p. 315.) Despite the popular but misleading name *glucokinase*, the sugar specificity of the enzyme is similar to that of other *hexokinase* isozymes.

**Figure 8.13**

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

a. Kinetics: *Glucokinase* differs from *hexokinase* in several important properties. For example, it has a much higher K_m , requiring a higher glucose concentration for half-saturation (see Figure 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 a carbohydrate-rich meal, and thus minimizes 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, thus rendering the enzyme inactive (Figure 8.14). 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 re-enters the cytosol where it phosphorylates glucose to glucose 6-phosphate. [Note: Fructose 1-phosphate inhibits formation of the *glucokinase*-GKRP complex.]

Glucokinase functions as a glucose sensor in the maintenance of blood glucose homeostasis. Mutations that decrease the activity of *glucokinase* are the cause of a rare form of diabetes, maturity onset diabetes of the young type 2 (MODY 2).

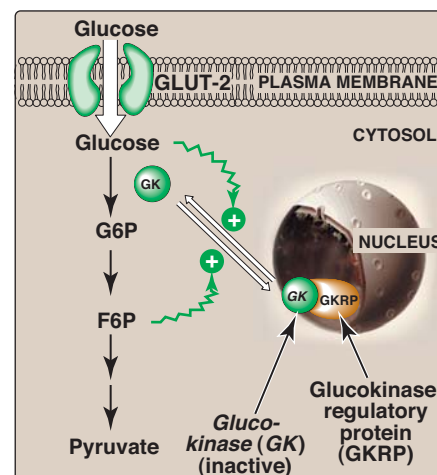


Figure 8.14

Regulation of *glucokinase* activity by glucokinase regulatory protein.

B. Isomerization of glucose 6-phosphate

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

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 8.16). *PFK-1* is controlled by the available concentrations of the substrates ATP and fructose 6-phosphate, and by regulatory substances described below.

- 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 (see p. 109), also inhibit *PFK-1*. Conversely, *PFK-1* is activated allosterically by high concentrations of AMP, which signal that the cell’s energy stores are depleted. [Note: Citrate inhibition favors the use of glucose for glycogen synthesis, see p.125.]
- 2. Regulation by fructose 2,6-bisphosphate:** Fructose 2,6-bisphosphate is the most potent activator of *PFK-1* (see Figure 8.16), and is able to activate the enzyme even when ATP levels are high. Fructose 2,6-bisphosphate is formed 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 a *phosphatase* activity that dephosphorylates fructose 2,6-bisphosphate back to fructose 6-phosphate. In liver, the *kinase* domain is active if dephosphorylated and is inactive if phosphorylated (Figure 8.17). [Note: Fructose 2,6-bisphos-

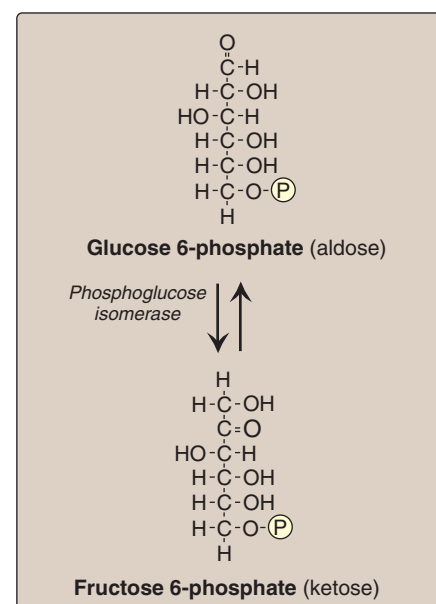
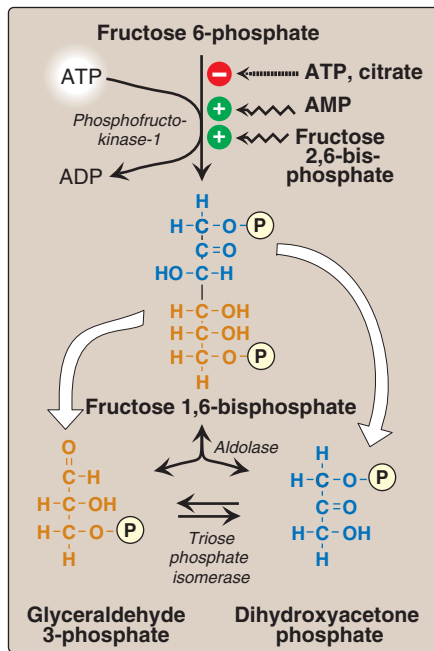


Figure 8.15

Aldose-ketose isomerization of glucose 6-phosphate to fructose 6-phosphate.

**Figure 8.16**

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

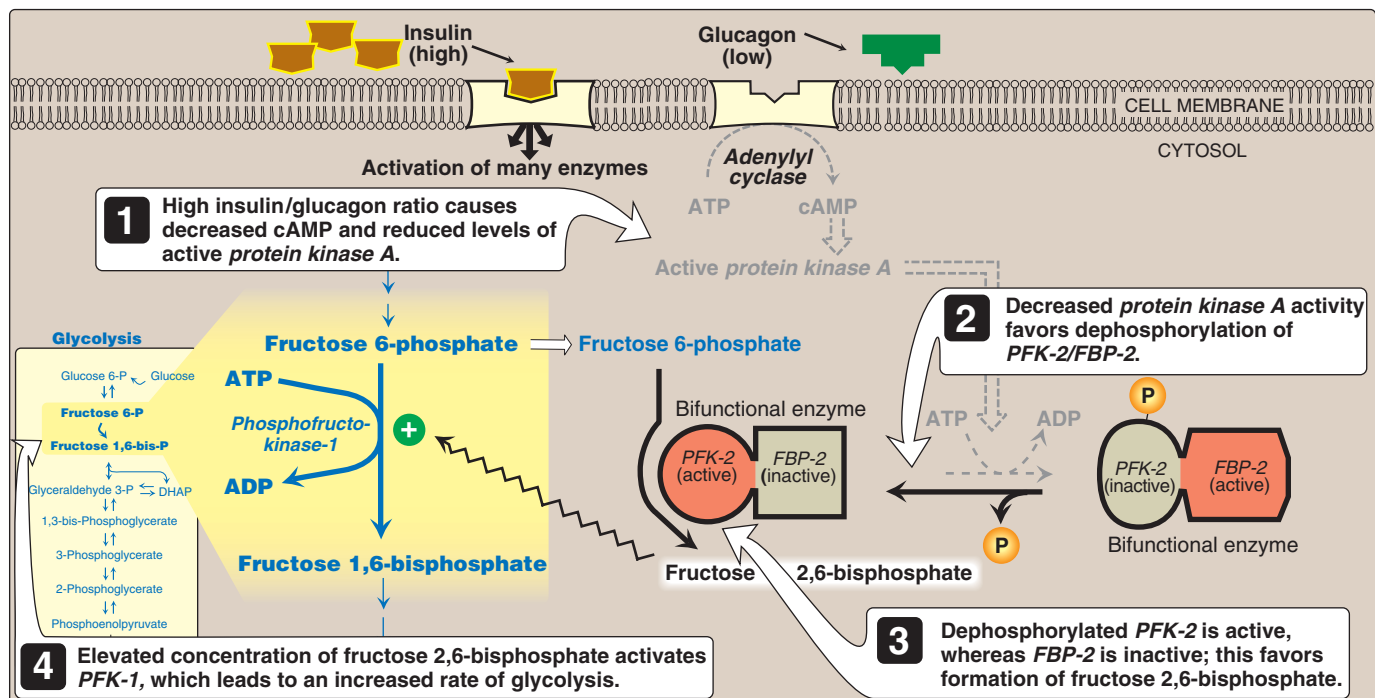
phate is an inhibitor of *fructose 1,6-bisphosphatase*, an enzyme of gluconeogenesis (see p. 120 for a discussion of the regulation of gluconeogenesis). The reciprocal actions of fructose 2,6-bisphosphate on glycolysis (activation) and gluconeogenesis (inhibition) ensure that both pathways are not fully 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 8.17). Fructose 2,6-bisphosphate, therefore, acts as an intracellular signal, indicating that glucose is abundant.

b. During starvation: Elevated levels of glucagon and low levels of insulin, such as occur during fasting (see p. 327), decrease the intracellular concentration of hepatic fructose 2,6-bisphosphate. This results in a decrease in the overall rate of glycolysis and an increase in gluconeogenesis.

D. Cleavage of fructose 1,6-bisphosphate

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

**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 bisphosphatase-2.

E. Isomerization of dihydroxyacetone phosphate

Triose phosphate isomerase interconverts dihydroxyacetone phosphate and glyceraldehyde 3-phosphate (see Figure 8.16). Dihydroxyacetone phosphate 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.

F. Oxidation of glyceraldehyde 3-phosphate

The conversion of glyceraldehyde 3-phosphate to 1,3-bisphosphoglycerate by *glyceraldehyde 3-phosphate dehydrogenase* is the first oxidation-reduction reaction of glycolysis (Figure 8.18). [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, see p. 96), and 2) oxidation of NADH via the respiratory chain (aerobic, see p. 75). The latter requires substrate shuttles (see p. 79).]

1. Synthesis of 1,3-bisphosphoglycerate (1,3-BPG): 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 explained primarily by the inhibition of enzymes such as *pyruvate dehydrogenase*, which require lipoic acid as a coenzyme (see p. 110). However, pentavalent arsenic (arsenate) also can prevent net ATP and NADH production by glycolysis, without inhibiting the pathway itself. The poison does so by competing with inorganic phosphate as a substrate for *glyceraldehyde 3-phosphate dehydrogenase*, forming a complex that spontaneously hydrolyzes to form 3-phosphoglycerate (see Figure 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: Arsenic also replaces P_i on the F_1 domain of *ATP synthase* (see p. 78), resulting in formation of ADP-arsenate that is rapidly hydrolyzed.]

3. Synthesis of 2,3-bisphosphoglycerate (2,3-BPG) in red blood cells: Some of the 1,3-BPG is converted to 2,3-BPG by the action of *bisphosphoglycerate mutase* (see Figure 8.18). 2,3-BPG, which is found in only trace amounts in most cells, is present at high concentration in red blood cells (increases 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 Figure 8.18). In the red blood cell, glycolysis is modified by inclusion of these “shunt” reactions.

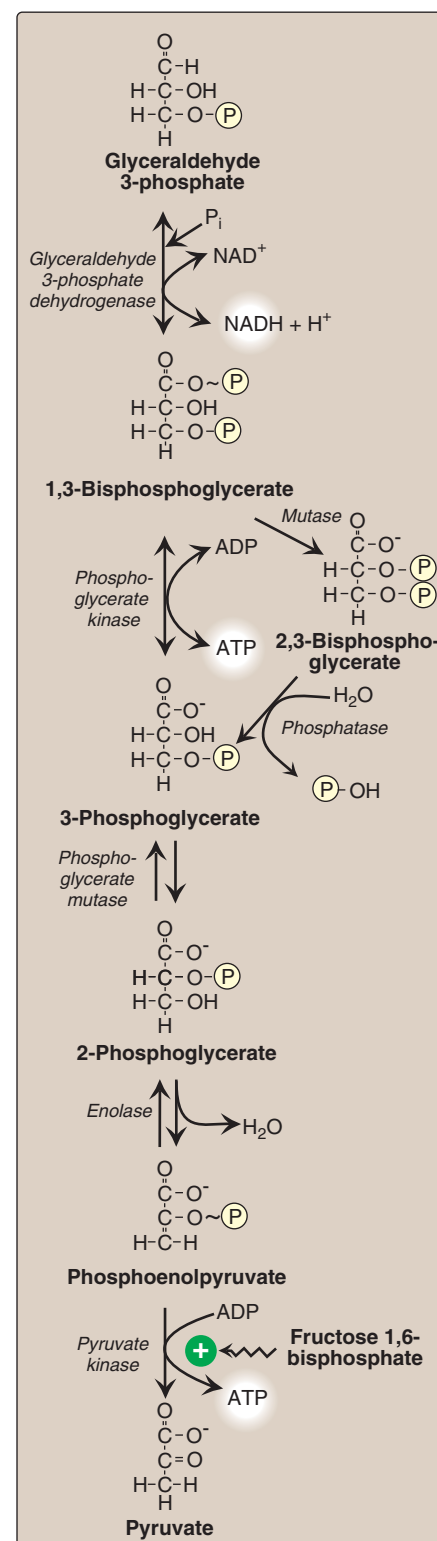


Figure 8.18

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

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 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 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 (see J. below and p. 113 for other examples).]

H. Shift of the phosphate group from carbon 3 to carbon 2

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 2-phosphoglycerate molecule, 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.

J. Formation of pyruvate producing ATP

The conversion of PEP to pyruvate is catalyzed by *pyruvate kinase*, the third irreversible reaction of glycolysis. The equilibrium of the *pyruvate kinase* reaction favors the formation of ATP (see Figure 8.18). [Note: This is another example of substrate-level phosphorylation.]

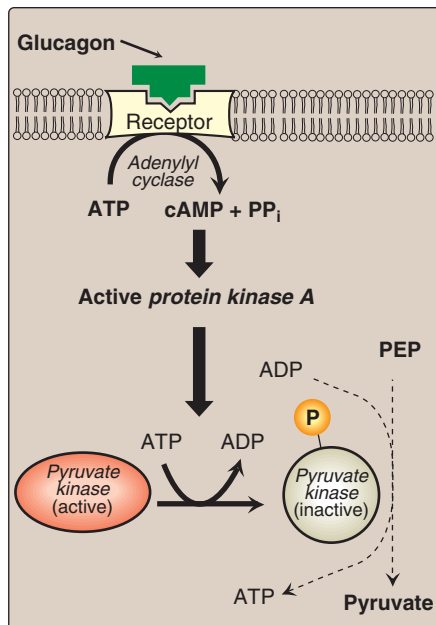


Figure 8.19
Covalent modification of hepatic *pyruvate kinase* results in inactivation of enzyme.

- 1. Feed-forward regulation:** In liver, *pyruvate kinase* is activated by fructose 1,6-bisphosphate, the product of the *phosphofructokinase* reaction. This feed-forward (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 *pyruvate kinase*.
- 2. Covalent modulation of pyruvate kinase:** Phosphorylation by a *cAMP-dependent protein kinase* leads to inactivation of *pyruvate kinase* in the liver (Figure 8.19). When blood glucose levels are low, elevated glucagon increases the intracellular level of cAMP, which causes the phosphorylation and inactivation of *pyruvate kinase*. Therefore, PEP is unable to continue in glycolysis, but instead enters the gluconeogenesis pathway. This, in part, explains the observed inhibition of hepatic glycolysis and stimulation of gluconeogenesis by glucagon. Dephosphorylation of *pyruvate kinase* by a *phosphoprotein phosphatase* results in reactivation of the enzyme.
- 3. Pyruvate kinase deficiency:** The normal, mature erythrocyte lacks mitochondria and is, therefore, completely dependent on glycolysis for production of ATP. This high-energy compound is required to meet the metabolic needs of the red blood cell, and also to fuel the pumps necessary for the maintenance of the biconcave, flexi-

ble shape of the cell, which allows it 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 red blood cell membrane lead to changes in the shape of the cell and, ultimately, to phagocytosis by the cells of the reticuloendothelial system, particularly macrophages of the spleen. The premature death and lysis of red blood cells results in hemolytic anemia. Among patients exhibiting the rare genetic defects of glycolytic enzymes, about 95% show a deficiency in *pyruvate kinase*, and 4% exhibit *phosphoglucose isomerase* deficiency. *PK* deficiency is restricted to the erythrocytes, and produces mild to severe chronic hemolytic anemia (erythrocyte destruction), with the severe form requiring regular cell transfusions. The severity of the disease depends both on the degree of enzyme deficiency (generally 5–25% of normal levels), and on the extent to which the individual's red blood cells compensate by synthesizing increased levels of 2,3-BPG (see p. 31). Almost all individuals with *PK* deficiency have a mutant enzyme that shows abnormal properties—most often altered kinetics (Figure 8.20).

Pyruvate kinase deficiency is the second most common cause (after *glucose 6-phosphate dehydrogenase* deficiency) of enzyme deficiency-related nonspherocytic hemolytic anemia.

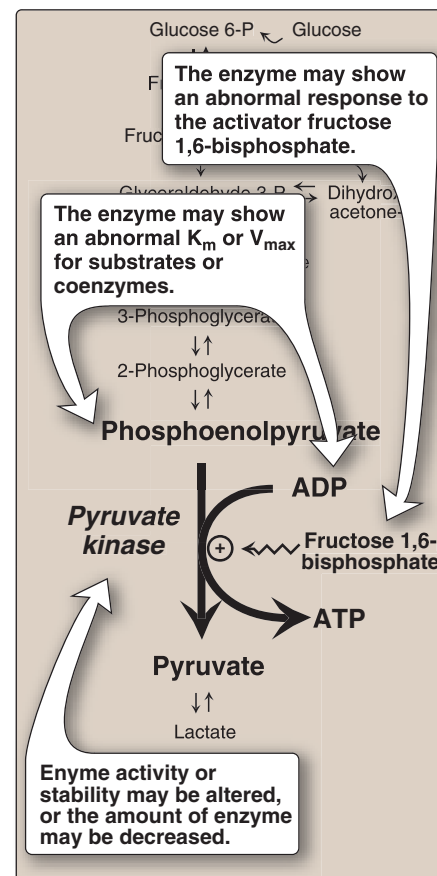


Figure 8.20

Alterations observed with various mutant forms of *pyruvate kinase*.

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 8.21). The formation of lactate is the major fate for pyruvate in lens and cornea of the eye, kidney medulla, testes, leukocytes and red blood cells, because these are all poorly vascularized and/or lack mitochondria.

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 citric acid cycle, see p. 112) 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 (see p. 118).

2. Lactate consumption: 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 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 CO₂ and H₂O via the citric acid cycle.

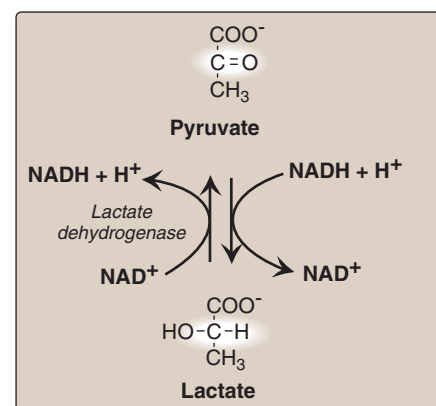


Figure 8.21

Interconversion of pyruvate and lactate.

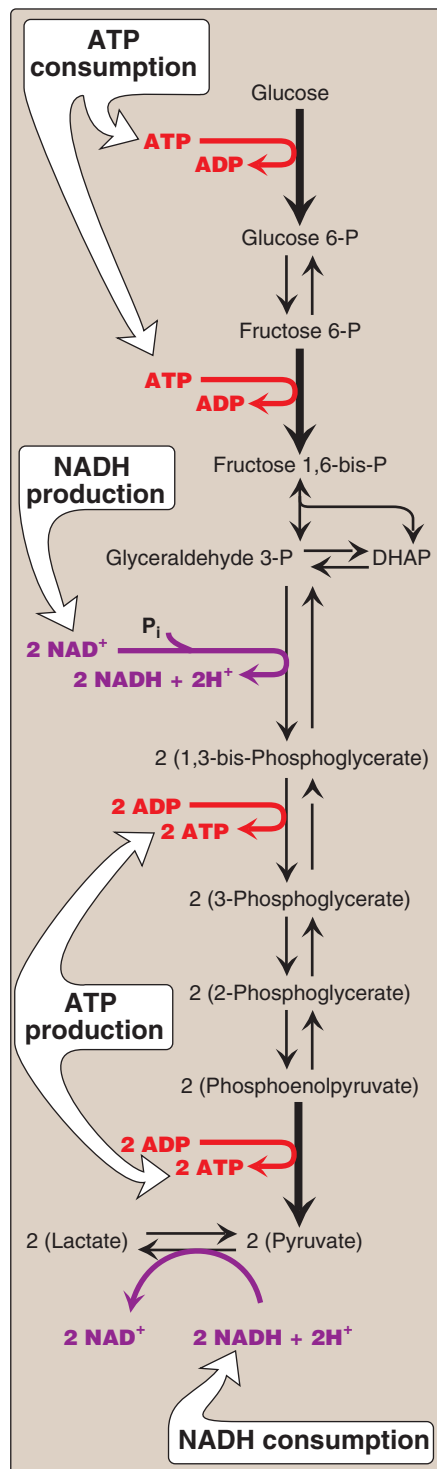


Figure 8.22

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.

3. Lactic acidosis: Elevated concentrations of lactate in the plasma, termed lactic 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 use anaerobic glycolysis as a backup system for generating ATP, producing lactic acid as the endproduct. [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 products, pyruvate or lactate, still contain most of the energy originally contained in glucose. The TCA cycle is required to release that energy completely (see p. 109).

- 1. Anaerobic glycolysis:** Two molecules of ATP are generated for each molecule of glucose converted to two molecules of lactate (Figure 8.22). There is no net production or consumption of NADH.
- 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 (see p. 77). [Note: NADH cannot cross the inner mitochondrial membrane, and substrate shuttles are required (see p. 79).]

VI. HORMONAL REGULATION OF GLYCOLYSIS

The regulation of glycolysis by allosteric activation or inhibition, or the 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 slower, and often more profound, hormonal influences on 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, which are described in

Chapter 10 (see p. 117). Regular consumption of meals rich in carbohydrate or administration of insulin initiates an increase in the amount of *glucokinase*, *phosphofructokinase*, and *pyruvate kinase* in liver (Figure 8.23). 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 well-fed state (see p. 321). Conversely, gene transcription and synthesis of *glucokinase*, *phosphofructokinase*, and *pyruvate kinase* are decreased when plasma glucagon is high and insulin is low, for example, as seen in fasting or diabetes.

VII. ALTERNATE FATES OF PYRUVATE

A. Oxidative decarboxylation of pyruvate

Oxidative decarboxylation of pyruvate by *pyruvate dehydrogenase complex* is an important pathway in tissues with a high oxidative capacity, such as cardiac muscle (Figure 8.24). *Pyruvate dehydrogenase* irreversibly converts pyruvate, the end product of glycolysis, into acetyl CoA, a major fuel for the TCA cycle (see p. 109) and the building block for fatty acid synthesis (see p. 183).

B. Carboxylation of pyruvate to oxaloacetate

Carboxylation of pyruvate to oxaloacetate (OAA) by *pyruvate carboxylase* is a biotin-dependent reaction (see Figure 8.24). This reaction is important because it replenishes the citric acid cycle intermediates, and provides substrate for gluconeogenesis (see p. 118).

C. Reduction of pyruvate to ethanol (microorganisms)

The conversion of pyruvate to ethanol occurs by the two reactions summarized in Figure 8.24. 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 a reaction similar to that described for *pyruvate dehydrogenase* (see p. 110).

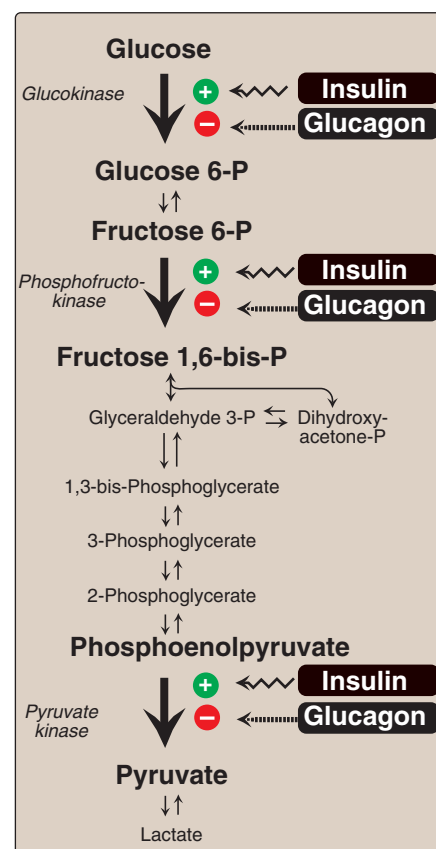


Figure 8.23

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

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 breakdown of ATP. The rate of a metabolic pathway can respond to **regulatory signals**, for example, **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** between cells, for example, by **hormones** or **neurotransmitters**. **Second messenger molecules** convey the intent of a chemical signal (hormone or neurotransmitter) to appropriate intracellular responders. **Adenylyl cyclase** is a membrane-bound enzyme that synthesizes **cAMP** in response to chemical signals, such

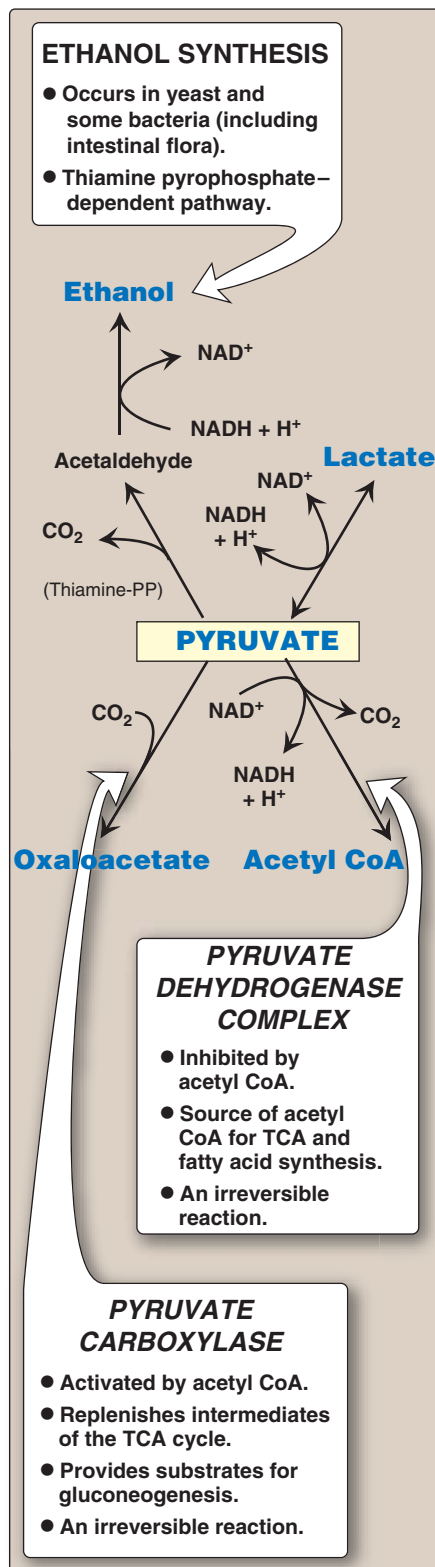


Figure 8.24
Summary of the metabolic fates of pyruvate.

as 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. **Anaerobic glycolysis**, in which **lactic acid** is the end product, occurs in cells that lack mitochondria, or 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 **brain**, **GLUT-4** (which is **insulin-dependent**) is found in **muscle** and **adipose tissue**, and **GLUT-2** is found in **liver** and the β cells of the pancreas. The conversion of glucose to pyruvate (**glycolysis**, Figure 8.25) occurs in two stages: an **energy investment phase** 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 K_m)** and a **small V_{max}** for glucose, and is **inhibited** by **glucose 6-phosphate**. **Glucokinase** has a **large K_m** and a **large V_{max}** for glucose. It is indirectly **inhibited** by **fructose 6-phosphate** and **activated** by **glucose**, and the **transcription** of the glucokinase gene is **enhanced** by **insulin**. Glucose 6-phosphate is isomerized to fructose 6-phosphate, which is phosphorylated to **fructose 1,6-bisphosphate** by **phosphofructokinase**. This enzyme is **allosterically inhibited** by **ATP** and **citrate**, and **activated** by **AMP**. **Fructose 2,6-bisphosphate**, whose synthesis is **activated** by **insulin**, is the most potent allosteric activator of this enzyme. 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**. This enzyme is **allosterically activated** by **fructose 1,6-bisphosphate**, and **hormonally activated** by **insulin** and **inhibited** by **glucagon** via the **cAMP pathway**. **Pyruvate kinase deficiency** accounts for 95% of all inherited defects in glycolytic enzymes. It is restricted to **erythrocytes**, and causes mild to severe **chronic hemolytic anemia**. In **anaerobic glycolysis**, NADH is reoxidized to NAD^+ by the **conversion of pyruvate to lactic acid**. 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 CoA**; 2) **carboxylated** to **oxaloacetate** (a TCA cycle intermediate) by **pyruvate carboxylase**; or 3) **reduced** by microorganisms to **ethanol** by **pyruvate decarboxylase**.

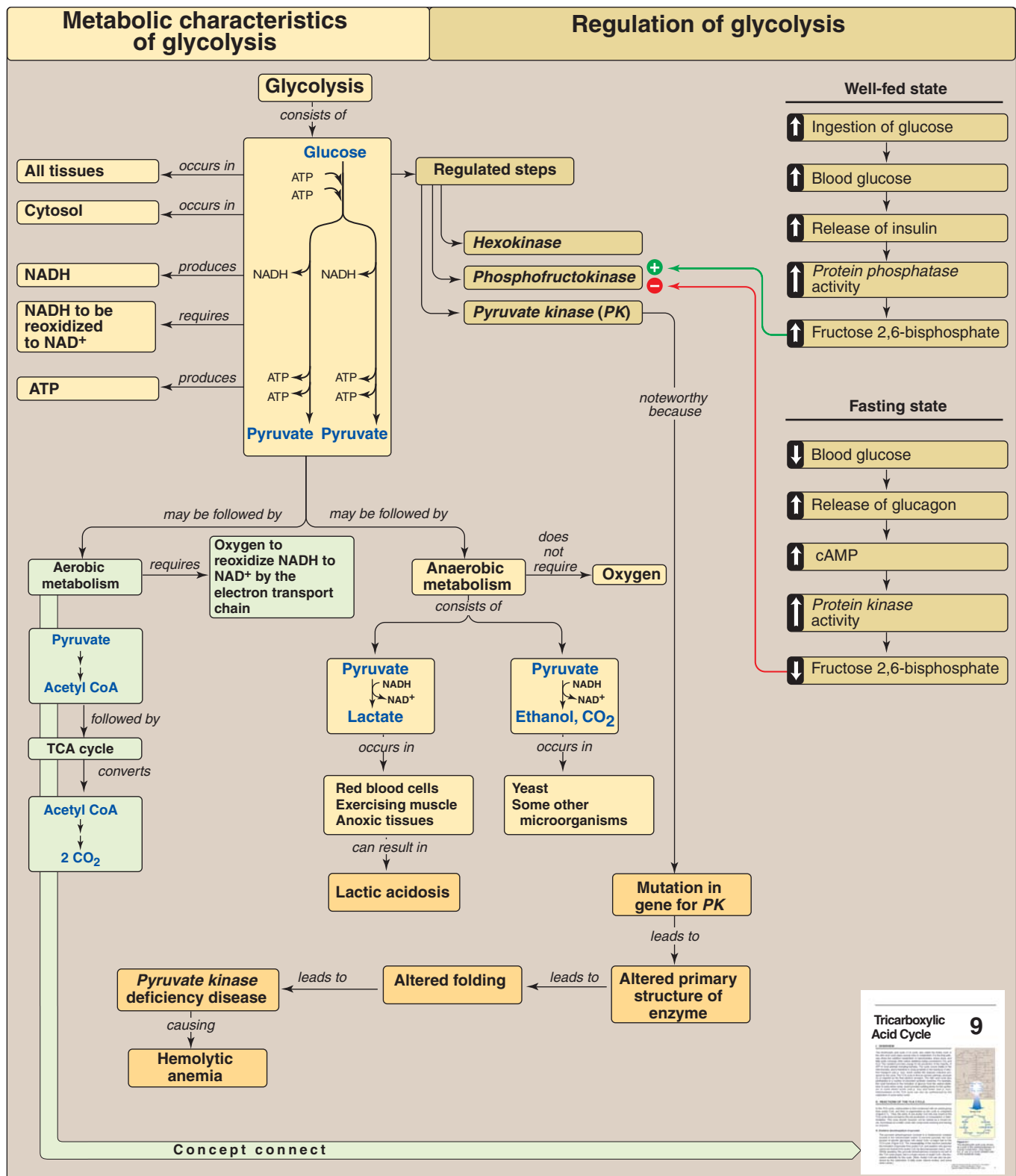


Figure 8.25

Key concept map for glycolysis.

Study Questions

Choose the ONE best answer.

8.1 Which one of the following statements concerning glycolysis is correct?

- A. The conversion of glucose to lactate requires the presence of oxygen.
- B. Hexokinase is important in hepatic glucose metabolism only in the absorptive period following consumption of a carbohydrate-containing meal.
- C. Fructose 2,6-bisphosphate is a potent inhibitor of phosphofructokinase.
- D. The regulated reactions are also the irreversible reactions.
- E. The conversion of glucose to lactate yields two ATP and two NADH.

Correct answer = D. Hexokinase, phosphofructokinase, and pyruvate kinase are all irreversible and are the regulated steps in glycolysis. The conversion of glucose to lactate (anaerobic glycolysis) is a process that does not involve a net oxidation or reduction and, thus, oxygen is not required. Glucokinase (not hexokinase) is important in hepatic glucose metabolism only in the absorptive period following consumption of a carbohydrate-containing meal. Fructose 2,6-bisphosphate is a potent activator (not inhibitor) of phosphofructokinase. The conversion of glucose to lactate yields two ATP but no net production of NADH.

8.2 The reaction catalyzed by phosphofructokinase-1:

- A. is activated by high concentrations of ATP and citrate.
- B. uses fructose 1-phosphate as substrate.
- C. is the rate-limiting reaction of the glycolytic pathway.
- D. is near equilibrium in most tissues.
- E. is inhibited by fructose 2,6-bisphosphate.

Correct answer = C. Phosphofructokinase-1 is the pace-setting enzyme of glycolysis. It is inhibited by ATP and citrate, uses fructose 6-phosphate as substrate, and catalyzes a reaction that is far from equilibrium. The reaction is activated by fructose 2,6-bisphosphate.

8.3 Compared with the resting state, vigorously contracting skeletal muscle shows:

- A. an increased conversion of pyruvate to lactate.
- B. decreased oxidation of pyruvate to CO_2 and water.
- C. a decreased NADH/NAD⁺ ratio.
- D. a decreased concentration of AMP.
- E. decreased levels of fructose 2,6-bisphosphate.

Correct answer = A. Vigorously contracting muscle shows an increased formation of lactate and an increased rate of pyruvate oxidation compared with resting skeletal muscle. The levels of AMP and NADH increase, whereas change in the concentration of fructose 2,6-bisphosphate is not a key regulatory factor in skeletal muscle.

8.4 A 43-year-old man presented with symptoms of weakness, fatigue, shortness of breath, and dizziness. His hemoglobin level was less than 7 g/dl (normal for a male being greater than 13.5 g/dl). Red blood cells isolated from the patient showed abnormally low level of lactate production. A deficiency of which one of the following enzymes would be the most likely cause of this patient's anemia?

- A. Phosphoglucose isomerase
- B. Phosphofructokinase
- C. Pyruvate kinase
- D. Hexokinase
- E. Lactate dehydrogenase

Correct answer = C. Decreased lactate production in the erythrocyte indicates a defect in glycolysis. Among patients exhibiting genetic defects of glycolytic enzymes, about 95% show a deficiency in pyruvate kinase. Pyruvate kinase deficiency is the second most common cause (after glucose 6-phosphate dehydrogenase deficiency) of enzyme deficiency-related hemolytic anemia.

Tricarboxylic Acid Cycle

9

I. OVERVIEW

The tricarboxylic acid cycle (TCA cycle, also called the Krebs cycle or the citric acid cycle) plays several roles in metabolism. It is the final pathway where the oxidative metabolism of carbohydrates, amino acids, and fatty acids converge, their carbon skeletons being converted to CO_2 . This oxidation provides energy for the production of the majority of ATP in most animals, including humans. The cycle occurs totally in the mitochondria and is, therefore, in close proximity to the reactions of electron transport (see p. 73), which oxidize the reduced coenzymes produced by the cycle. The TCA cycle is an aerobic pathway, because O_2 is required as the final electron acceptor. Most of the body's catabolic pathways converge on the TCA cycle (Figure 9.1). Reactions such as the catabolism of some amino acids generate intermediates of the cycle and are called anaplerotic reactions. The citric acid cycle also supplies intermediates for a number of important synthetic reactions. For example, the cycle functions in the formation of glucose from the carbon skeletons of some amino acids, and it provides building blocks for the synthesis of some amino acids (see p. 267) and heme (see p. 278). Therefore, this cycle should not be viewed as a closed circle, but instead as a traffic circle with compounds entering and leaving as required.

II. REACTIONS OF THE TCA CYCLE

In the TCA cycle, oxaloacetate is first condensed with an acetyl group from acetyl coenzyme A (CoA), and then is regenerated as the cycle is completed (Figure 9.1). Thus, the entry of one acetyl CoA into one round of the TCA cycle does not lead to the net production or consumption of intermediates. [Note: Two carbons entering the cycle as acetyl CoA are balanced by two CO_2 exiting.]

A. Oxidative decarboxylation of pyruvate

Pyruvate, the endproduct of aerobic glycolysis, must be transported into the mitochondrion before it can enter the TCA cycle. This is accomplished by a specific pyruvate transporter that helps pyruvate cross the inner mitochondrial membrane. Once in the matrix, pyruvate is converted to acetyl CoA by the *pyruvate dehydrogenase complex*, which is a multienzyme complex. Strictly speaking, the *pyruvate dehydrogenase complex* is not part of the TCA cycle proper, but is a major source of acetyl CoA—the two-carbon substrate for the cycle.

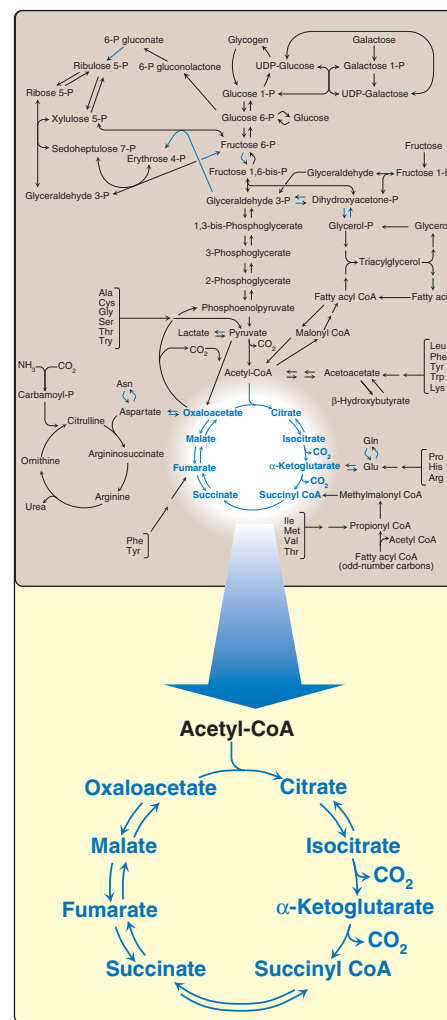


Figure 9.1

The tricarboxylic acid cycle shown as a part of the central pathways of energy metabolism. (See Figure 8.2, p. 92 for a more detailed view of the metabolic map.)

- 1. Component enzymes:** The *pyruvate dehydrogenase complex* (*PDH complex*) is a multimolecular aggregate of three enzymes, *pyruvate dehydrogenase* (*PDH* or E_1 , also called a *decarboxylase*), *dihydrolipoyl transacetylase* (E_2), and *dihydrolipoyl dehydrogenase* (E_3). Each catalyzes a part of the overall reaction (Figure 9.2). Their physical association links the reactions in proper sequence without the release of intermediates. In addition to the enzymes participating in the conversion of pyruvate to acetyl CoA, the complex also contains two tightly bound regulatory enzymes, *pyruvate dehydrogenase kinase* and *pyruvate dehydrogenase phosphatase*.
- 2. Coenzymes:** The *PDH complex* contains five coenzymes that act as carriers or oxidants for the intermediates of the reactions shown in Figure 9.2. E_1 requires thiamine pyrophosphate (TPP), E_2 requires lipoic acid and CoA, and E_3 requires FAD and NAD^+ .

Deficiencies of thiamine or niacin can cause serious central nervous system problems. This is because brain cells are unable to produce sufficient ATP (via the TCA cycle) if the *PDH complex* is inactive. Wernicke-Korsakoff, an encephalopathy-psychosis syndrome due to thiamine deficiency, may be seen with alcohol abuse.

- 3. Regulation of the pyruvate dehydrogenase complex:** Covalent modification by the two regulatory enzymes that are part of the complex alternately activate and inactivate E_1 (*PDH*). The cyclic AMP-independent *PDH kinase* phosphorylates and, thereby, inhibits E_1 , whereas *PDH phosphatase* dephosphorylates and

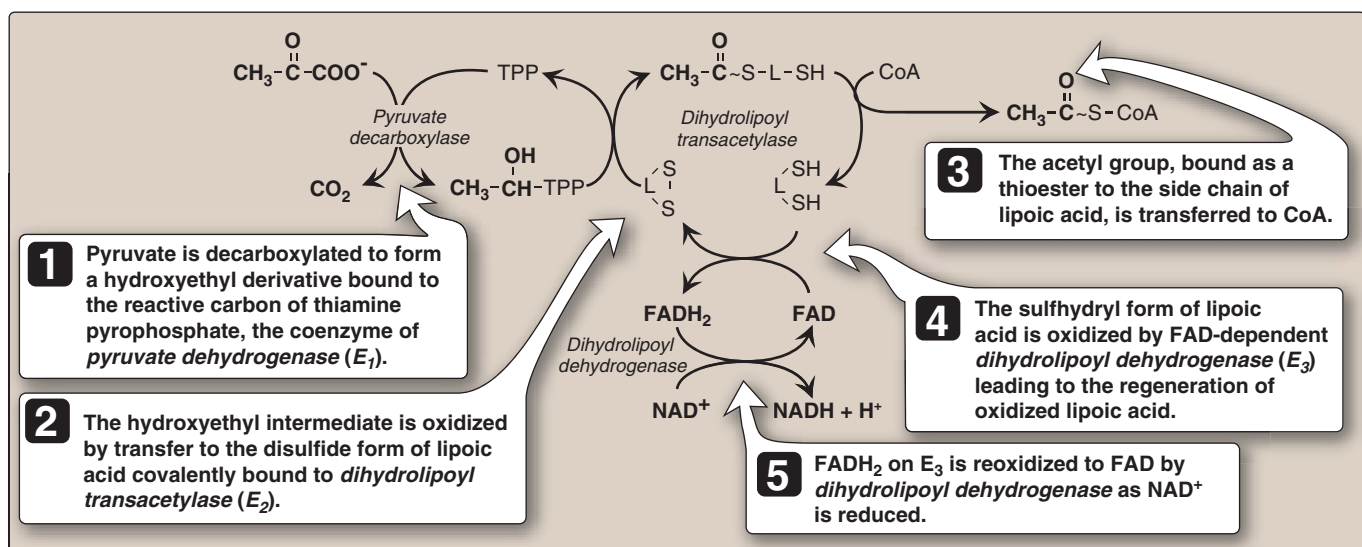


Figure 9.2

Mechanism of action of the *pyruvate dehydrogenase complex*. TPP = thiamine pyrophosphate; L = lipoic acid.

activates E_1 (Figure 9.3). The *kinase* itself is allosterically activated by ATP, acetyl CoA, and NADH. Therefore, in the presence of these high-energy signals, the *PDH complex* is turned off. Pyruvate is a potent inhibitor of *PDH kinase*. Therefore, if pyruvate concentrations are elevated, E_1 will be maximally active. Calcium is a strong activator of *PDH phosphatase*, stimulating E_1 activity. This is particularly important in skeletal muscle, where release of Ca^{2+} during contraction stimulates the *PDH complex*, and thereby energy production. [Note: Although covalent regulation by the *kinase* and *phosphatase* is key, the complex is also subject to product (NADH, acetyl CoA) inhibition.]

- 4. Pyruvate dehydrogenase deficiency:** A deficiency in the E_1 component of the *PDH complex*, although rare, is the most common biochemical cause of congenital lactic acidosis. This enzyme deficiency results in an inability to convert pyruvate to acetyl CoA, causing pyruvate to be shunted to lactic acid via *lactate dehydrogenase* (see p. 103). This causes particular problems for the brain, which relies on the TCA cycle for most of its energy, and is particularly sensitive to acidosis. Symptoms are variable and include neurodegeneration, muscle spasticity and, in the neonatal onset form, early death. The E_1 defect is X-linked, but because of the importance of the enzyme in the brain, it affects both males and females. Therefore, the defect is classified as X-linked dominant. There is no proven treatment for *pyruvate dehydrogenase* deficiency: however, dietary restriction of carbohydrate and supplementation with TPP may reduce symptoms in select patients.

Leigh syndrome (subacute necrotizing encephalomyelopathy) is a rare, progressive neurological disorder that is the result of defects in mitochondrial ATP production, primarily as a result of mutations in the *PDH complex*, the electron transport chain, or *ATP synthase*. Both nuclear and mtDNA can be affected.

- 5. Mechanism of arsenic poisoning:** As previously described (see p. 101), arsenic can interfere with glycolysis at the *glyceraldehyde 3-phosphate* step, thereby decreasing ATP production. “Arsenic poisoning” is, however, due primarily to inhibition of enzymes that require lipoic acid as a coenzyme, including E_2 of the *PDH complex*, α -ketoglutarate dehydrogenase (see below), and *branched-chain α -keto acid dehydrogenase* (see p. 266). Arsenite (the trivalent form of arsenic) forms a stable complex with the thiol ($-\text{SH}$) groups of lipoic acid, making that compound unavailable to serve as a coenzyme. When it binds to lipoic acid in the *PDH complex*, pyruvate (and consequently lactate) accumulates. Like *pyruvate dehydrogenase* deficiency, this particularly affects the brain, causing neurologic disturbances and death.

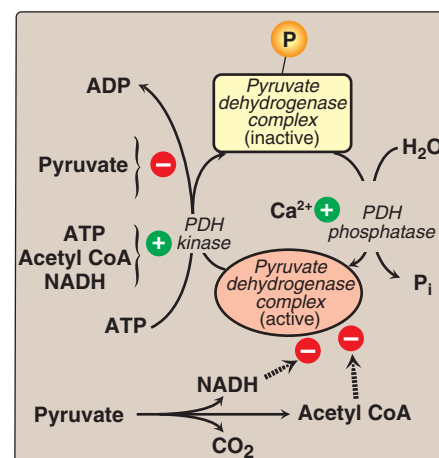


Figure 9.3
Regulation of *pyruvate dehydrogenase complex*.
[\dashrightarrow denotes product inhibition.]

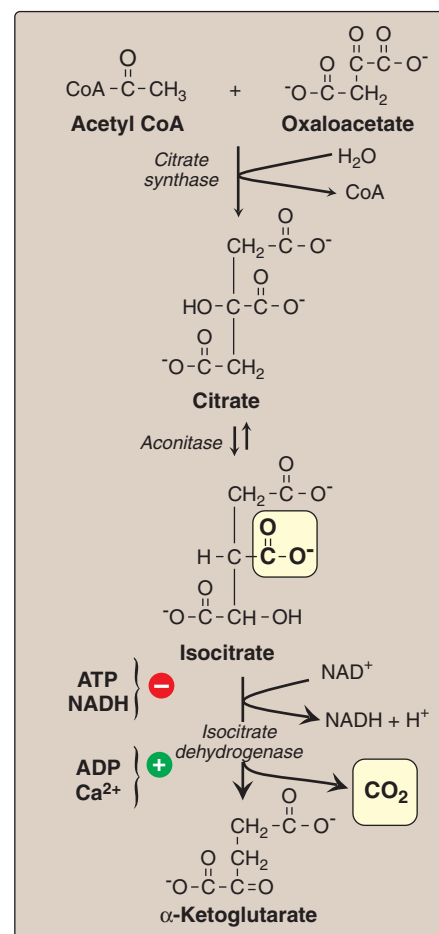
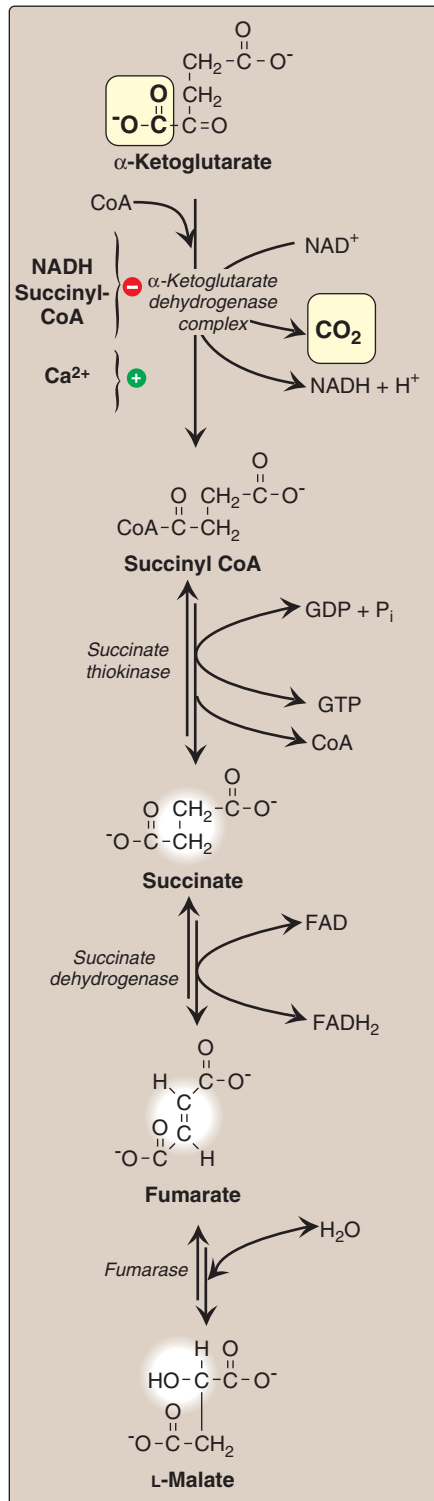


Figure 9.4
Formation of α -ketoglutarate from acetyl CoA and oxaloacetate.

**Figure 9.5**

Formation of malate from α -ketoglutarate.

B. Synthesis of citrate from acetyl CoA and oxaloacetate

The condensation of acetyl CoA and oxaloacetate to form citrate (a tricarboxylic acid) is catalyzed by *citrate synthase* (Figure 9.4). This aldol condensation has an equilibrium far in the direction of citrate synthesis. In humans, *citrate synthase* is not an allosteric enzyme. It is inhibited by its product, citrate. Substrate availability is another means of regulation for *citrate synthase*. The binding of oxaloacetate causes a conformational change in the enzyme that generates a binding site for acetyl CoA. [Note: Citrate, in addition to being an intermediate in the TCA cycle, provides a source of acetyl CoA for the cytosolic synthesis of fatty acids (see p. 183). Citrate also inhibits *phosphofructokinase*, the rate-limiting enzyme of glycolysis (see p. 99), and activates *acetyl CoA carboxylase* (the rate-limiting enzyme of fatty acid synthesis; see p. 183).]

C. Isomerization of citrate

Citrate is isomerized to isocitrate by *aconitase*, an Fe-S protein (see Figure 9.4). [Note: *Aconitase* is inhibited by fluoroacetate, a compound that is used as a rat poison. Fluoroacetate is converted to fluoroacetyl CoA, which condenses with oxaloacetate to form fluorocitrate—a potent inhibitor of *aconitase*—resulting in citrate accumulation.]

D. Oxidation and decarboxylation of isocitrate

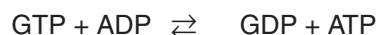
Isocitrate dehydrogenase catalyzes the irreversible oxidative decarboxylation of isocitrate, yielding the first of three NADH molecules produced by the cycle, and the first release of CO_2 (see Figure 9.4). This is one of the rate-limiting steps of the TCA cycle. The enzyme is allosterically activated by ADP (a low-energy signal) and Ca^{2+} , and is inhibited by ATP and NADH, whose levels are elevated when the cell has abundant energy stores.

E. Oxidative decarboxylation of α -ketoglutarate

The conversion of α -ketoglutarate to succinyl CoA is catalyzed by the α -ketoglutarate dehydrogenase complex, a multimolecular aggregate of three enzymes (Figure 9.5). The mechanism of this oxidative decarboxylation is very similar to that used for the conversion of pyruvate to acetyl CoA by the *PDH complex*. The reaction releases the second CO_2 and produces the second NADH of the cycle. The coenzymes required are thiamine pyrophosphate, lipoic acid, FAD, NAD^+ , and CoA. Each functions as part of the catalytic mechanism in a way analogous to that described for the *PDH complex* (see p. 110). The equilibrium of the reaction is far in the direction of succinyl CoA—a high-energy thioester similar to acetyl CoA. α -Ketoglutarate dehydrogenase complex is inhibited by its products, NADH and succinyl CoA, and activated by Ca^{2+} . However, it is not regulated by phosphorylation/dephosphorylation reactions as described for *PDH complex*. [Note: α -Ketoglutarate is also produced by the oxidative deamination (see p. 252) or transamination of the amino acid, glutamate (see p. 250).]

F. Cleavage of succinyl CoA

Succinate thiokinase (also called *succinyl CoA synthetase*—named for the reverse reaction) cleaves the high-energy thioester bond of succinyl CoA (see Figure 9.5). This reaction is coupled to phosphorylation of guanosine diphosphate (GDP) to guanosine triphosphate (GTP). GTP and ATP are energetically interconvertible by the *nucleoside diphosphate kinase* reaction:



The generation of GTP by *succinate thiokinase* is another example of substrate-level phosphorylation (see p. 102). [Note: Succinyl CoA is also produced from propionyl CoA derived from the metabolism of fatty acids with an odd number of carbon atoms (see p. 193), and from the metabolism of several amino acids (see pp. 265–266).]

G. Oxidation of succinate

Succinate is oxidized to fumarate by *succinate dehydrogenase*, as FAD (its coenzyme) is reduced to FADH_2 (see Figure 9.5). *Succinate dehydrogenase* is the only enzyme of the TCA cycle that is embedded in the inner mitochondrial membrane. As such, it functions as Complex II of the electron transport chain (see p. 75). [Note: FAD, rather than NAD^+ , is the electron acceptor because the reducing power of succinate is not sufficient to reduce NAD^+ .]

H. Hydration of fumarate

Fumarate is hydrated to malate in a freely reversible reaction catalyzed by *fumarase* (also called *fumarate hydratase*, see Figure 9.5). [Note: Fumarate is also produced by the urea cycle (see p. 254), in purine synthesis (see p. 294), and during catabolism of the amino acids, phenylalanine and tyrosine (see p. 263).]

I. Oxidation of malate

Malate is oxidized to oxaloacetate by *malate dehydrogenase* (Figure 9.6). This reaction produces the third and final NADH of the cycle. The ΔG^0 of the reaction is positive, but the reaction is driven in the direction of oxaloacetate by the highly exergonic *citrate synthase* reaction. [Note: Oxaloacetate is also produced by the transamination of the amino acid, aspartic acid (see p. 250).]

III. ENERGY PRODUCED BY THE TCA CYCLE

Two carbon atoms enter the cycle as acetyl CoA and leave as CO_2 . The cycle does not involve net consumption or production of oxaloacetate or of any other intermediate. Four pairs of electrons are transferred during one turn of the cycle: three pairs of electrons reducing three NAD^+ to NADH and one pair reducing FAD to FADH_2 . Oxidation of one NADH by the electron transport chain leads to formation of approximately three ATP, whereas oxidation of FADH_2 yields approximately two ATP (see p. 77). The total yield of ATP from the oxidation of one acetyl CoA is shown in Figure 9.7. Figure 9.8 summarizes the reactions of the TCA cycle.

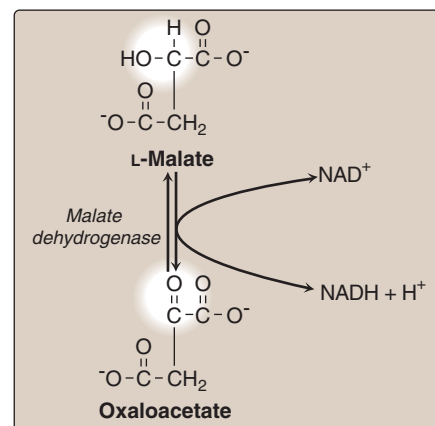


Figure 9.6

Formation of oxaloacetate from malate.

Energy producing reaction	Number of ATP produced
$3 \text{ NADH} \longrightarrow 3 \text{ NAD}^+$	9
$\text{FADH}_2 \longrightarrow \text{FAD}$	2
$\text{GDP} + \text{P}_i \longrightarrow \text{GTP}$	1
<hr/>	
12 ATP/acetyl CoA oxidized	

Figure 9.7

Number of ATP molecules produced from the oxidation of one molecule of acetyl CoA (using both substrate-level and oxidative phosphorylation).

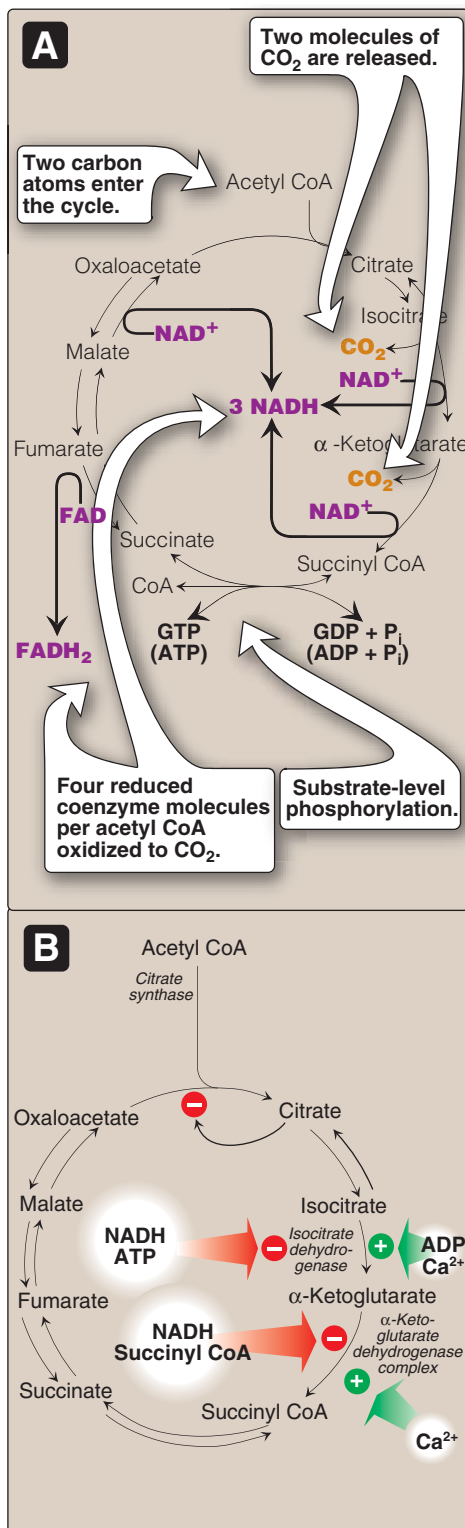


Figure 9.8

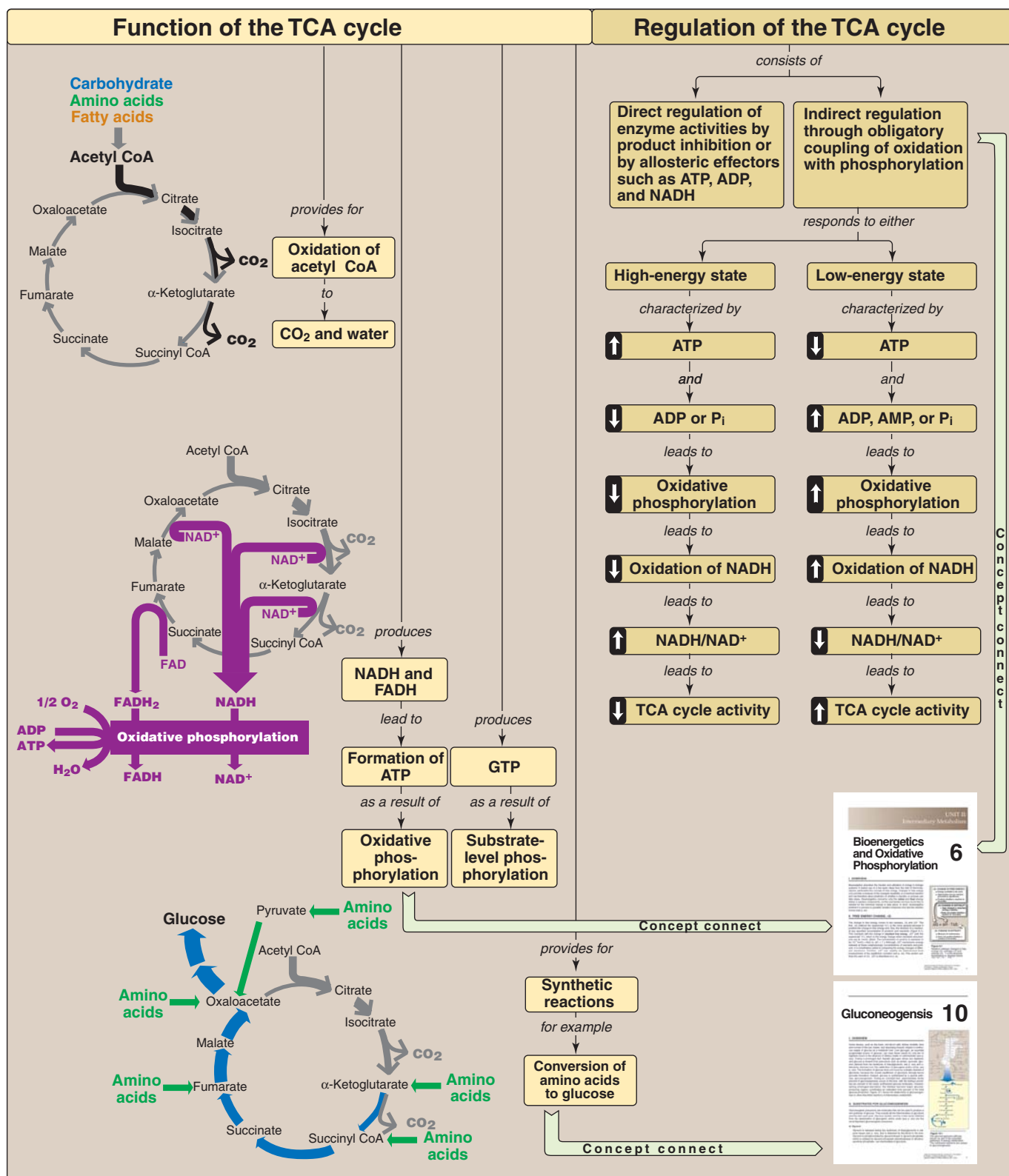
A. Production of reduced coenzymes, ATP, and CO₂ in the citric acid cycle. B. Inhibitors and activators of the cycle.

IV. REGULATION OF THE TCA CYCLE

In contrast to glycolysis, which is regulated primarily by *phosphofructokinase*, the TCA cycle is controlled by the regulation of several enzyme activities (see Figure 9.8). The most important of these regulated enzymes are those that catalyze reactions with highly negative ΔG^0 : *citrate synthase*, *isocitrate dehydrogenase*, and *α-ketoglutarate dehydrogenase complex*. Reducing equivalents needed for oxidative phosphorylation are generated by the *pyruvate dehydrogenase complex* and the TCA cycle, and both processes are upregulated in response to a rise in ADP.

V. CHAPTER SUMMARY

Pyruvate is oxidatively decarboxylated by pyruvate dehydrogenase (PDH) complex, producing **acetyl CoA**, which is the major fuel for the tricarboxylic acid cycle (TCA cycle, Figure 9.9). This multienzyme complex requires five coenzymes: **thiamine pyrophosphate**, **lipoic acid**, **FAD**, **NAD⁺**, and **coenzyme A**. PDH complex is regulated by covalent modification of E₁ (pyruvate dehydrogenase, PDH) by PDH kinase and PDH phosphatase: phosphorylation inhibits PDH. PDH kinase is allosterically activated by ATP, acetyl CoA, and NADH and inhibited by pyruvate; the phosphatase is activated by Ca²⁺. **Pyruvate dehydrogenase deficiency** is the most common biochemical cause of **congenital lactic acidosis**. The central nervous system is particularly affected in this is **X-linked dominant** disorder. **Arsenic poisoning** causes inactivation of PDH complex by binding to lipoic acid. **Citrate** is synthesized from **oxaloacetate** and **acetyl CoA** by **citrate synthase**. This enzyme is subject to product inhibition by citrate. Citrate is isomerized to **isocitrate** by **aconitase**. **Isocitrate** is oxidized and decarboxylated by **isocitrate dehydrogenase** to **α-ketoglutarate**, producing CO₂ and NADH. The enzyme is inhibited by ATP and NADH, and activated by ADP and Ca²⁺. **α-Ketoglutarate** is oxidatively decarboxylated to **succinyl CoA** by the **α-ketoglutarate dehydrogenase complex**, producing CO₂ and NADH. The enzyme is very similar to pyruvate dehydrogenase and uses the same coenzymes. **α-Ketoglutarate dehydrogenase complex** is activated by calcium and inhibited by NADH and succinyl CoA, but is not covalently regulated. **Succinyl CoA** is cleaved by **succinate thiokinase** (also called **succinyl CoA synthetase**), producing **succinate** and **GTP**. This is an example of **substrate-level phosphorylation**. **Succinate** is oxidized to **fumarate** by **succinate dehydrogenase**, producing **FADH₂**. **Fumarate** is hydrated to **malate** by **fumarase** (**fumarate hydratase**), and **malate** is oxidized to **oxaloacetate** by **malate dehydrogenase**, producing NADH. **Three NADH**, **one FADH₂**, and **one GTP** (whose terminal phosphate can be transferred to ADP by nucleoside diphosphate kinase, producing ATP) are produced by one round of the TCA cycle. The generation of acetyl CoA by the oxidation of pyruvate via the PDH complex also produces an NADH. Oxidation of these NADHs and FADH₂ by the electron transport chain yields 14 ATP. An additional ATP (GTP) comes from substrate level phosphorylation in the TCA cycle. Therefore, a total of 15 ATPs are produced from the complete mitochondrial oxidation of pyruvate to CO₂.

**Figure 9.9**

Key concept map for the tricarboxylic acid (TCA) cycle.

Study Questions

Choose the ONE correct answer.

9.1 The conversion of pyruvate to acetyl CoA and CO₂:

- A. is reversible.
- B. involves the participation of lipoic acid.
- C. is activated when pyruvate dehydrogenase (PDH, E₁) of the pyruvate dehydrogenase complex is phosphorylated by PDH kinase in the presence of ATP.
- D. occurs in the cytosol.
- E. depends on the coenzyme biotin.

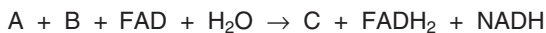
Correct answer = B. Lipoic acid is an intermediate acceptor of the acetyl group formed in the reaction. Pyruvate dehydrogenase complex catalyzes an irreversible reaction that is inhibited when the PDH (E₁) component is phosphorylated. The enzyme complex is located in the mitochondrial matrix. Biotin is utilized by carboxylases.

9.2 Which one of the following conditions decreases the oxidation of acetyl CoA by the citric acid cycle?

- A. A low ATP/ADP ratio
- B. A low NADH concentration due to rapid oxidation to NAD⁺ through the respiratory chain
- C. A low NAD⁺/NADH ratio
- D. A high concentration of AMP
- E. A low GTP/GDP ratio

Correct answer = C. A low NAD⁺/NADH ratio limits the rates of the NAD⁺-requiring dehydrogenases. A low ATP/ADP or GTP/GDP ratio stimulates the cycle. AMP does not directly affect the cycle.

9.3 The following is the sum of three steps in the citric acid cycle.



Choose the lettered answer that corresponds to the missing "A", "B", and "C" in the equation.

Reactant A	Reactant B	Reactant C
A. Succinyl CoA	GDP	Succinate
B. Succinate	NAD ⁺	Oxaloacetate
C. Fumarate	NAD ⁺	Oxaloacetate
D. Succinate	NAD ⁺	Malate
E. Fumarate	GTP	Malate

Correct answer = B. Succinate + NAD⁺ + FAD → oxaloacetate + NADH + FADH₂

9.4 A 1-month-old male showed abnormalities of the nervous system and lactic acidosis. Enzyme assay for pyruvate dehydrogenase (PDH) activity on extracts of cultured skin fibroblasts showed 5% of normal activity, with a low concentration (1 × 10⁻⁴ mM) of thiamine pyrophosphate (TPP), but 80% of normal activity when the assay contained a high (0.4 mM) concentration of TPP. Which one of the following statements concerning this patient is most correct?

- A. Elevated levels of lactate and pyruvate in the blood reliably predict the presence of PDH deficiency.
- B. The patient is expected to show disturbances in fatty acid degradation.
- C. A diet consisting of high carbohydrate intake would be expected to be beneficial in this patient.
- D. Alanine concentration in the blood is expected to be less than normal.
- E. Administration of thiamine is expected to reduce his serum lactate concentration and improve his clinical symptoms.

Correct answer = E. The patient appears to have a thiamine-responsive PDH deficiency. The enzyme fails to bind thiamine pyrophosphate at low concentration, but shows significant activity at a high concentration of the coenzyme. This mutation, which affects the K_m of the enzyme for the coenzyme, is present in some, but not all, cases of PDH deficiency. All inborn errors of PDH are associated with elevated levels of lactate, pyruvate, and alanine (the transamination product of pyruvate). Patients routinely show neuroanatomic defects, developmental delay, and often early death. Elevated lactate and pyruvate are also observed in pyruvate carboxylase deficiency, another rare defect in pyruvate metabolism. Because PDH is an integral part of carbohydrate metabolism, a diet low in carbohydrates would be expected to blunt the effects of the enzyme deficiency. By contrast, fatty acid degradation occurs via conversion to acetyl CoA by β-oxidation, a process that does not involve pyruvate as an intermediate. Thus, fatty acid metabolism is not disturbed in this enzyme deficiency.

Gluconeogenesis

10

I. OVERVIEW

Some tissues, such as the brain, red blood cells, kidney medulla, lens and cornea of the eye, testes, and exercising muscle, require a continuous supply of glucose as a metabolic fuel. Liver glycogen, an essential postprandial source of glucose, can meet these needs for only 10–18 hours in the absence of dietary intake of carbohydrate (see p. 329). During a prolonged fast, however, hepatic glycogen stores are depleted, and glucose is formed from precursors such as lactate, pyruvate, glycerol (derived from the backbone of triacylglycerols, see p. 190), and α -ketoacids (derived from the catabolism of glucogenic amino acids, see p. 261). The formation of glucose does not occur by a simple reversal of glycolysis, because the overall equilibrium of glycolysis strongly favors pyruvate formation. Instead, glucose is synthesized by a special pathway, gluconeogenesis, that requires both mitochondrial and cytosolic enzymes. During an overnight fast, approximately 90% of gluconeogenesis occurs in the liver, with the kidneys providing 10% of the newly synthesized glucose molecules. However, during prolonged fasting, the kidneys become major glucose-producing organs, contributing an estimated 40% of the total glucose production. Figure 10.1 shows the relationship of gluconeogenesis to other important reactions of intermediary metabolism.

II. SUBSTRATES FOR GLUCONEOGENESIS

Gluconeogenic precursors are molecules that can be used to produce a net synthesis of glucose. They include intermediates of glycolysis and the tricarboxylic acid (TCA) cycle. Glycerol, lactate, and the α -keto acids obtained from the transamination of glucogenic amino acids are the most important gluconeogenic precursors.

A. Glycerol

Glycerol is released during the hydrolysis of triacylglycerols in adipose tissue (see p. 190), and is delivered by the blood to the liver. Glycerol is phosphorylated by *glycerol kinase* to glycerol phosphate, which is oxidized by *glycerol phosphate dehydrogenase* to dihydroxyacetone phosphate—an intermediate of glycolysis. [Note: Adipocytes cannot phosphorylate glycerol because they essentially lack *glycerol kinase*.]

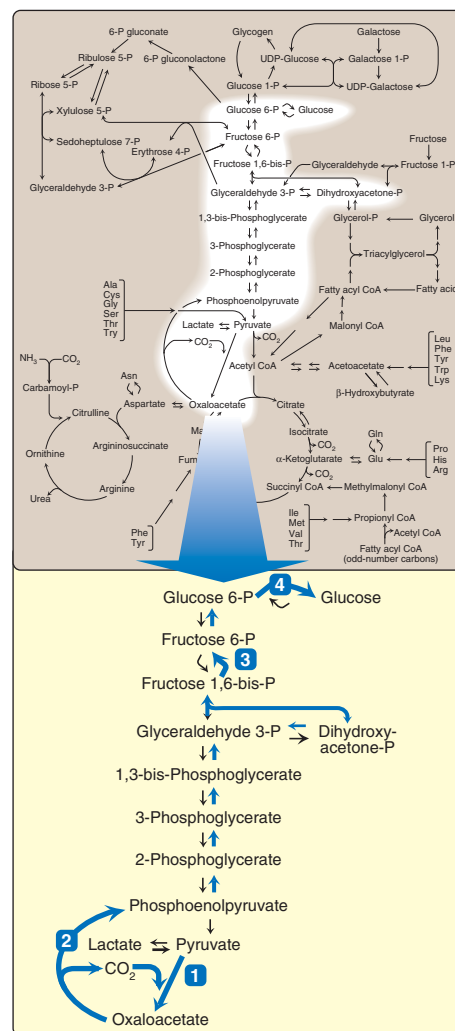


Figure 10.1

The gluconeogenesis pathway shown as part of the essential pathways of energy metabolism. The numbered reactions are unique to gluconeogenesis. (See Figure 8.2, p. 92, for a more detailed map of metabolism.)

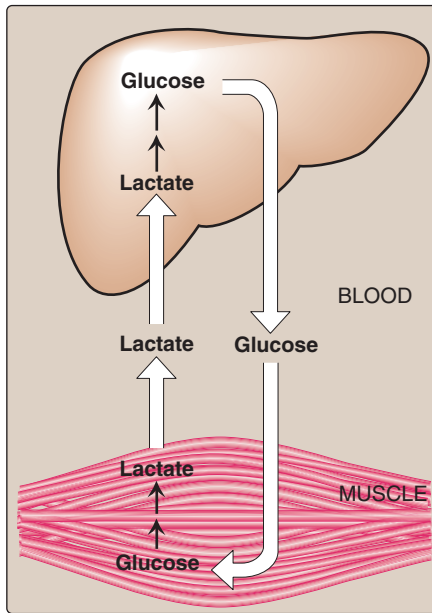


Figure 10.2
The Cori cycle.

B. Lactate

Lactate is released into the blood by exercising skeletal muscle, and by cells that lack mitochondria, such as red blood cells. In the Cori cycle, bloodborne glucose is converted by exercising muscle to lactate, which diffuses into the blood. This lactate is taken up by the liver and reconverted to glucose, which is released back into the circulation (Figure 10.2).

C. Amino acids

Amino acids derived from hydrolysis of tissue proteins are the major sources of glucose during a fast. α -Ketoacids, such as α -keto-glutarate, are derived from the metabolism of glucogenic amino acids (see p. 261). These α -ketoacids can enter the TCA cycle and form oxaloacetate (OAA)—a direct precursor of phosphoenolpyruvate (PEP). [Note: Acetyl coenzyme A (CoA) and compounds that give rise only to acetyl CoA (for example, acetoacetate and amino acids such as lysine and leucine) cannot give rise to a net synthesis of glucose. This is due to the irreversible nature of the *pyruvate dehydrogenase* reaction, which converts pyruvate to acetyl CoA (see p. 109). These compounds give rise instead to ketone bodies (see p. 195) and are therefore termed ketogenic.]

III. REACTIONS UNIQUE TO GLUCONEOGENESIS

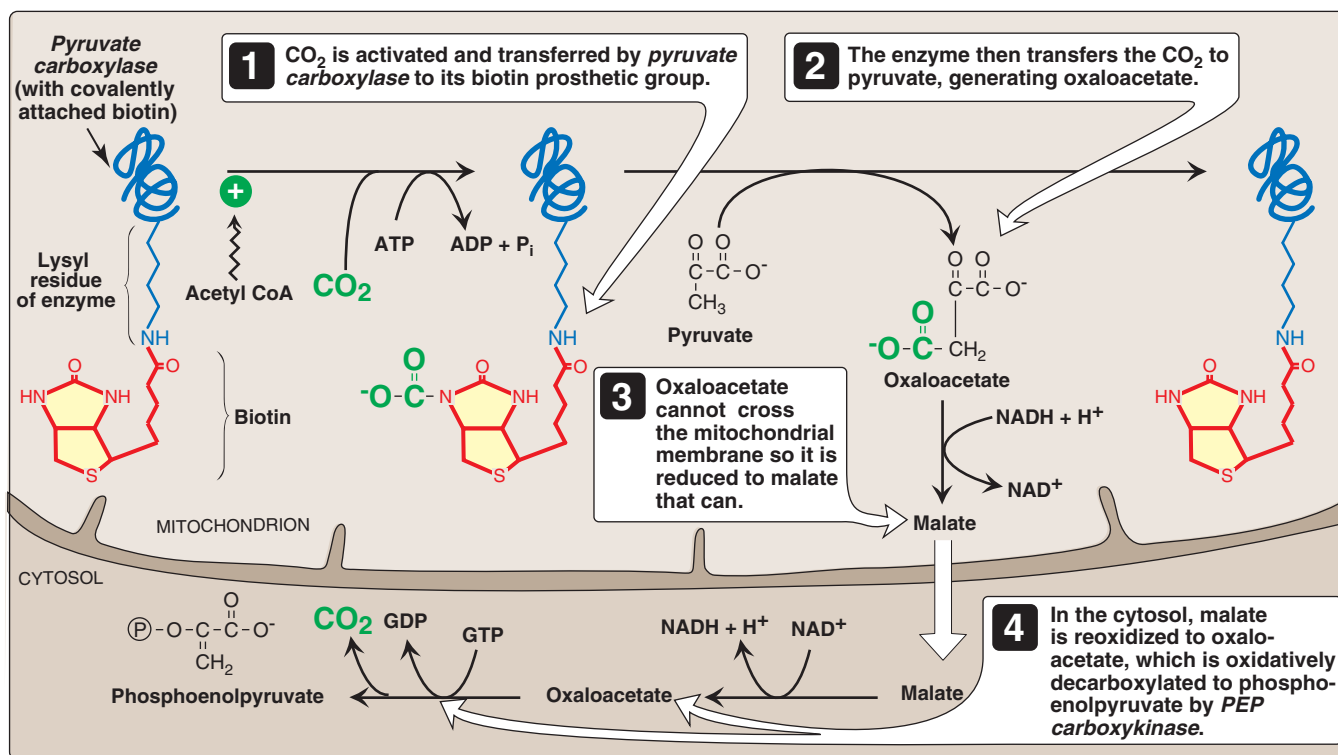
Seven glycolytic reactions are reversible and are used in the synthesis of glucose from lactate or pyruvate. However, three of the reactions are irreversible and must be circumvented by four alternate reactions that energetically favor the synthesis of glucose. These reactions, unique to gluconeogenesis, are described below.

A. Carboxylation of pyruvate

The first “roadblock” to overcome in the synthesis of glucose from pyruvate is the irreversible conversion in glycolysis of PEP to pyruvate by *pyruvate kinase*. In gluconeogenesis, pyruvate is first carboxylated by *pyruvate carboxylase* to OAA, which is then converted to PEP by the action of *PEP-carboxykinase* (Figure 10.3).

1. **Biotin is a coenzyme:** *Pyruvate carboxylase* requires biotin (see p. 381) covalently bound to the ϵ -amino group of a lysine residue in the enzyme (see Figure 10.3). Hydrolysis of ATP drives the formation of an enzyme–biotin– CO_2 intermediate. This high-energy complex subsequently carboxylates pyruvate to form OAA. [Note: This reaction occurs in the mitochondria of liver and kidney cells, and has two purposes: to provide an important substrate for gluconeogenesis, and to provide OAA that can replenish the TCA cycle intermediates that may become depleted, depending on the synthetic needs of the cell. Muscle cells also contain *pyruvate carboxylase*, but use the OAA produced only for the latter purpose—they do not synthesize glucose.]

Pyruvate carboxylase is one of several *carboxylases* that require biotin. Others include *acetyl CoA carboxylase* (p. 183), *propionyl CoA carboxylase* (p. 194), and *methylcrotonyl CoA carboxylase* (p. 266).

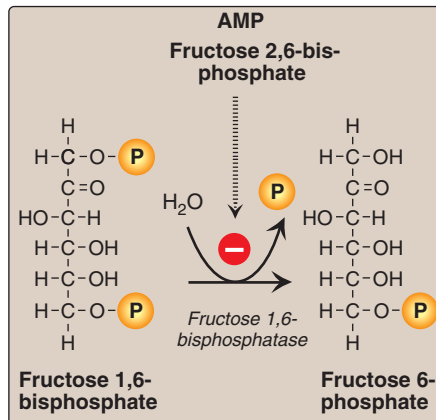
**Figure 10.3**

Activation and transfer of CO_2 to pyruvate, followed by transport of oxaloacetate to the cytosol and subsequent decarboxylation. Alternatively, OAA can be converted to PEP that is transported out of the mitochondria.

2. Allosteric regulation: *Pyruvate carboxylase* is allosterically activated by acetyl CoA. Elevated levels of acetyl CoA in mitochondria signal a metabolic state in which the increased synthesis of OAA is required. For example, this occurs during fasting, when OAA is used for the synthesis of glucose by gluconeogenesis in the liver and kidney. Conversely, at low levels of acetyl CoA, *pyruvate carboxylase* is largely inactive, and pyruvate is primarily oxidized by the *pyruvate dehydrogenase complex* to produce acetyl CoA that can be further oxidized by the TCA cycle (see p. 109).

B. Transport of oxaloacetate to the cytosol

OAA must be converted to PEP for gluconeogenesis to continue. The enzyme that catalyzes this conversion is found in both the mitochondria and the cytosol in humans. The PEP that is generated in the mitochondria is transported to the cytosol by a specific transporter, whereas that generated in the cytosol requires the transport of OAA from the mitochondria to the cytosol. However, OAA is unable to directly cross the inner mitochondrial membrane; it must first be reduced to malate by mitochondrial *malate dehydrogenase*. Malate can be transported from the mitochondria to the cytosol, where it is reoxidized to oxaloacetate by cytosolic *malate dehydrogenase* as NAD^+ is reduced (see Figure 10.3). The NADH produced is used in the reduction of 1,3-BPG to glyceraldehyde 3-phosphate (see p. 101), a step common to both glycolysis and gluconeogenesis.

**Figure 10.4**

Dephosphorylation of fructose 1,6-bisphosphate.

C. Decarboxylation of cytosolic oxaloacetate

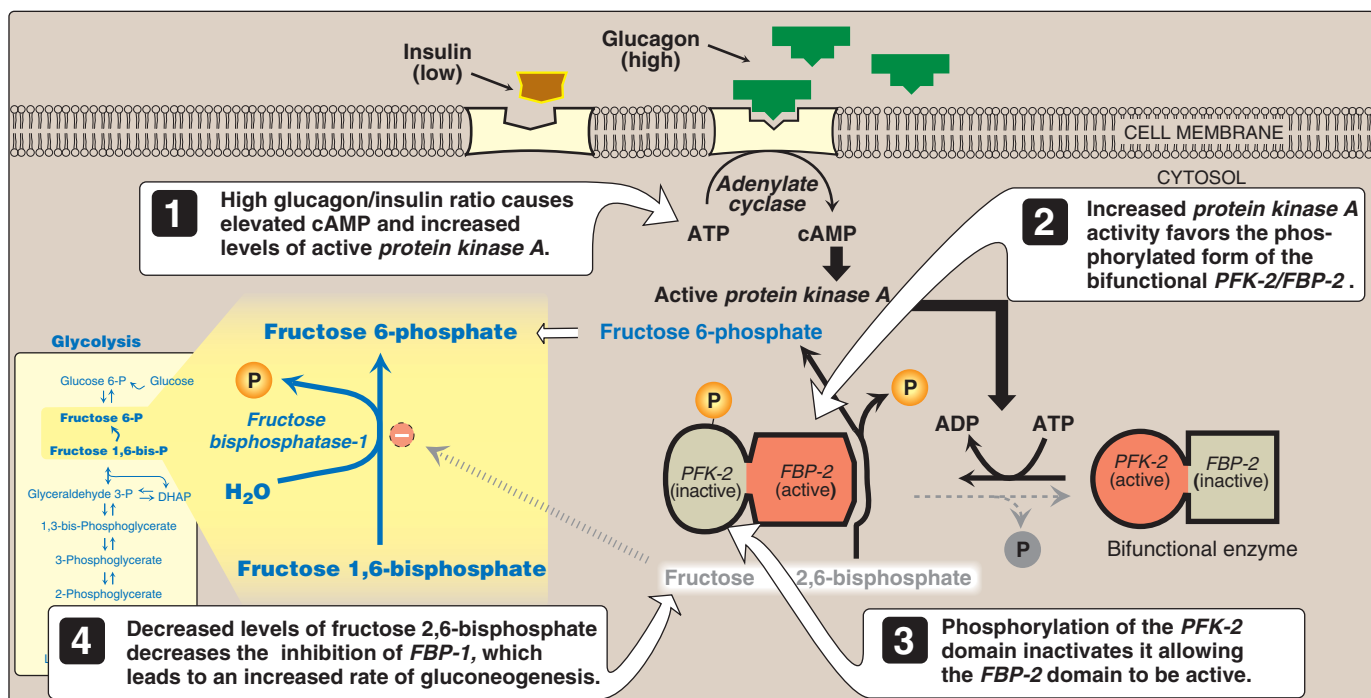
Oxaloacetate is decarboxylated and phosphorylated to PEP in the cytosol by *PEP-carboxykinase* (also referred to as *PEPCK*). The reaction is driven by hydrolysis of guanosine triphosphate (GTP, see Figure 10.3). The combined actions of *pyruvate carboxylase* and *PEP-carboxykinase* provide an energetically favorable pathway from pyruvate to PEP. Then, PEP is acted on by the reactions of glycolysis running in the reverse direction until it becomes fructose 1,6-bisphosphate.

The pairing of carboxylation with decarboxylation, as seen in gluconeogenesis, drives reactions that would otherwise be energetically unfavorable. A similar strategy is used in fatty acid synthesis (see pp. 183–184).

D. Dephosphorylation of fructose 1,6-bisphosphate

Hydrolysis of fructose 1,6-bisphosphate by *fructose 1,6-bisphosphatase* bypasses the irreversible *phosphofructokinase-1* reaction, and provides an energetically favorable pathway for the formation of fructose 6-phosphate (Figure 10.4). This reaction is an important regulatory site of gluconeogenesis.

1. Regulation by energy levels within the cell: *Fructose 1,6-bisphosphatase* is inhibited by elevated levels of adenosine monophosphate (AMP), which signal an “energy-poor” state in the cell.

**Figure 10.5**

Effect of elevated glucagon on the intracellular concentration of fructose 2,6-bisphosphate in the liver. PFK-2 = phosphofructokinase-2; FBP-2 = fructose bisphosphatase-2.

Conversely, high levels of ATP and low concentrations of AMP stimulate gluconeogenesis, an energy-requiring pathway.

- 2. Regulation by fructose 2,6-bisphosphate:** *Fructose 1,6-bisphosphatase*, found in liver and kidney, is inhibited by fructose 2,6-bisphosphate, an allosteric effector whose concentration is influenced by the level of circulating glucagon (Figure 10.5). [Note: The signals that inhibit (low energy, high fructose 2,6-bisphosphate) or favor (high energy, low fructose 2,6-bisphosphate) gluconeogenesis have the opposite effect on glycolysis, providing reciprocal control of the pathways that synthesize and oxidize glucose (see p. 100).]

E. Dephosphorylation of glucose 6-phosphate

Hydrolysis of glucose 6-phosphate by *glucose 6-phosphatase* bypasses the irreversible *hexokinase* reaction, and provides an energetically favorable pathway for the formation of free glucose (Figure 10.6). Liver and kidney are the only organs that release free glucose from glucose 6-phosphate. This process actually requires two proteins: *glucose 6-phosphate translocase*, which transports glucose 6-phosphate across the endoplasmic reticulum (ER) membrane, and the ER enzyme, *glucose 6-phosphatase* (found only in gluconeogenic cells), which removes the phosphate, producing free glucose (see Figure 10.6). [Note: These proteins are also required for the final step of glycogen degradation (see p. 130). Type Ia glycogen storage disease (see p. 130), due to an inherited deficiency of *glucose 6-phosphatase*, is characterized by severe fasting hypoglycemia, because free glucose is unable to be produced from either gluconeogenesis or glycogenolysis.] Specific transporters are responsible for releasing free glucose and phosphate back into the cytosol and, for glucose, into blood. [Note: Muscle lacks *glucose 6-phosphatase*, and therefore muscle glycogen can not be used to maintain blood glucose levels.]

F. Summary of the reactions of glycolysis and gluconeogenesis

Of the 11 reactions required to convert pyruvate to free glucose, seven are catalyzed by reversible glycolytic enzymes (Figure 10.7). The irreversible reactions of glycolysis catalyzed by *hexokinase*, *phosphofructokinase-1*, and *pyruvate kinase* are circumvented by *glucose 6-phosphatase*, *fructose 1,6-bisphosphatase*, and *pyruvate carboxylase/PEP-carboxykinase*. In gluconeogenesis, the equilibria of the seven reversible reactions of glycolysis are pushed in favor of glucose synthesis as a result of the essentially irreversible formation of PEP, fructose 6-phosphate, and glucose catalyzed by the gluconeogenic enzymes. [Note: The stoichiometry of gluconeogenesis from pyruvate couples the cleavage of six high-energy phosphate bonds and the oxidation of two NADH with the formation of each molecule of glucose (see Figure 10.7).]

IV. REGULATION OF GLUCONEOGENESIS

The moment-to-moment regulation of gluconeogenesis is determined primarily by the circulating level of glucagon, and by the availability of gluconeogenic substrates. In addition, slow adaptive changes in

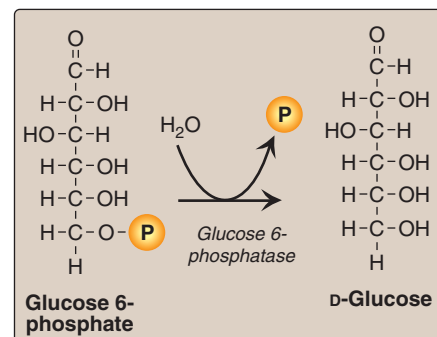


Figure 10.6

Dephosphorylation of glucose 6-phosphate allows release of free glucose from liver and kidney into blood.

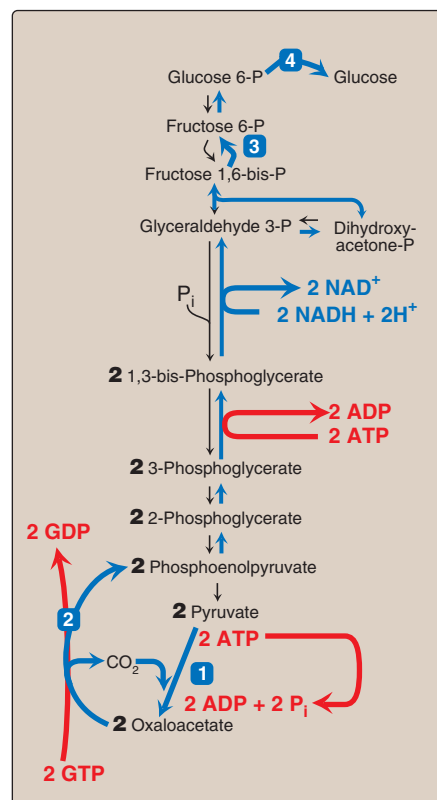


Figure 10.7

Summary of the reactions of glycolysis and gluconeogenesis, showing the energy requirements of gluconeogenesis.

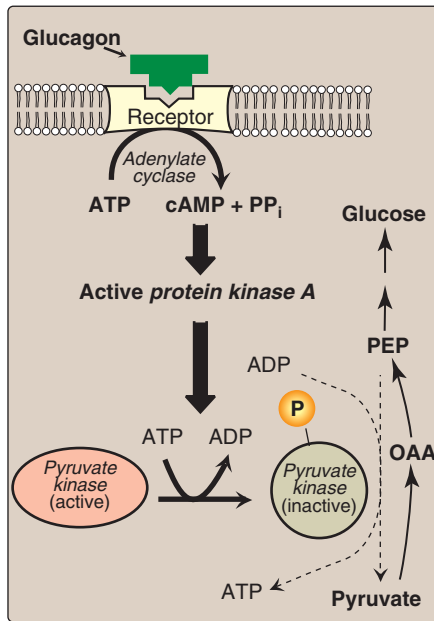


Figure 10.8

Covalent modification of *pyruvate kinase* results in inactivation of the enzyme. OAA = oxaloacetate. [Note: Only the hepatic isozyme is subject to covalent regulation.]

enzyme activity result from an alteration in the rate of enzyme synthesis or degradation, or both. [Note: Hormonal control of the glucoregulatory system is presented in Chapter 23.]

A. Glucagon

This hormone from the α cells of pancreatic islets (see p. 313) stimulates gluconeogenesis by three mechanisms.

- 1. Changes in allosteric effectors:** Glucagon lowers the level of fructose 2,6-bisphosphate, resulting in activation of *fructose 1,6-bisphosphatase* and inhibition of *phosphofructokinase-1*, thus favoring gluconeogenesis over glycolysis (see Figure 10.5). [Note: See p. 100 for the role of fructose 2,6-bisphosphate in the regulation of glycolysis.]
- 2. Covalent modification of enzyme activity:** Glucagon binds its G protein-coupled receptor (see p. 95) and, via an elevation in cyclic AMP (cAMP) level and *cAMP-dependent protein kinase* activity, stimulates the conversion of hepatic *pyruvate kinase* to its inactive (phosphorylated) form. This decreases the conversion of PEP to pyruvate, which has the effect of diverting PEP to the synthesis of glucose (Figure 10.8).
- 3. Induction of enzyme synthesis:** Glucagon increases the transcription of the gene for *PEP-carboxykinase*, thereby increasing the availability of this enzyme as levels of its substrate rise during fasting. [Note: Insulin causes decreased transcription of the mRNA for this enzyme.]

B. Substrate availability

The availability of gluconeogenic precursors, particularly glucogenic amino acids, significantly influences the rate of hepatic glucose synthesis. Decreased levels of insulin favor mobilization of amino acids from muscle protein, and provide the carbon skeletons for gluconeogenesis. In addition, ATP and NADH, coenzymes-cosubstrates required for gluconeogenesis, are primarily provided by the catabolism of fatty acids.

C. Allosteric activation by acetyl CoA

Allosteric activation of hepatic *pyruvate carboxylase* by acetyl CoA occurs during fasting. As a result of increased lipolysis in adipose tissue, the liver is flooded with fatty acids (see p. 330). The rate of formation of acetyl CoA by β -oxidation of these fatty acids exceeds the capacity of the liver to oxidize it to CO_2 and H_2O . As a result, acetyl CoA accumulates and leads to activation of *pyruvate carboxylase*. [Note: Acetyl CoA inhibits *pyruvate dehydrogenase* (by activating *PDH kinase*, see p. 111). Thus, this single compound can divert pyruvate toward gluconeogenesis and away from the TCA cycle.]

D. Allosteric inhibition by AMP

Fructose 1,6-bisphosphatase is inhibited by AMP—a compound that activates *phosphofructokinase-1*. This results in a reciprocal regulation of glycolysis and gluconeogenesis seen previously with fructose 2,6-bisphosphate (see p. 121). [Note: Elevated AMP thus stimulates pathways that oxidize nutrients to provide energy for the cell.]

V. CHAPTER SUMMARY

Gluconeogenic precursors include the **intermediates of glycolysis** and the **citric acid cycle**, **glycerol** released during the hydrolysis of triacylglycerols in adipose tissue, **lactate** released into the blood by cells that lack mitochondria and by exercising skeletal muscle, and **α -ketoacids** derived from the metabolism of glucogenic amino acids (Figure 10.9). Seven of the reactions of glycolysis are reversible and are used for gluconeogenesis in the liver and kidneys. Three reactions are **physiologically irreversible** and must be circumvented. These reactions are catalyzed by the glycolytic enzymes **pyruvate kinase**, **phosphofructokinase**, and **hexokinase**. **Pyruvate** is converted to oxaloacetate (OAA) and then to **phosphoenolpyruvate (PEP)** by **pyruvate carboxylase** and **PEP-carboxykinase**. The carboxylase requires **biotin** and **ATP**, and is allosterically activated by **acetyl CoA**. PEP-carboxykinase requires **GTP**. The transcription of its mRNA is increased by glucagon and decreased by insulin. **Fructose 1,6-bisphosphate** is converted to **fructose 6-phosphate** by **fructose 1,6-bisphosphatase**. This enzyme is **inhibited** by elevated levels of **AMP** and **activated** when **ATP** levels are elevated. The enzyme is also **inhibited** by **fructose 2,6-bisphosphate**, the primary allosteric activator of glycolysis. **Glucose 6-phosphate** is converted to **glucose** by **glucose 6-phosphatase**. This enzyme of the ER is required for the final step in gluconeogenesis, as well as hepatic and renal glycogen degradation. A deficiency of this enzyme results in severe, fasting hypoglycemia.

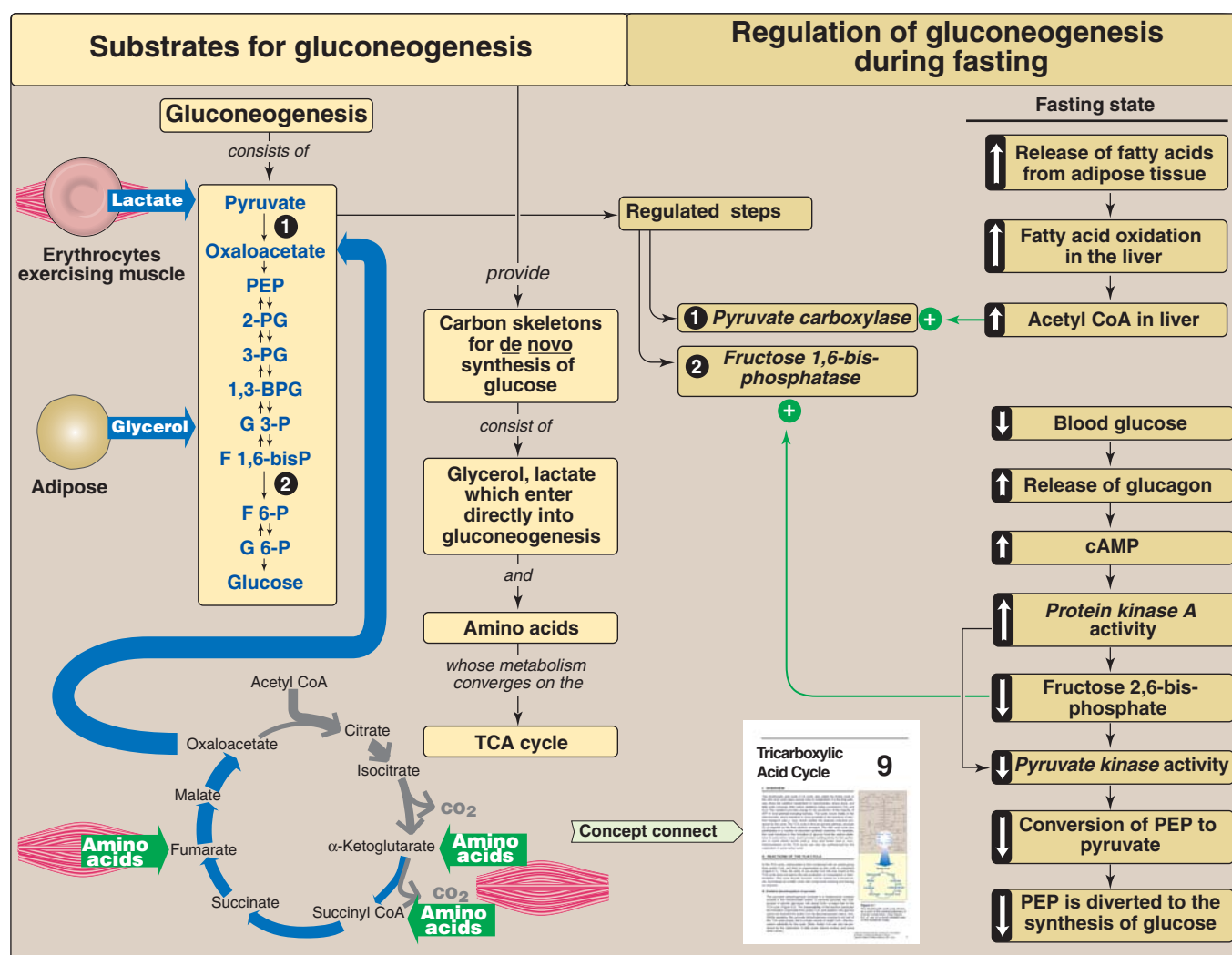


Figure 10.9

Key concept map for gluconeogenesis.

Study Questions

Choose the ONE correct answer.

10.1 The synthesis of glucose from pyruvate by gluconeogenesis:

- A. occurs exclusively in the cytosol.
- B. is inhibited by an elevated level of glucagon.
- C. requires the participation of biotin.
- D. involves lactate as an intermediate.
- E. requires the oxidation/reduction of FAD.

Correct answer = C. Biotin is the coenzyme-prosthetic group of pyruvate carboxylase. The carboxylation of pyruvate occurs in the mitochondria. Glucagon stimulates gluconeogenesis. Lactate is not an intermediate in the conversion of pyruvate to glucose; however, pyruvate can be produced from lactate. FAD is not involved in gluconeogenesis.

10.2 Which one of the following statements concerning gluconeogenesis is correct?

- A. It occurs in muscle.
- B. It is stimulated by fructose 2,6-bisphosphate.
- C. It is inhibited by elevated levels of acetyl CoA.
- D. It is important in maintaining blood glucose during the normal overnight fast.
- E. It uses carbon skeletons provided by degradation of fatty acids.

Correct answer = D. During the overnight fast, glycogen is partially depleted and gluconeogenesis provides blood glucose. Gluconeogenesis is inhibited by fructose 2,6-bisphosphate and stimulated by elevated levels of acetyl CoA. Degradation of fatty acids yields acetyl CoA, which cannot be converted to glucose. This is because there is no net gain of carbons from acetyl CoA in the TCA cycle, and the PDH reaction is physiologically irreversible. Carbon skeletons of most amino acids are, however, gluconeogenic.

10.3 Which one of the following reactions is unique to gluconeogenesis?

- A. Lactate \rightarrow pyruvate
- B. Phosphoenolpyruvate \rightarrow pyruvate
- C. Oxaloacetate \rightarrow phosphoenolpyruvate
- D. Glucose 6-phosphate \rightarrow fructose 6-phosphate
- E. 1,3-Bis-phosphoglycerate \rightarrow 3-phosphoglycerate

Correct answer = C. The other reactions are common to both gluconeogenesis and glycolysis.

10.4 The metabolism of ethanol by alcohol dehydrogenase (ADH) produces NADH. What effect is the change in the NAD^+/NADH expected to have on gluconeogenesis? Explain.

The increase in NADH as ethanol is oxidized, will decrease the availability of OAA because the reversible oxidation of malate to OAA by malate dehydrogenase of the TCA cycle is driven in the reverse direction by the high availability of NADH. Additionally, the reversible reduction of pyruvate to lactate by lactate dehydrogenase of glycolysis is driven in the forward direction by NADH. Thus, two important gluconeogenic substrates, OAA and pyruvate, are decreased as a result of the increase in NADH during ethanol metabolism. This will result in a decrease in gluconeogenesis.

10.5 Given that acetyl CoA cannot be a substrate for gluconeogenesis, why is its production in fatty acid oxidation essential for gluconeogenesis?

Acetyl CoA inhibits pyruvate dehydrogenase and activates pyruvate carboxylase, pushing pyruvate to gluconeogenesis.

10.6 What effect does AMP have on gluconeogenesis and glycolysis? What enzymes are affected?

AMP inhibits gluconeogenesis through inhibition of fructose 1,6-bisphosphatase and favors glycolysis through activation of phosphofructokinase-1. (Fructose 2,6-bisphosphate has a similar effect on these enzymes.)

Glycogen Metabolism

11

I. OVERVIEW

A constant source of blood glucose is an absolute requirement for human life. Glucose is the greatly preferred energy source for the brain, and the required energy source for cells with few or no mitochondria, such as mature erythrocytes. Glucose is also essential as an energy source for exercising muscle, where it is the substrate for anaerobic glycolysis. Blood glucose can be obtained from three primary sources: the diet, degradation of glycogen, and gluconeogenesis. Dietary intake of glucose and glucose precursors, such as starch, monosaccharides, and disaccharides, is sporadic and, depending on the diet, is not always a reliable source of blood glucose. In contrast, gluconeogenesis (see p. 117) can provide sustained synthesis of glucose, but it is somewhat slow in responding to a falling blood glucose level. Therefore, the body has developed mechanisms for storing a supply of glucose in a rapidly mobilizable form, namely, glycogen. In the absence of a dietary source of glucose, this sugar is rapidly released from liver and kidney glycogen. Similarly, muscle glycogen is extensively degraded in exercising muscle to provide that tissue with an important energy source. When glycogen stores are depleted, specific tissues synthesize glucose *de novo*, using amino acids from the body's proteins as a primary source of carbons for the gluconeogenic pathway. Figure 11.1 shows the reactions of glycogen synthesis and degradation as part of the essential pathways of energy metabolism.

II. STRUCTURE AND FUNCTION OF GLYCOGEN

The main stores of glycogen in the body are found in skeletal muscle and liver, although most other cells store small amounts of glycogen for their own use. The function of muscle glycogen is to serve as a fuel reserve for the synthesis of adenosine triphosphate (ATP) during muscle contraction. That of liver glycogen is to maintain the blood glucose concentration, particularly during the early stages of a fast (Figure 11.2, and see p. 329).

A. Amounts of liver and muscle glycogen

Approximately 400 g of glycogen make up 1–2% of the fresh weight of resting muscle, and approximately 100 g of glycogen make up to 10% of the fresh weight of a well-fed adult liver. What limits the production of glycogen at these levels is not clear. However, in some

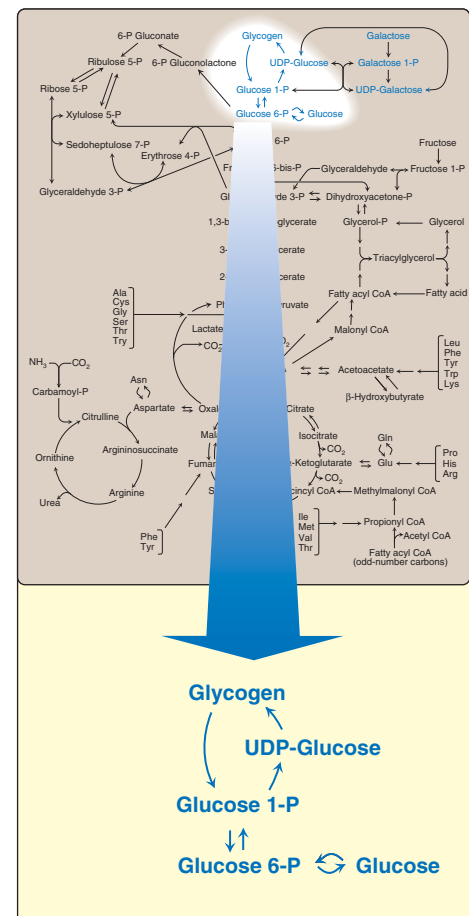


Figure 11.1

Glycogen synthesis and degradation shown as a part of the essential reactions of energy metabolism (see Figure 8.2, p. 92, for a more detailed view of the overall reactions of metabolism).

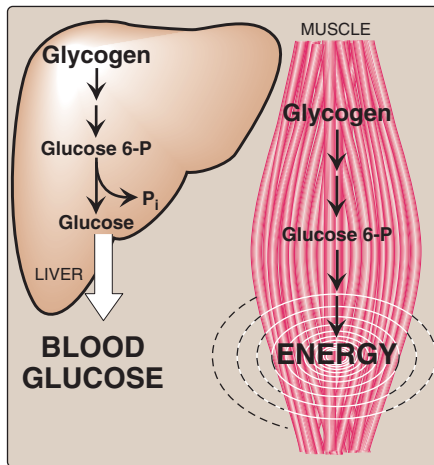


Figure 11.2
Functions of muscle and liver glycogen.

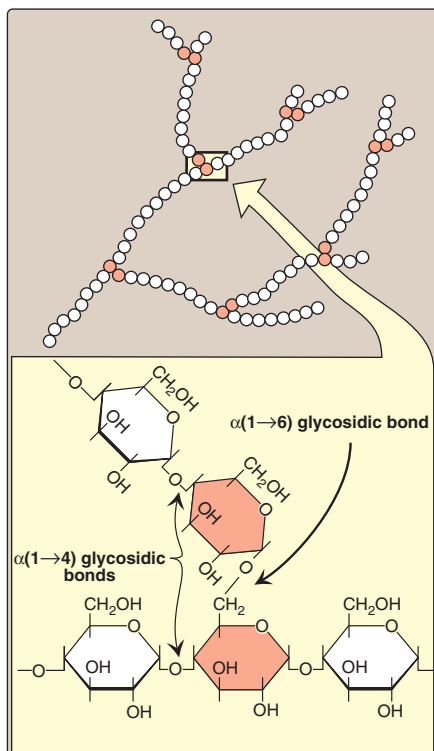


Figure 11.3
Branched structure of glycogen, showing $\alpha(1 \rightarrow 4)$ and $\alpha(1 \rightarrow 6)$ glycosidic bonds.

glycogen storage diseases (see Figure 11.8), the amount of glycogen in the liver and/or muscle can be significantly higher.

B. Structure of glycogen

Glycogen is a branched-chain polysaccharide made exclusively from α -D-glucose. The primary glycosidic bond is an $\alpha(1 \rightarrow 4)$ linkage. After an average of eight to ten glucosyl residues, there is a branch containing an $\alpha(1 \rightarrow 6)$ linkage (Figure 11.3). A single molecule of glycogen can have a molecular mass of up to 10^8 daltons. These molecules exist in discrete cytoplasmic granules that also contain most of the enzymes necessary for glycogen synthesis and degradation.

C. Fluctuation of glycogen stores

Liver glycogen stores increase during the well-fed state (see p. 323), and are depleted during a fast (see p. 329). Muscle glycogen is not affected by short periods of fasting (a few days) and is only moderately decreased in prolonged fasting (weeks). Muscle glycogen is synthesized to replenish muscle stores after they have been depleted following strenuous exercise. [Note: Glycogen synthesis and degradation are cytosolic processes that go on continuously. The differences between the rates of these two processes determine the levels of stored glycogen during specific physiologic states.]

III. SYNTHESIS OF GLYCOGEN (GLYCOGENESIS)

Glycogen is synthesized from molecules of α -D-glucose. The process occurs in the cytosol, and requires energy supplied by ATP (for the phosphorylation of glucose) and uridine triphosphate (UTP).

A. Synthesis of UDP-glucose

α -D-Glucose attached to uridine diphosphate (UDP) is the source of all the glucosyl residues that are added to the growing glycogen molecule. UDP-glucose (Figure 11.4) is synthesized from glucose 1-phosphate and UTP by *UDP-glucose pyrophosphorylase* (Figure 11.5). The high-energy bond in pyrophosphate (PP_i), the second product of the reaction, is hydrolyzed to two inorganic phosphates (P_i) by *pyrophosphatase*, which ensures that the *UDP-glucose pyrophosphorylase* reaction proceeds in the direction of UDP-glucose production. [Note: Glucose 6-phosphate is converted to glucose 1-phosphate by *phosphoglucomutase*. Glucose 1,6-bisphosphate is an obligatory intermediate in this reaction (Figure 11.6).]

B. Synthesis of a primer to initiate glycogen synthesis

Glycogen synthase is responsible for making the $\alpha(1 \rightarrow 4)$ linkages in glycogen. This enzyme cannot initiate chain synthesis using free glucose as an acceptor of a molecule of glucose from UDP-glucose. Instead, it can only elongate already existing chains of glucose. Therefore, a fragment of glycogen can serve as a primer in cells whose glycogen stores are not totally depleted. In the absence of a

glycogen fragment, a protein, called *glycogenin*, can serve as an acceptor of glucose residues from UDP-glucose (see Figure 11.5). The side chain hydroxyl group of a specific tyrosine serves as the site at which the initial glucosyl unit is attached. The reaction is catalyzed by *glycogenin* itself via autoglucosylation; thus, *glycogenin* is an enzyme. *Glycogenin* then catalyzes the transfer of the next few molecules of glucose from UDP-glucose, producing a short, $\alpha(1\rightarrow4)$ -linked glucosyl chain. This short chain serves as a primer that is able to be elongated by *glycogen synthase* as described below [Note: *Glycogenin* stays associated with and forms the core of a glycogen granule.]

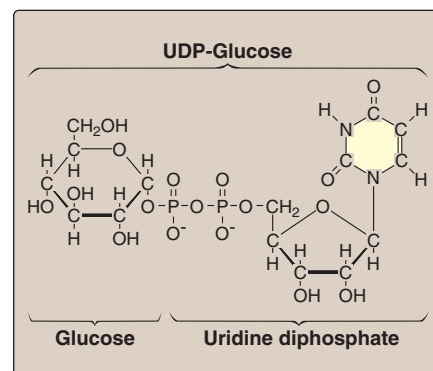


Figure 11.4

The structure of UDP-glucose, a nucleotide sugar.

C. Elongation of glycogen chains by glycogen synthase

Elongation of a glycogen chain involves the transfer of glucose from UDP-glucose to the nonreducing end of the growing chain, forming a new glycosidic bond between the anomeric hydroxyl of carbon 1 of the activated glucose and carbon 4 of the accepting glucosyl residue (see Figure 11.5). [Note: The nonreducing end of a carbohydrate chain is one in which the anomeric carbon of the terminal sugar is linked by a glycosidic bond to another compound, making the terminal sugar nonreducing (see p. 84).] The enzyme responsible for making the $\alpha(1\rightarrow4)$ linkages in glycogen is *glycogen synthase*. [Note: The UDP released when the new $\alpha(1\rightarrow4)$ glycosidic bond is made can be phosphorylated to UTP by *nucleoside diphosphate kinase* ($\text{UDP} + \text{ATP} \rightleftharpoons \text{UTP} + \text{ADP}$, see p. 296).]

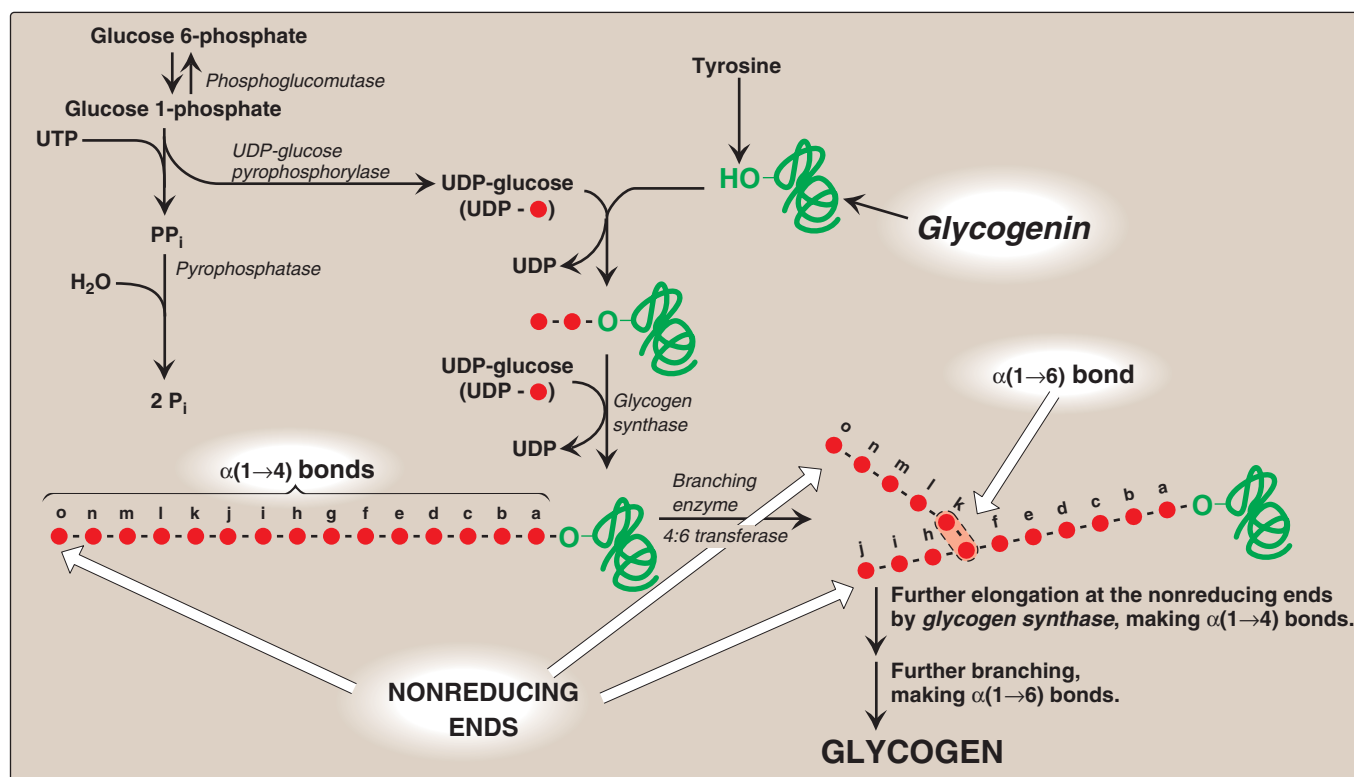
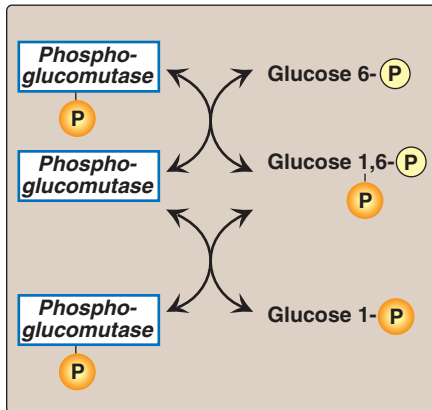
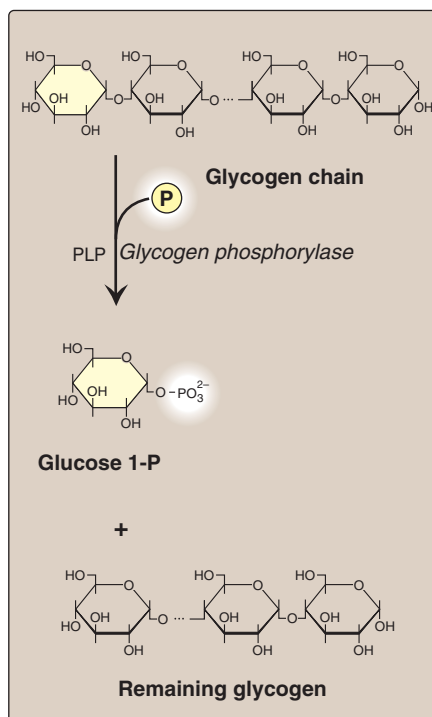


Figure 11.5
Glycogen synthesis.

**Figure 11.6**

Interconversion of glucose 6-phosphate and glucose 1-phosphate by *phosphoglucomutase*.

**Figure 11.7**

Cleavage of an $\alpha(1 \rightarrow 4)$ -glycosidic bond. PLP= pyridoxal phosphate.

D. Formation of branches in glycogen

If no other synthetic enzyme acted on the chain, the resulting structure would be a linear (unbranched) molecule of glucosyl residues attached by $\alpha(1 \rightarrow 4)$ linkages. Such a compound is found in plant tissues, and is called amylose. In contrast, glycogen has branches located, on average, eight glucosyl residues apart, resulting in a highly branched, tree-like structure (see Figure 11.3) that is far more soluble than the unbranched amylose. Branching also increases the number of nonreducing ends to which new glucosyl residues can be added (and also, as described later, from which these residues can be removed), thereby greatly accelerating the rate at which glycogen synthesis can occur, and dramatically increasing the size of the molecule.

1. Synthesis of branches: Branches are made by the action of the branching enzyme, *amylo- $\alpha(1 \rightarrow 4) \rightarrow \alpha(1 \rightarrow 6)$ -transglucosidase*. This enzyme removes a chain of six to eight glucosyl residues from the nonreducing end of the glycogen chain, breaking an $\alpha(1 \rightarrow 4)$ bond to another residue on the chain, and attaches it to a non-terminal glucosyl residue by an $\alpha(1 \rightarrow 6)$ linkage, thus functioning as a *4:6 transferase*. The resulting new, nonreducing end (see “j” in Figure 11.5), as well as the old nonreducing end from which the six to eight residues were removed (see “o” in Figure 11.5), can now be further elongated by *glycogen synthase*.

2. Synthesis of additional branches: After elongation of these two ends has been accomplished by *glycogen synthase*, their terminal six to eight glucosyl residues can be removed and used to make additional branches.

IV. DEGRADATION OF GLYCOGEN (GLYCOGENOLYSIS)

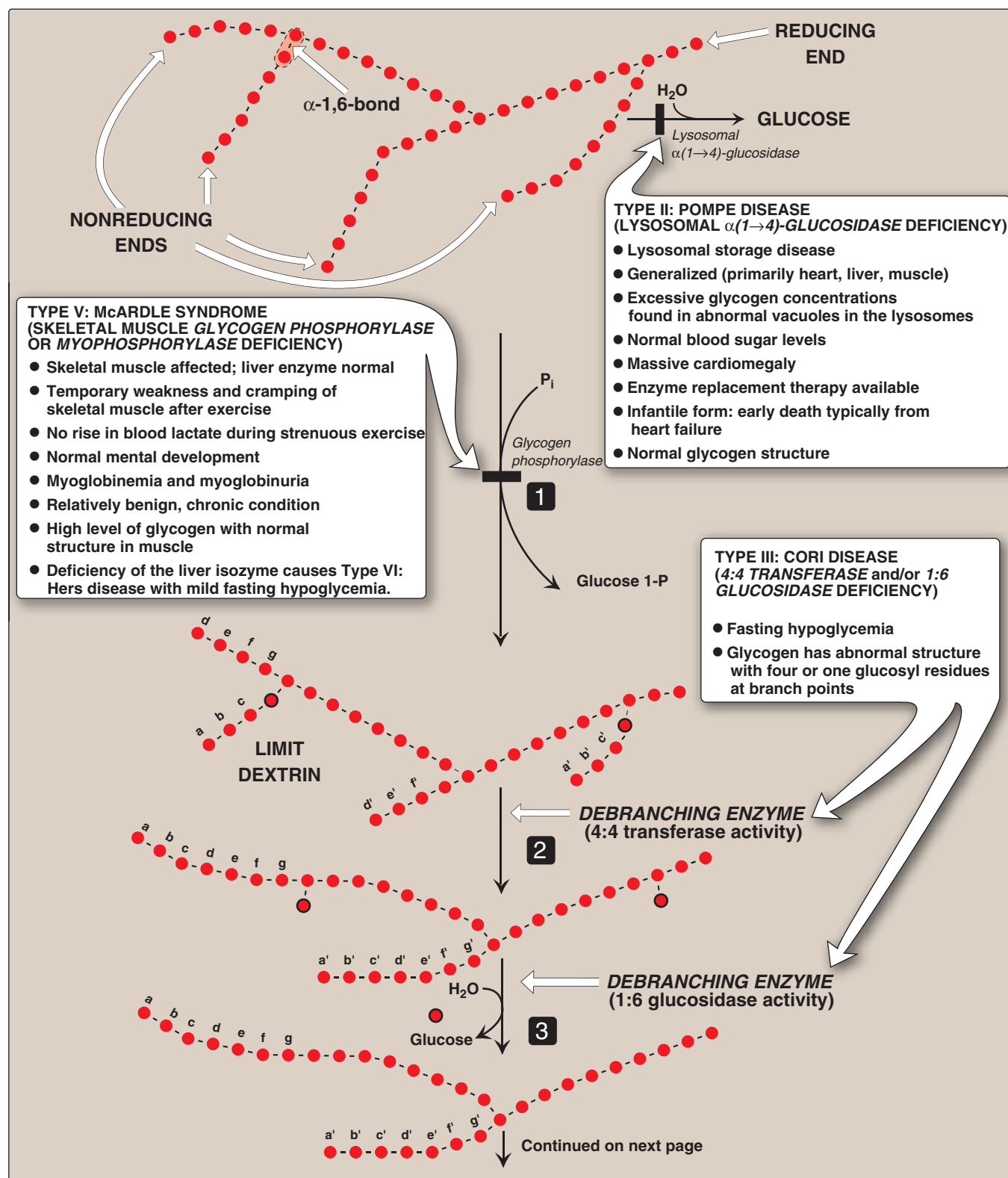
The degradative pathway that mobilizes stored glycogen in liver and skeletal muscle is not a reversal of the synthetic reactions. Instead, a separate set of cytosolic enzymes is required. When glycogen is degraded, the primary product is glucose 1-phosphate, obtained by breaking $\alpha(1 \rightarrow 4)$ glycosidic bonds. In addition, free glucose is released from each $\alpha(1 \rightarrow 6)$ -linked glucosyl residue.

A. Shortening of chains

Glycogen phosphorylase sequentially cleaves the $\alpha(1 \rightarrow 4)$ glycosidic bonds between the glucosyl residues at the nonreducing ends of the glycogen chains by simple phosphorolysis (producing glucose 1-phosphate) until four glucosyl units remain on each chain before a branch point (Figure 11.7). [Note: This enzyme contains a molecule of covalently bound pyridoxal phosphate (PLP) that is required as a coenzyme.] The resulting structure is called a limit dextrin, and *phosphorylase* cannot degrade it any further (Figure 11.8).

B. Removal of branches

Branches are removed by the two enzymic activities of a single bifunctional protein, the debranching enzyme (see Figure 11.8). First, *oligo- $\alpha(1 \rightarrow 4) \rightarrow \alpha(1 \rightarrow 4)$ -glucan transferase* activity removes the

**Figure 11.8**

Glycogen degradation, showing some of the glycogen storage diseases (GSD). [Note: A GSD can also be caused by defects in *branching enzyme*, an enzyme of synthesis, resulting in Type IV: Andersen disease and causing death in early childhood.](Continued on next page.)

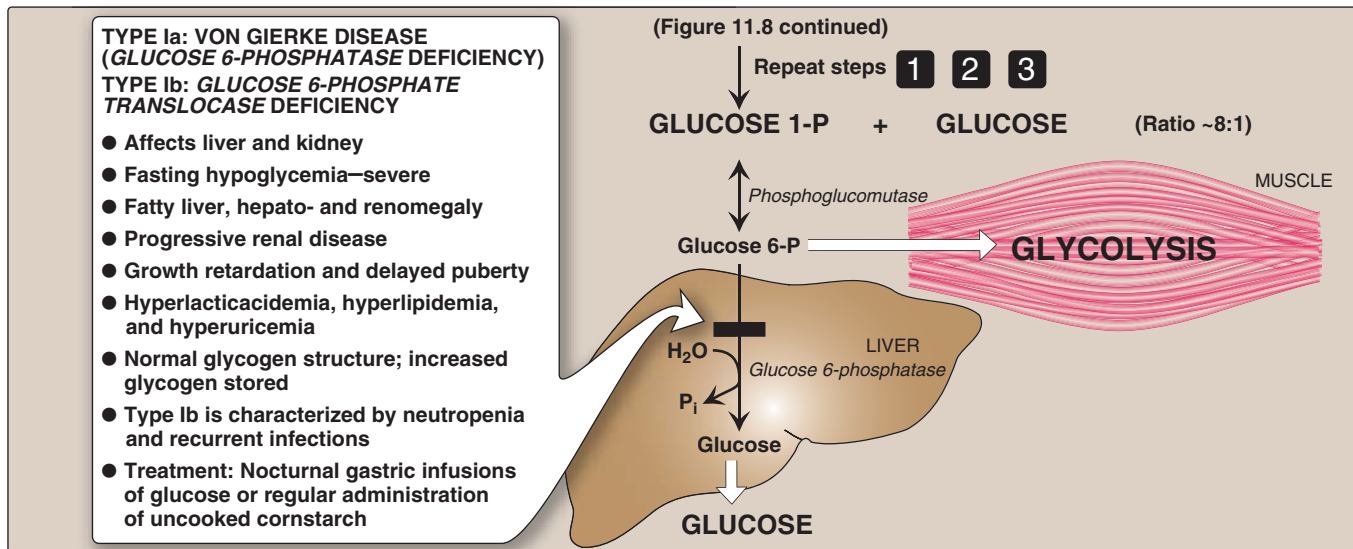


Figure 11.8 (Continued)

outer three of the four glucosyl residues attached at a branch. It next transfers them to the nonreducing end of another chain, lengthening it accordingly. Thus, an $\alpha(1\rightarrow4)$ bond is broken and an $\alpha(1\rightarrow4)$ bond is made, and the enzyme functions as a *4:4 transferase*. Next, the remaining single glucose residue attached in an $\alpha(1\rightarrow6)$ linkage is removed hydrolytically by *amylol- $\alpha(1\rightarrow6)$ -glucosidase* activity, releasing free glucose. The glucosyl chain is now available again for degradation by *glycogen phosphorylase* until four glucosyl units from the next branch are reached.

C. Conversion of glucose 1-phosphate to glucose 6-phosphate

Glucose 1-phosphate, produced by *glycogen phosphorylase*, is converted in the cytosol to glucose 6-phosphate by *phosphoglucomutase*—a reaction that produces glucose 1,6-bisphosphate as a temporary but essential intermediate (see Figure 11.6). In the liver, glucose 6-phosphate is transported into the endoplasmic reticulum (ER) by *glucose 6-phosphate translocase*. There it is converted to glucose by *glucose 6-phosphatase*—the same enzyme used in the last step of gluconeogenesis (see p. 121). The glucose then moves from the ER to the cytosol. Hepatocytes release glycogen-derived glucose into the blood to help maintain blood glucose levels until the gluconeogenic pathway is actively producing glucose. [Note: In the muscle, glucose 6-phosphate cannot be dephosphorylated because of a lack of *glucose 6-phosphatase*. Instead, it enters glycolysis, providing energy needed for muscle contraction.]

D. Lysosomal degradation of glycogen

A small amount (1–3%) of glycogen is continuously degraded by the lysosomal enzyme, *$\alpha(1\rightarrow4)$ -glucosidase (acid maltase)*. The purpose of this pathway is unknown. However, a deficiency of this enzyme causes accumulation of glycogen in vacuoles in the lysosomes, resulting in the serious glycogen storage disease Type II: Pompe disease (see Figure 11.8). [Note: Type II: Pompe disease is the only glycogen storage disease that is a lysosomal storage disease.]

Lysosomal storage diseases are genetic disorders characterized by the accumulation of abnormal amounts of carbohydrates or lipids primarily due to their decreased lysosomal degradation.

V. REGULATION OF GLYCOGEN SYNTHESIS AND DEGRADATION

Because of the importance of maintaining blood glucose levels, the synthesis and degradation of its glycogen storage form are tightly regulated. In the liver, glycogenesis accelerates during periods when the body has been well fed, whereas glycogenolysis accelerates during periods of fasting. In skeletal muscle, glycogenolysis occurs during active exercise, and glycogenesis begins as soon as the muscle is again at rest. Regulation of glycogen synthesis and degradation is accomplished on two levels. First, *glycogen synthase* and *glycogen phosphorylase* are hormonally regulated to meet the needs of the body as a whole. Second, the pathways of glycogen synthesis and degradation are allosterically controlled to meet the needs of a particular tissue.

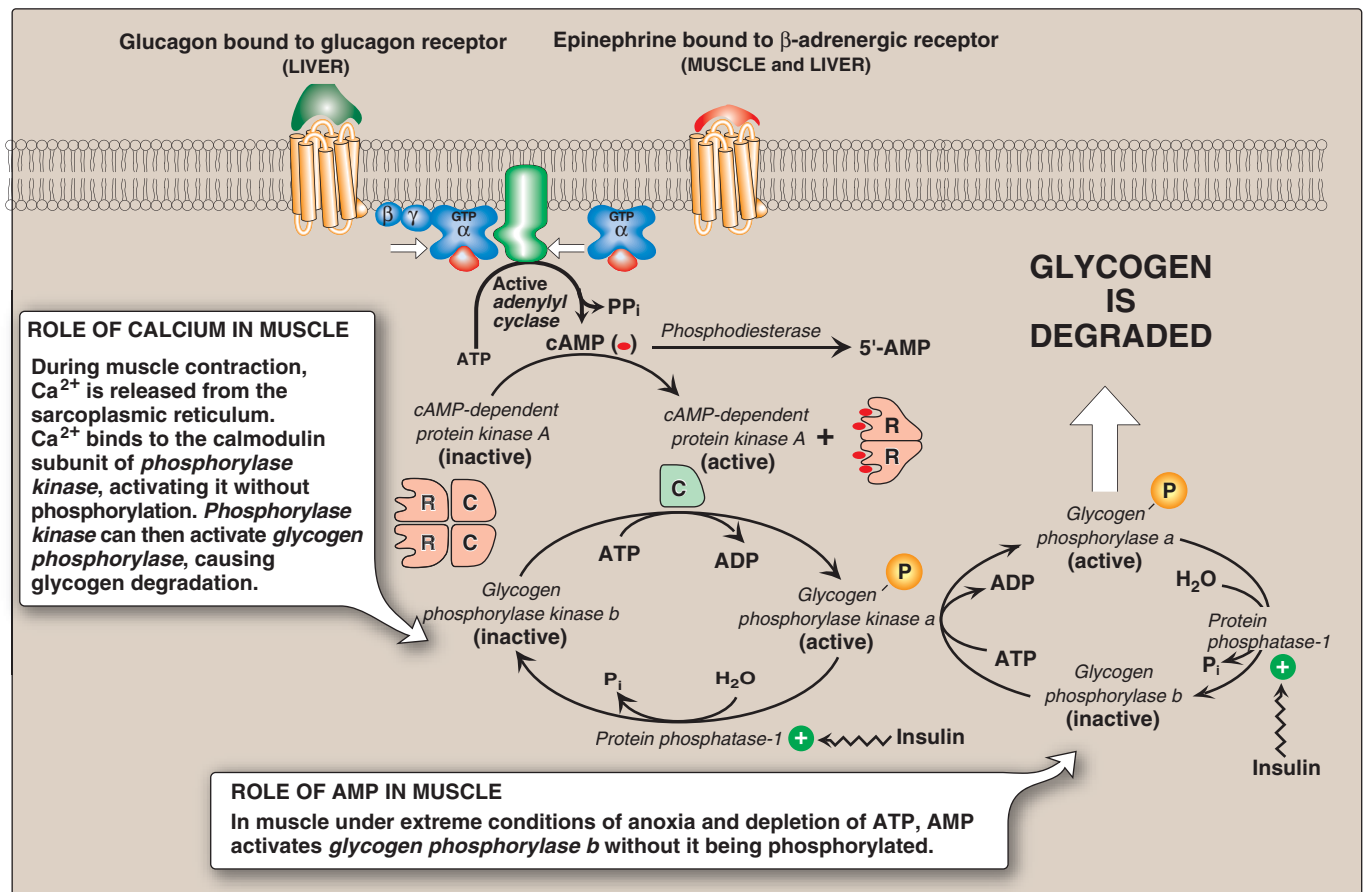


Figure 11.9

Stimulation and inhibition of glycogen degradation.

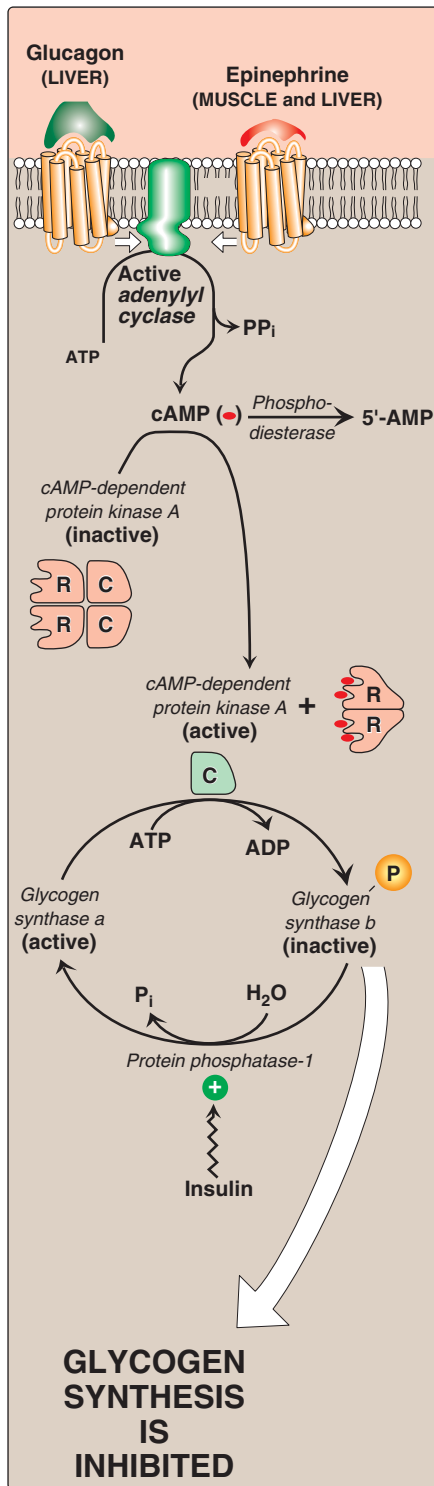


Figure 11.10

Hormonal regulation of glycogen synthesis. [Note: In contrast to *glycogen phosphorylase*, *glycogen synthase* is inactivated by phosphorylation.]

A. Activation of glycogen degradation by cAMP-directed pathway

The binding of hormones, such as glucagon or epinephrine, to plasma membrane G protein-coupled receptors (GPCR) signals the need for glycogen to be degraded—either to elevate blood glucose levels or to provide energy for exercising muscle.

1. Activation of protein kinase A: Binding of glucagon or epinephrine to their specific hepatocyte GPCR, or of epinephrine to specific myocyte GPCR, results in the G protein-mediated activation of *adenylyl cyclase*. This enzyme catalyzes the synthesis of cAMP, which activates *cAMP-dependent protein kinase A (PKA)*, as described on page 95. *PKA* is a tetramer, having two regulatory subunits (R) and two catalytic subunits (C). cAMP binds to the regulatory subunit dimer, releasing individual catalytic subunits that are active (Figure 11.9). *PKA* then phosphorylates several enzymes of glycogen metabolism, affecting their activity. [Note: When cAMP is removed, the inactive tetramer, R₂C₂, is again formed.]

2. Activation of phosphorylase kinase: *Phosphorylase kinase* exists in two forms: an inactive “b” form and an active “a” form. Active *PKA* phosphorylates the inactive “b” form of *phosphorylase kinase*, producing the active “a” form (see Figure 11.9). [Note: The phosphorylated enzyme can be inactivated by the hydrolytic removal of its phosphate by *protein phosphatase-1*. This enzyme is activated by a signal cascade initiated by insulin (see p. 311). Insulin also activates the *phosphodiesterase* that degrades cAMP, thus opposing the effects of glucagon and epinephrine.]

3. Activation of glycogen phosphorylase: *Glycogen phosphorylase* also exists in two forms: the dephosphorylated, inactive “b” form and the phosphorylated, active “a” form. Active *phosphorylase kinase* phosphorylates *glycogen phosphorylase b* to its active “a” form, which then begins glycogenolysis (see Figure 11.9). *Phosphorylase a* is reconverted to *phosphorylase b* by the hydrolysis of its phosphate by *protein phosphatase-1*. [Note: *Protein phosphatase-1* is inactivated by inhibitor proteins that bind in response to their phosphorylation and activation by *PKA*.]

4. Summary of the regulation of glycogen degradation: The cascade of reactions listed above results in glycogenolysis. The large number of sequential steps serves to amplify the effect of the hormonal signal, that is, a few hormone molecules binding to their receptors result in a number of *protein kinase A* molecules being activated that can each activate many *phosphorylase kinase* molecules. This causes the production of many active *glycogen phosphorylase a* molecules that can degrade glycogen.

B. Inhibition of glycogen synthesis by a cAMP-directed pathway

The regulated enzyme in glycogenesis is *glycogen synthase*. It also exists in two forms, the active “a” form and the inactive “b” form. However, for *glycogen synthase*, in contrast to *phosphorylase kinase* and *glycogen phosphorylase*, the active form is dephosphorylated

whereas the inactive form is phosphorylated (Figure 11.10). *Glycogen synthase a* is converted to the inactive “b” form by phosphorylation at several sites on the enzyme, with the level of inactivation proportional to its degree of phosphorylation. This conversion process is catalyzed by several different *protein kinases* that are regulated by cAMP or other signaling mechanisms (see C below). *Glycogen synthase b* can be reconverted to the “a” form by *protein phosphatase-1*, which removes the phosphate groups hydrolytically.

C. Allosteric regulation of glycogen synthesis and degradation

In addition to hormonal signals, *glycogen synthase* and *glycogen phosphorylase* respond to the levels of metabolites and energy needs of the cell. Glycogenesis is stimulated when substrate availability and energy levels are high, whereas glycogenolysis is increased when glucose and energy levels are low. This allosteric regulation allows a rapid response to the needs of a cell, and can override the effects of hormone-mediated covalent regulation.

1. Regulation of glycogen synthesis and degradation in the well-fed state:

In the well-fed state, *glycogen synthase b* in both liver and muscle is allosterically activated by glucose 6-phosphate which is present in elevated concentrations (Figure 11.11). In contrast, *glycogen phosphorylase a* is allosterically inhibited by glucose 6-phosphate, as well as by ATP, a high-energy signal in the cell. [Note: In liver, but not muscle, non-phosphorylated glucose is also an allosteric inhibitor of *glycogen phosphorylase a*, making it a better substrate for *protein phosphatase-1*.]

2. Activation of glycogen degradation by calcium: Ca^{2+} is released into the cytoplasm in muscle in response to neural stimulation and in liver in response to epinephrine binding to α_1 -adrenergic receptors. The Ca^{2+} binds to calmodulin, the most widely distributed member of a family of small, calcium-binding proteins. The binding of four molecules of Ca^{2+} to calmodulin triggers a conformational change such that the activated Ca^{2+} -calmodulin complex binds to and activates protein molecules—often enzymes—that are inactive in the absence of this complex (see Figure 11.12). Thus, calmodulin functions as an essential subunit of many complex proteins. One such protein is *phosphorylase kinase b*, which is activated by the Ca^{2+} -calmodulin complex without the need for the *kinase* to be phosphorylated by *PKA*. [Note: Epinephrine at β -adrenergic receptors signals through a rise in cAMP, not calcium (see p. 131).]

a. Calcium activation of muscle phosphorylase kinase: During muscle contraction, there is a rapid and urgent need for ATP. This energy is supplied by the degradation of muscle glycogen to glucose, which can then enter glycolysis. Nerve impulses cause membrane depolarization, which promotes Ca^{2+} release from the sarcoplasmic reticulum into the sarcoplasm of myocytes. The Ca^{2+} binds calmodulin and the complex activates muscle *phosphorylase kinase b* (see Figure 11.9).

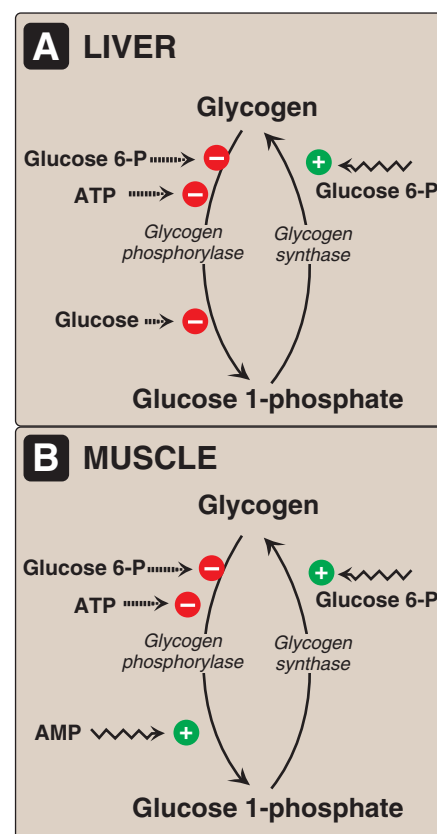


Figure 11.11

Allosteric regulation of glycogen synthesis and degradation. A. Liver. B. Muscle. [Note: Ca^{2+} indirectly activates phosphorylase in both muscle and liver by directly activating *phosphorylase kinase*.]

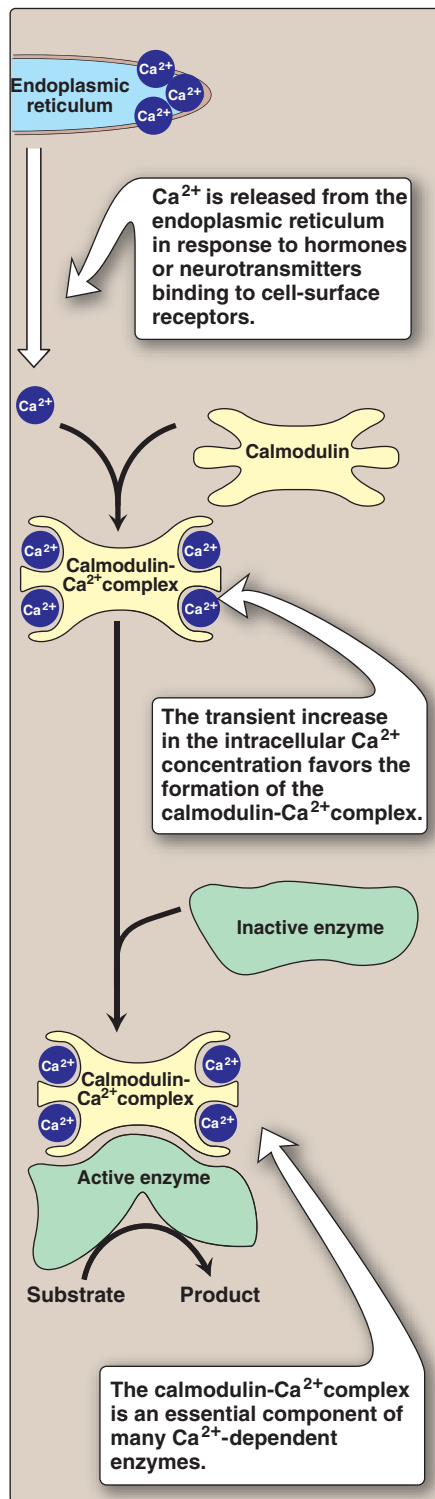


Figure 11.12

Calmodulin mediates many effects of intracellular calcium.

b. Calcium activation of liver phosphorylase kinase: During “fight or flight” situations, epinephrine is released from the adrenal medulla and signals the need for blood glucose. This glucose initially comes from hepatic glycogenolysis. Binding of epinephrine to hepatocyte α -adrenergic G protein-coupled receptors activates a phospholipid-dependent cascade (see p. 205) that results in movement of Ca^{2+} from the ER into the cytoplasm. A Ca^{2+} -calmodulin complex forms and activates hepatic *phosphorylase kinase b*. [Note: The released Ca^{2+} also helps to activate *protein kinase C* that can phosphorylate (thus inactivate) *glycogen synthase a*.]

3. Activation of glycogen degradation in muscle by AMP: Muscle *glycogen phosphorylase* is active in the presence of the high AMP concentrations that occur in the muscle under extreme conditions of anoxia and ATP depletion. AMP binds to *glycogen phosphorylase b*, causing its activation without phosphorylation (see Figure 11.9). [Note: Recall that AMP also activates *PFK-1* of glycolysis (see p. 99).]

VI. GLYCOGEN STORAGE DISEASES

These are a group of genetic diseases that result from a defect in an enzyme required for glycogen synthesis or degradation. They result either in formation of glycogen that has an abnormal structure, or in the accumulation of excessive amounts of normal glycogen in specific tissues as a result of impaired degradation. A particular enzyme may be defective in a single tissue, such as liver (resulting in hypoglycemia) or muscle (muscle weakness), or the defect may be more generalized, affecting liver, muscle, kidney, intestine, and myocardium. The severity of the glycogen storage diseases (GSDs) ranges from fatal in infancy to mild disorders that are not life-threatening. Some of the more prevalent GSDs are illustrated in Figure 11.8.

VII. CHAPTER SUMMARY

The **main stores** of glycogen in the body are found in **skeletal muscle**, where they serve as a **fuel reserve** for the synthesis of ATP during **muscle contraction**, and in the **liver**, where they are used to **maintain the blood glucose** concentration, particularly during the **early stages of a fast**. Glycogen is a **highly branched** polymer of α -D-glucose. The primary glycosidic bond is an $\alpha(1 \rightarrow 4)$ linkage. After about eight to ten glucosyl residues, there is a **branch** containing an $\alpha(1 \rightarrow 6)$ linkage. **UDP-glucose**, the **building block** of glycogen, is synthesized from **glucose 1-phosphate** and **UTP** by **UDP-glucose pyrophosphorylase** (Figure 11.13). Glucose from UDP-glucose is transferred to the nonreducing ends of glycogen chains by primer-requiring **glycogen synthase**, which makes $\alpha(1 \rightarrow 4)$ linkages. The primer is made by **glycogenin**. **Branches** are formed by **amylo- $\alpha(1 \rightarrow 4) \rightarrow \alpha(1 \rightarrow 6)$ -transglucosidase**, which transfers a chain of six to eight glucosyl residues from the nonreducing end of the glycogen chain (**breaking an $\alpha(1 \rightarrow 4)$ linkage**), and attaches it with an $\alpha(1 \rightarrow 6)$ linkage to another residue in the chain.

PLP-requiring **glycogen phosphorylase** cleaves the $\alpha(1\rightarrow4)$ bonds between glucosyl residues at the **nonreducing ends** of the glycogen chains, producing **glucose 1-phosphate**. This sequential degradation continues until four glucosyl units remain on each chain before a branch point. The resulting structure is called a **limit dextrin** that is degraded by the bifunctional debranching enzyme. **Oligo- $\alpha(1\rightarrow4)\rightarrow\alpha(1\rightarrow4)$ -glucan transferase** (common name, **glucosyl 4:4 transferase**) removes the outer three of the four glucosyl residues attached at a branch, and transfers them to the nonreducing end of another chain where they can be converted to glucose 1-phosphate by glycogen phosphorylase. Next, the remaining single glucose residue attached in an $\alpha(1\rightarrow4)$ linkage is removed hydrolytically by the **amyl $\alpha(1\rightarrow6)$ glucosidase** activity of debranching enzyme, releasing **free glucose**. **Glucose 1-phosphate** is converted to **glucose 6-phosphate** by **phosphoglucomutase**. In the **muscle**, glucose 6-phosphate enters glycolysis. In the **liver**, the phosphate is removed by **glucose 6-phosphatase**, releasing **free glucose** that can be used to maintain blood glucose levels at the beginning of a fast. A **deficiency** of the **phosphatase** causes **glycogen storage disease Type 1a (Von Gierke disease)**. This disease results in an inability of the liver to provide free glucose to the body during a fast. It affects both glycogen degradation and gluconeogenesis. Glycogen synthesis and degradation are reciprocally regulated to meet whole-body needs by the same hormonal signals, namely, an **elevated insulin** level results in overall **increased glycogenesis** and **decreased glycogenolysis**, whereas an **elevated glucagon** (or **epinephrine**) level causes **increased glycogenolysis** and **decreased glycogenesis**. Key enzymes are phosphorylated by a family of **protein kinases**, some of which are **cAMP-dependent** (a compound increased by glucagon and epinephrine). Phosphate groups are removed by protein phosphatase-1 (activated when insulin levels are elevated). **Glycogen synthase**, **phosphorylase kinase** and **phosphorylase** are also **allosterically regulated** to meet tissues needs. In the well-fed state, **glycogen synthase** is **activated** by **glucose 6-phosphate**, but **glycogen phosphorylase** is **inhibited** by **glucose 6-phosphate**, as well as by **ATP**. In the liver, glucose also serves as an allosteric inhibitor of glycogen phosphorylase. The Ca^{2+} released from the endoplasmic reticulum in muscle during exercise and in liver in response to epinephrine **activates phosphorylase kinase** by binding to the enzyme's **calmodulin** subunit. This allows the enzyme to activate **glycogen phosphorylase**, thereby causing glycogen degradation.

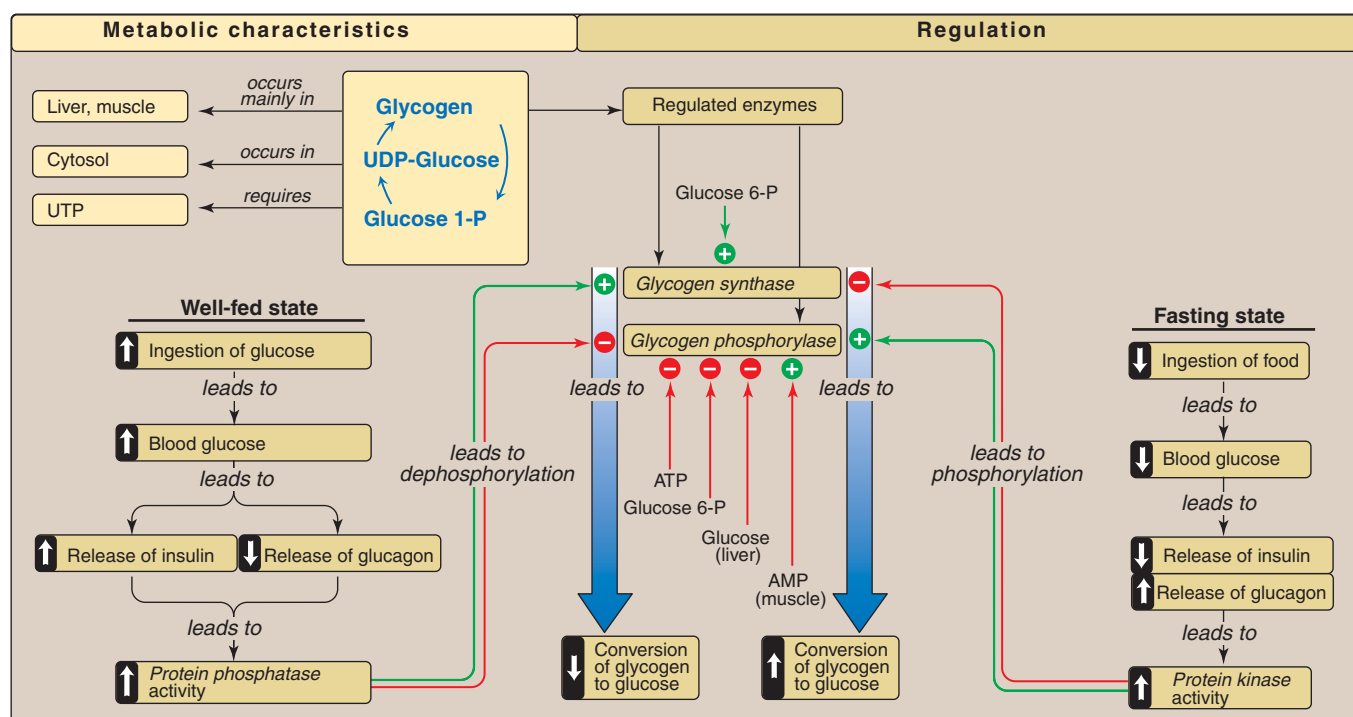


Figure 11.13

Key concept map for glycogen metabolism in liver. [Note: *Glycogen phosphorylase* is phosphorylated by *phosphorylase kinase*, the "b" form of which can be activated by Ca^{2+} .]

Study Questions

Choose the ONE correct answer.

11.1 A 2-year-old boy was brought into the emergency room, suffering from severe fasting hypoglycemia. On physical examination, he was found to have hepatomegaly. Laboratory tests indicated that he also had hyperlacticacidemia and hyperuricemia. A liver biopsy indicated that hepatocytes contained greater than normal amounts of glycogen that was of normal structure. Enzyme assay likely confirmed a deficiency in which of the following enzymes?

- A. Glycogen synthase
- B. Glycogen phosphorylase
- C. Glucose 6-phosphatase
- D. Amylo- $\alpha(1\rightarrow6)$ -glucosidase
- E. Amylo- $\alpha(1\rightarrow4)\rightarrow\alpha(1\rightarrow6)$ -transglucosidase

11.2 The hormones, epinephrine and glucagon have which one of the following effects on glycogen metabolism in the liver?

- A. The net synthesis of glycogen is increased.
- B. Glycogen phosphorylase is phosphorylated and active, whereas glycogen synthase is phosphorylated and inactive.
- C. Both glycogen phosphorylase and glycogen synthase are activated by phosphorylation but at significantly different rates.
- D. Glycogen phosphorylase is inactivated by a rise in Ca^{2+} , whereas glycogen synthase is activated.
- E. cAMP-dependent protein kinase A is activated, whereas phosphorylase kinase is inactivated.

11.3 In contracting skeletal muscle, a sudden elevation of the cytosolic Ca^{2+} concentration will result in:

- A. activation of cAMP-dependent protein kinase A.
- B. dissociation of cAMP-dependent protein kinase A into catalytic and regulatory subunits.
- C. inactivation of phosphorylase kinase caused by the action of protein phosphatase-1.
- D. direct activation of phosphorylase kinase b.
- E. direct activation of glycogen phosphorylase b.
- F. conversion of cAMP to AMP by phosphodiesterase.

11.4 Explain why the hypoglycemia seen with Type Ia glycogen storage disease (glucose 6-phosphatase deficiency) is severe, whereas that seen with Type VI (liver phosphorylase deficiency) is mild.

Correct answer = C. A deficiency of glucose 6-phosphatase (Von Gierke disease) prevents the liver from releasing free glucose into the blood, causing severe fasting hypoglycemia, hyperlacticacidemia, and hyperuricemia. A deficiency of glycogen phosphorylase would result in a decrease in glycogen degradation, causing fasting hypoglycemia, but not the other symptoms. A deficiency of glycogen synthase would result in lower amounts of stored glycogen. Amylo- $\alpha(1\rightarrow6)$ -glucosidase removes single glucosyl residues attached to the glycogen chain through an $\alpha(1\rightarrow6)$ -glycosidic bond. A deficiency in this enzyme would result in a decreased ability of the cell to completely degrade glycogen branches. Amylo- $\alpha(1\rightarrow4)\rightarrow\alpha(1\rightarrow6)$ -transglucosidase deficiency would decrease the ability of the cell to make branches.

Correct answer = B. Epinephrine and glucagon both cause increased glycogen degradation in the liver through covalent modification (phosphorylation) of key enzymes of glycogen metabolism. Glycogen phosphorylase is phosphorylated and active ("a" form), whereas glycogen synthase is phosphorylated and inactive ("b" form). cAMP-dependent protein kinase A is active and phosphorylates (and activates) its substrate, phosphorylase kinase. It is phosphorylase kinase that directly phosphorylates and activates phosphorylase.

Correct answer = D. Ca^{2+} released from the sarcoplasmic reticulum during exercise binds to the calmodulin subunit of phosphorylase kinase, thereby allosterically activating the "b" form of this enzyme. The other choices are not caused by an elevation of cytosolic calcium.

With Type Ia, the liver is unable to generate free glucose either from glycogenolysis or gluconeogenesis because both processes produce glucose 6-phosphate. With Type VI, liver is still able to produce free glucose from gluconeogenesis; glycogenolysis, however, is inhibited.