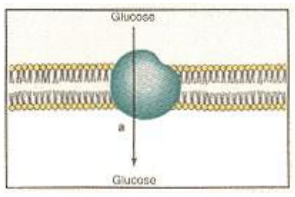
Chapter 7—
Carbohydrate Metabolism I:
Major Metabolic Pathways and their Control

Robert A. Harris



7.1— Overview

The major pathways of carbohydrate metabolism either begin or end with glucose (Figure 7.1). This chapter describes the utilization of glucose as a source of energy, formation of glucose from noncarbohydrate precursors, storage of glucose in the form of glycogen for later use, and release of glucose from glycogen for use by cells. An understanding of the pathways and their regulation is necessary because of the important role played by glucose in the body. Glucose is the major form in which carbohydrate absorbed from the intestinal tract is presented to cells of the body. Glucose is the only fuel used to any significant extent by a few specialized cells and the major fuel used by the brain. Indeed, glucose is so important to these specialized cells and the brain that several of the major tissues of the body work together to ensure a continuous supply of this essential substrate. Glucose metabolism is defective in two very common metabolic diseases, obesity and diabetes, which contribute in development of a number of major medical problems, including atherosclerosis, hypertension, small vessel disease, kidney disease, and blindness.

The discussion begins with **glycolysis,** a pathway used by all cells of the body to extract part of the chemical energy inherent in the glucose molecule. This pathway also converts glucose to pyruvate and sets the stage for complete oxidation of glucose to CO_2 and H_2O. The *de novo* synthesis of glucose, that is, gluconeogenesis, is a function of the liver and kidneys and can conveniently be discussed following glycolysis because it makes use of some of the same enzymes used in the glycolytic pathway, although the reactions catalyzed are in the opposite direction. In contrast to glycolysis, which produces ATP, gluconeogenesis requires ATP and is therefore an energy-requiring process. The consequence is that only some of the enzyme-catalyzed steps can be common to both the glycolytic and gluconeogenic pathways. Indeed, additional enzymes including some in mitochondria become involved to make the overall process of gluconeogenesis exergonic. Regulation of the rate-limiting and key enzyme-catalyzed steps will be stressed throughout the chapter. This will be particularly

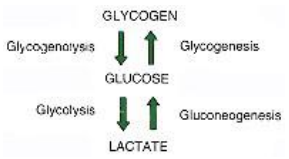


Figure 7.1
Relationship of glucose to the major pathways of carbohydrate metabolism.

true for glycogen synthesis **(glycogenesis)** and degradation **(glycogenolysis).** Many cells store glycogen for the purpose of having glucose available for later use. The liver is less selfish, storing glycogen not for its own use, but for maintenance of blood glucose levels that ensure that other tissues, especially the brain, have an adequate supply of this important substrate. Regulation of the synthesis and degradation of glycogen is a model for our understanding of how hormones work and how other metabolic pathways may be regulated. This subject contributes to our understanding of the diabetic condition, starvation, and how tissues of the body respond to stress, severe trauma, and injury. The Appendix presents the nomenclature and chemistry of the carbohydrates.

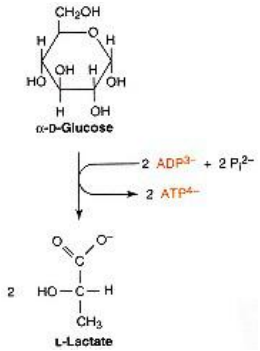


Figure 7.2
Overall balanced equation for the sum of the reactions of the glycolytic pathway.

7.2— Glycolysis

Glycolysis Occurs in All Human Cells

The Embden–Meyerhof or glycolytic pathway represents an ancient process, possessed by all cells of the human body, in which anaerobic degradation of glucose to lactate occurs. This is one example of anaerobic **fermentation,** a term used to refer to pathways by which organisms extract chemical energy from high-energy fuels in the absence of molecular oxygen. For many tissues glycolysis is an emergency energy-yielding pathway, capable of yielding 2 mol of ATP from 1 mol of glucose in the absence of molecular oxygen (Figure 7.2). Thus when the oxygen supply to a tissue is shut off, ATP levels can still be maintained by glycolysis for at least a short period of time. Many examples could be given, but the capacity to use glycolysis as a source of energy is particularly important to the human being at birth. With the exception of the brain, circulation of blood decreases to most parts of the body of the neonate during delivery. The brain is not normally deprived of oxygen during delivery, but other tissues must depend on glycolysis for their supply of ATP until circulation returns to normal and oxygen becomes available again. This conserves oxygen for use by the **brain,** illustrating one of many mechanisms that have evolved to assure survival of brain tissue in times of stress. Glycolysis sets the stage for aerobic oxidation of carbohydrate. Oxygen is not necessary for glycolysis, and the presence of oxygen can indirectly suppress glycolysis, a phenomenon called the **Pasteur effect** that is considered later. Nevertheless, glycolysis can and does occur in cells with an abundant supply of molecular oxygen. Provided cells also contain mitochondria, the end product of glycolysis in the presence of oxygen is pyruvate rather than lactate. Pyruvate can then be completely oxidized to CO_2 and H_2O by enzymes housed within the mitochondria. The overall process of glycolysis plus the subsequent mitochondrial oxidation of pyruvate to CO_2 and H_2O has the following equation:

$$\underset{(C_6H_{12}O_6)}{\text{D-Glucose}} + 6\,O_2 + 38\,ADP^{3-} + 38P_i^{2-} + 38\,H^+ \rightarrow 6\,CO_2 + 6\,H_2O + 38\,ATP^{4-}$$

Much more ATP is produced in complete oxidation of glucose to CO_2 and H_2O than in the conversion of glucose to lactate. This has important consequences, which are considered in detail later. For glucose to be completely oxidized to CO_2 and H_2O, it must first be converted to pyruvate by glycolysis (Figure 7.3). The importance of glycolysis as a preparatory pathway is best exemplified by

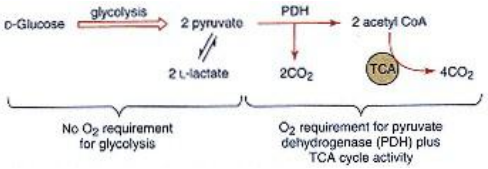


Figure 7.3
Glycolysis is a preparatory pathway for aerobic metabolism of glucose.
TCA refers to the tricarboxylic acid cycle.

the brain. This tissue has an absolute need for glucose and processes most of it via glycolysis. Pyruvate produced is then oxidized to CO_2 and H_2O in mitochondria. An adult human **brain** uses approximately 120 g of glucose each day in order to meet its need for ATP. In contrast, glycolysis with lactate as the end product is the major mechanism of ATP production in a number of other tissues. **Red blood cells** lack mitochondria and therefore are unable to convert pyruvate to CO_2 and H_2O. The **cornea, lens,** and regions of the **retina** have a limited blood supply and also lack mitochondria (because mitochondria would absorb and scatter light) and depend on glycolysis as the major mechanism for ATP production. **Kidney medulla, testis, leukocytes,** and white **muscle fibers** are almost totally dependent on glycolysis as a source of ATP, because these tissues have relatively few mitochondria. Tissues dependent primarily on glycolysis for ATP production consume about 40 g of glucose per day in a normal adult.

Major dietary sources of glucose are indicated in Chapter 26. **Starch** is the storage form of glucose in plants and contains α-1,4-glycosidic linkages along with α-1,6-glycosidic branches. **Glycogen** is the storage form of glucose in animal tissues and contains the same type of glycosidic linkages and branches. Exogenous glycogen refers to that which we eat and digest; endogenous glycogen is that synthesized or stored in our tissues. Exogenous starch or glycogen is hydrolyzed in the intestinal tract with the production of glucose, whereas stored glycogen endogenous to our tissues is converted to glucose or glucose 6-phosphate by enzymes present within the cells. Disaccharides that serve as important sources of glucose in our diet include milk sugar (lactose) and grocery store sugar (sucrose). Hydrolysis of these sugars by enzymes of the brush border of the intestinal tract is discussed on page 1075. Glucose can be used as a source of energy for cells of the intestinal tract. However, these cells do not depend on glucose to any great extent; most of their energy requirement is met by glutamine catabolism (see p. 450). Most of the glucose passes through the cells of the intestinal tract into the portal blood, then the general circulation, to be used by other tissues. Liver is the first major tissue to have an opportunity to remove glucose from the portal blood. When blood glucose is high, the liver removes glucose for the glucose-consuming processes of glycogenesis and glycolysis. When blood glucose is low, the liver supplies the blood with glucose by the glucose-producing processes of glycogenolysis and gluconeogenesis. The liver is also the first organ exposed to the blood flowing from the pancreas and therefore is exposed to the highest concentrations of the hormones released from this endocrine tissue—**glucagon** and **insulin.** These important hormonal regulators of blood glucose levels have effects on enzyme-catalyzed steps in the liver.

Glucose Is Metabolized Differently in Various Cells

After penetrating the plasma membrane by mediated transport on the **glucose transport protein GLUT-1,** glucose is metabolized mainly by glycolysis in red blood cells (Figure 7.4*a*). Since red blood cells lack mitochondria, the end

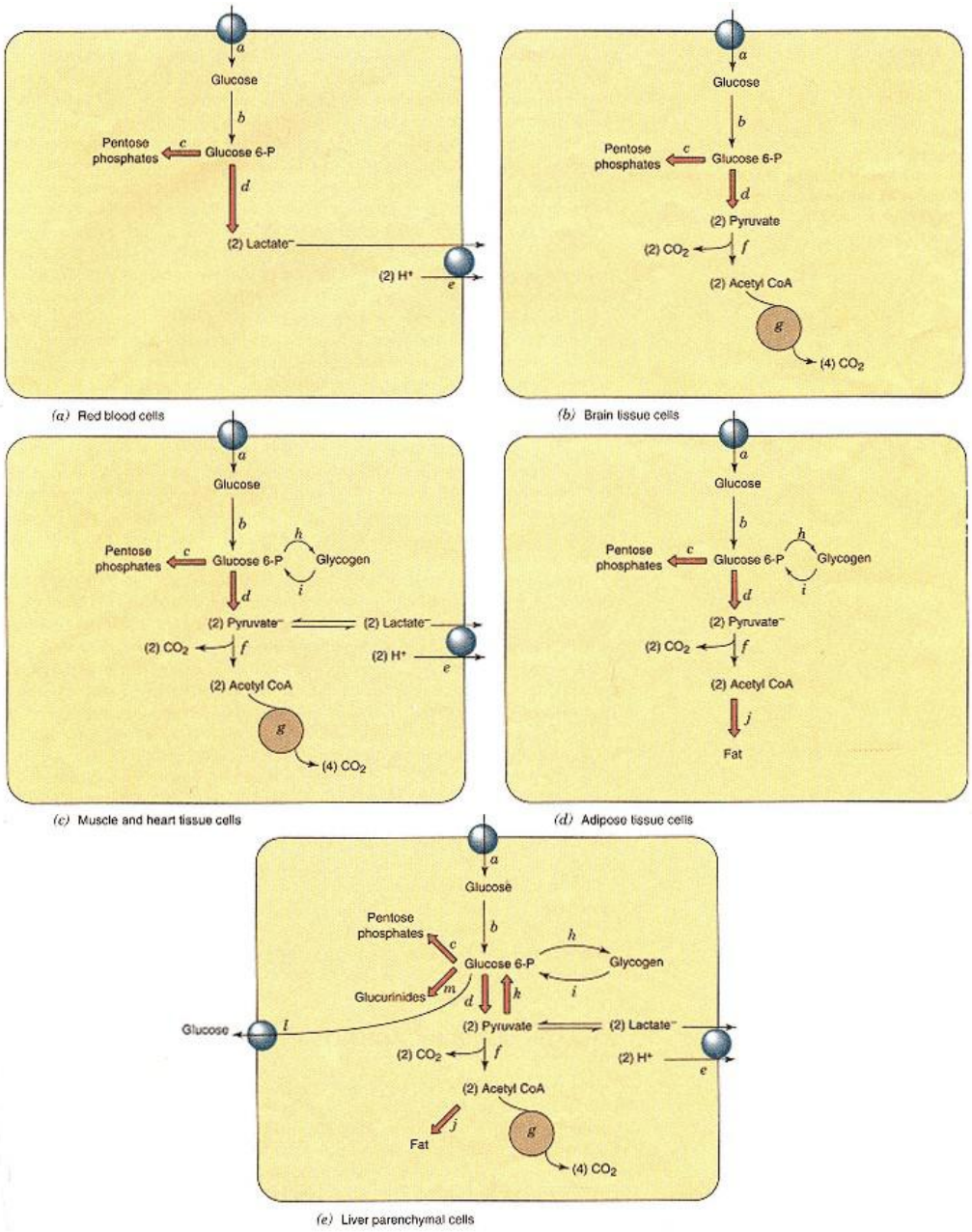


Figure 7.4
Overviews of the major ways in which glucose is metabolized within cells of selected tissues of the body.
(a) Glucose transport into the cell by a glucose transport protein (GLUT);
(b) glucose phosphorylation by hexokinase;
(c) the pentose phosphate pathway;
(d) glycolysis;
(e) lactic acid transport out of ther cell;
(f) pyruvate decarboxylation by pyruvate dehydrogenase;
(g) TCA cycle;
(h) glycogenesis;
(i) glycogenolysis;
(j) lipogenesis;
(k) gluconeogenesis;
(l) hydrolysis of glucose 6-phosphate and release of glucose from the cell into the blood;
(m) formation of glucuronides (drug and bilirubin detoxification by conjugation)
by the glucuronic acid pathway.

product of glycolysis is lactic acid, which is released into the blood. Glucose used by the pentose phosphate pathway in red blood cells provides NADPH to keep **glutathione** in the reduced state, which has an important role in the destruction of organic peroxides and H_2O_2 (Figure 7.5). Peroxides cause irreversible damage to membranes, DNA, and numerous other cellular components and must be removed to prevent cell death.

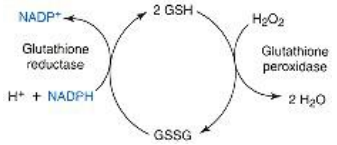


Figure 7.5
Destruction of H_2O_2 is dependent on reduction of oxidized glutathione by NADPH generated by the pentose phosphate pathway.

The brain takes up glucose by mediated transport in an insulin-independent manner by glucose transport protein **GLUT-3** (Figure 7.4*b*). Glycolysis in the brain yields pyruvate, which is oxidized to CO_2 and H_2O. The pentose phosphate pathway is active in these cells, generating part of the NADPH needed for reductive synthesis and the maintenance of glutathione in the reduced state.

Muscle and heart cells readily utilize glucose (Figure 7.4*c*). Insulin stimulates transport of glucose into these cells by way of glucose transport protein **GLUT-4.** Once in these cells, glucose can be utilized by glycolysis to give pyruvate, which is used by the pyruvate dehydrogenase complex and the TCA cycle to provide ATP. Muscle and heart, in contrast to the tissues just considered, are capable of synthesizing significant quantities of glycogen, an important process in these cells. Adipose tissue also transports glucose by the GLUT-4 protein, again in an insulin-dependent mechanism (Figure 7.4*d*). Pyruvate, as in other cells, is generated by glycolysis and is oxidized by the pyruvate dehydrogenase complex to give acetyl CoA, which is used primarily for *de novo* fatty acid synthesis. Generation of NADPH by the pentose phosphate pathway is important in adipose tissue because NADPH is necessary for the reductive steps of **fatty acid synthesis.** Adipose tissue has the capacity for glycogenesis and glycogenolysis, but these processes are much more limited in this tissue than in muscle and heart.

Liver has the greatest number of ways to utilize glucose (Figure 7.4*e*). Uptake of glucose by the liver occurs independent of insulin by means of a low-affinity, high-capacity glucose transport protein, **GLUT-2.** Glucose is used rather extensively by the pentose phosphate pathway for the production of NADPH, which is needed for reductive synthesis, maintenance of reduced glutathione, and numerous reactions catalyzed by endoplasmic reticulum enzyme systems. A quantitatively less important but nevertheless vital function of the pentose phosphate pathway is the provision of ribose phosphate, required for the synthesis of nucleotides such as ATP and those in DNA and RNA. Glucose is also used for glycogen synthesis, making glycogen storage an important feature of the liver. Glucose can also be used in the **glucuronic acid pathway,** which is important in **drug** and **bilirubin detoxification** (see Chapter 23). The liver is also capable of glycolysis, the pyruvate produced being used as a source of acetyl CoA for complete oxidation by the TCA cycle and for the synthesis of fat by the process of *de novo* **fatty acid synthesis.** In contrast to the other tissues, the liver is unique in that it has the capacity to convert three-carbon precursors, such as lactate, pyruvate, glycerol, and alanine, into glucose by the process of **gluconeogenesis,** to meet the need for glucose of other cells.

7.3— The Glycolytic Pathway

Glucose is combustible and will burn in a test tube to yield heat and light but, of course, no ATP. Cells use some 30 steps to take glucose to CO_2 and H_2O, a seemingly inefficient process, since it can be done in a single step in a test tube. However, side reactions and some of the actual steps used by the cell to "burn" glucose to CO_2 and H_2O lead to the conservation of a significant amount of energy in the form of ATP. In other words, ATP is produced by the controlled "burning" of glucose in the cell, glycolysis representing only the first few steps, shown in Figure 7.6, in the overall process.

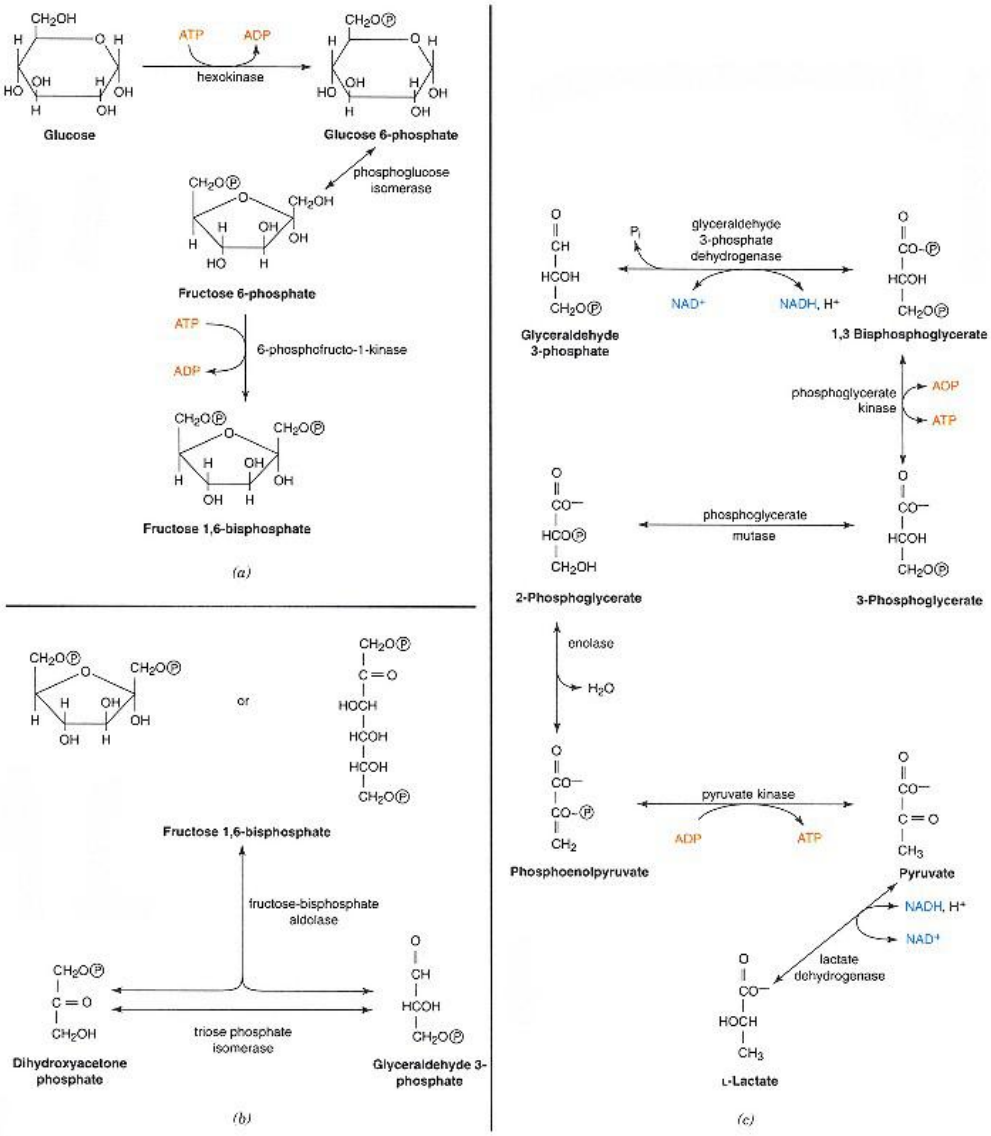


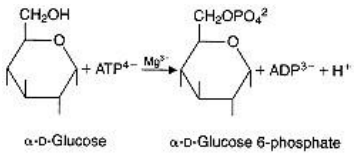
Figure 7.6
The glycolytic pathway, divided into its three stages.
The symbol P refers to the phosphoryl group PO_3^{2-}; ~ indicates a high-energy phosphate bond.
(a) Priming stage.
(b) Splitting stage.
(c) Oxidoreduction–phosphorylation stage.

Glycolysis Occurs in Three Stages

Glycolysis can conveniently be pictured as occurring in three major stages (also see Figure 7.6).

Priming stage:

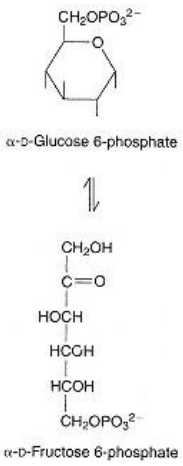
D-Glucose + 2 ATP^{4-} → D-fructose 1,6-bisphosphate^{4-} + 2 ADP^{3-} + 2 H^+



Step 1

Splitting stage:

D-Fructose 1,6-bisphosphate^{4-} → 2 D-glyceraldehyde 3-phosphate^{2-}



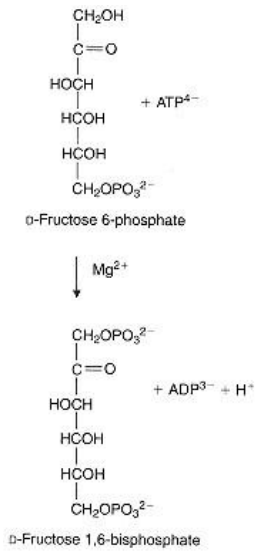
Step 2

Oxidoreduction–phosphorylation stage:

2 D-Glyceraldehyde 3-phosphate^{2-} + 4 ADP^{3-} + 2 P_i^{2-} + 2 H^+ →
2 L-lactate$^-$ + 4 ATP^{4-}

Sum:

D-Glucose + 2 ADP^{3-} + 2 P_i^{2-} → 2 L-lactate$^-$ + 2 ATP^{4-}



Step 3

Priming stage involves input of two molecules of ATP to convert glucose into a molecule of fructose 1,6-bisphosphate. ATP is therefore "invested" in the priming stage of glycolysis. However, ATP beyond this investment is gained from the glycolytic process. The **splitting stage** "splits" the six-carbon molecule fructose 1,6-bisphosphate into two molecules of glyceraldehyde 3-phosphate. In the **oxidoreduction–phosphorylation stage** two molecules of glyceraldehyde 3-phosphate are converted into two molecules of lactate with the production of four molecules of ATP. The overall process of glycolysis generates two molecules of lactate and two molecules of ATP at the expense of one molecule of glucose.

Stage One Primes the Glucose Molecule

Hexokinase catalyzes the first step of glycolysis (see Figure 7.6*a* and Step 1). Although this reaction consumes ATP, it gets glycolysis off to a good start by trapping glucose as glucose 6-phosphate (G6P) within the cytosol of the cell where all of the glycolytic enzymes are located. Phosphate esters are charged hydrophilic compounds that do not readily penetrate cell membranes. The phosphorylation of glucose with ATP is a thermodynamically favorable reaction, requiring the use of one high-energy phosphate bond. It is irreversible under cellular conditions. It is not, however, a way to synthesize ATP or to hydrolyze G6P to give glucose by the reverse reaction. Hydrolysis of G6P is accomplished by a different reaction, catalyzed by **glucose 6-phosphatase:**

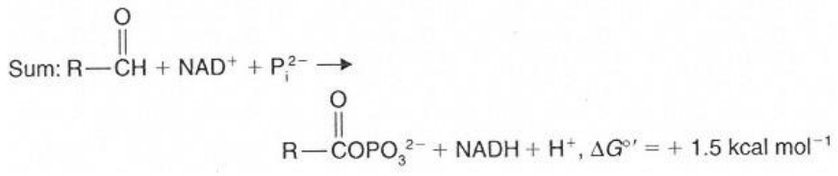
Glucose 6-phosphate^{2-} + H_2O → glucose + P_i^{2-}

This reaction is thermodynamically favorable in the direction written and cannot be used in cells for the synthesis of G6P from glucose. (A common mistake is to note that ATP and ADP are involved in the hexokinase reaction but not to note that they are not involved in the glucose 6-phosphatase reaction.) Glucose 6-phosphatase is an important enzyme in liver, functioning to produce free glucose from G6P in the last step of both gluconeogenesis and glycogenolysis; it has no role in glycolysis.

The next reaction is a readily reversible step of the glycolytic pathway, catalyzed by **phosphoglucose isomerase** (Step 2). This step is not subject to regulation and, since it is readily reversible, functions in both glycolysis and gluconeogenesis.

6-Phosphofructo-1-kinase (or phosphofructokinase-1) catalyzes the next reaction, an ATP-dependent phosphorylation of fructose 6-phosphate (F6P) to give fructose 1,6-bisphosphate (FBP) (Step 3). This is a favorite enzyme of many students of biochemistry, being subject to regulation by several effectors and often considered the rate-limiting enzyme of the glycolytic pathway. The reac-

tion is irreversible under intracellular conditions; that is, it represents a way to produce FBP but not a way to produce ATP or F6P by the reverse reaction. This reaction utilizes the second ATP needed to "prime" glucose, thereby completing the first stage of glycolysis.



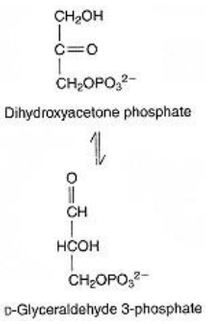
Step 4

Stage Two Is Splitting of a Phosphorylated Intermediate

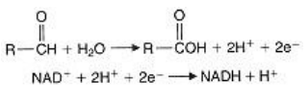
Fructose 1,6-bisphosphate **aldolase** catalyzes the cleavage of fructose 1,6-bis-phosphate into a molecule each of dihydroxyacetone phosphate and glyceralde-hyde 3-phosphate (GAP) (Figure 7.6*b*) (Step 4). This is a reversible reaction, the enzyme being called aldolase because the overall reaction is a variant of an aldol cleavage in one direction and an aldol condensation in the other. **Triose phosphate isomerase** then catalyzes the reversible interconversion of dihydroxyacetone phosphate and GAP to complete the splitting stage of glycolysis (Step 5). With the transformation of dihydroxyacetone phosphate (DHAP) into GAP, one molecule of glucose is converted into two molecules of GAP.

Stage Three Involves Oxidoreduction Reactions and the Synthesis of ATP

The first reaction of the last stage of glycolysis (Figure 7.6*c*) is catalyzed by **glyceraldehyde-3-phosphate dehydrogenase** (Step 6). This reaction is of considerable interest because of what is accomplished in a single enzyme-catalyzed step. An aldehyde (glyceraldehyde 3-phosphate) is oxidized to a carboxylic acid with the reduction of NAD^+ to NADH. In addition to NADH, the reaction produces 1,3-bisphosphoglycerate, a mixed anhydride of a carboxylic acid and phosphoric acid. 1,3-Bisphosphoglycerate has a large negative free energy of hydrolysis, enabling it to participate in a subsequent reaction that yields ATP. The overall reaction catalyzed by glyceraldehyde-3-phosphate dehydrogenase can be visualized as the coupling of a very favorable exergonic reaction with an unfavorable endergonic reaction. The exergonic reaction can be thought of as being composed of a half-reaction in which an aldehyde is oxidized to a carboxylic acid, which is then coupled with a half-reaction in which NAD^+ is reduced to NADH:

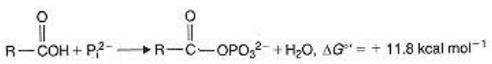


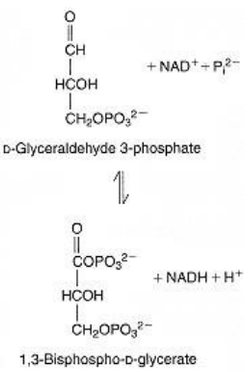
Step 5



The overall reaction (sum of the half-reactions) is quite exergonic, with the aldehyde being oxidized to a carboxylic acid and NAD^+ being reduced to NADH:

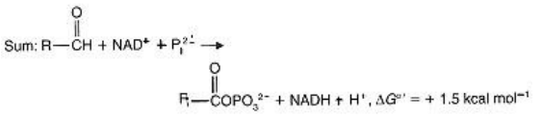
The endergonic component of the reaction corresponds to the formation of a mixed anhydride between the carboxylic acid and phosphoric acid:





Step 6

The overall reaction involves coupling of the endergonic and exergonic components to give an overall standard free-energy change of +1.5 kcal mol^{-1}.



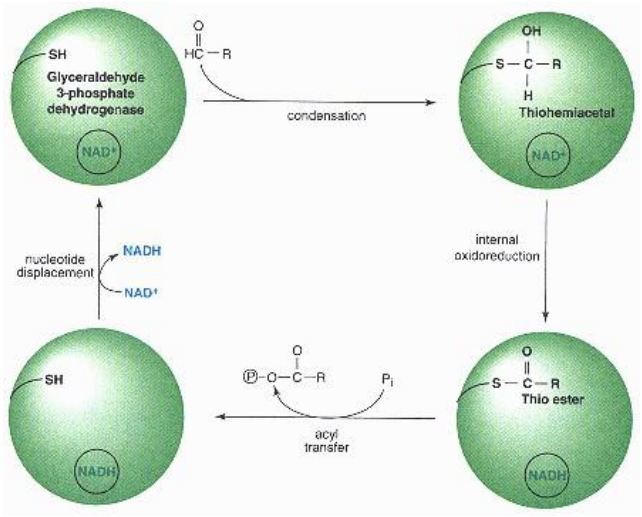
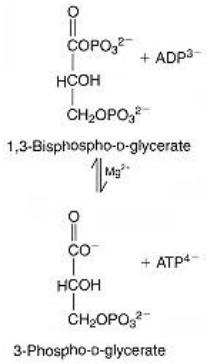


Figure 7.7
Mechanism of action of glyceraldehyde-3-phosphate dehydrogenase.
Large sphere represents the enzyme; small circle, the binding site for NAD^+; RCH, glyceraldehyde 3-phosphate; –SH, the sulfhydryl group of the cysteine residue located at the active site; and ~P, the high-energy phosphate bond of 1,3-bisphosphoglycerate.

The reaction is freely reversible in cells and is used in both the glycolytic and gluconeogenic pathways. The proposed mechanism for the enzyme-catalyzed reaction is shown in Figure 7.7. Glyceraldehyde 3-phosphate reacts with a sulfhydryl group of a cysteine residue of the enzyme to generate a thiohemiacetal. An internal oxidation–reduction reaction occurs in which bound NAD^+ is reduced to NADH and the thiohemiacetal is oxidized to give a high-energy thiol ester. The high-energy thiol ester reacts with P_i to form the mixed anhydride and regenerate the free sulfhydryl group. The mixed anhydride dissociates from the enzyme. Exogenous NAD^+ then replaces the bound NADH. Note that a carboxylic acid (RCOOH) is not an intermediate in the actual reaction. Instead, the enzyme generates a high-energy thiol ester, which is converted into another high-energy compound, a mixed anhydride of carboxylic and phosphoric acids.

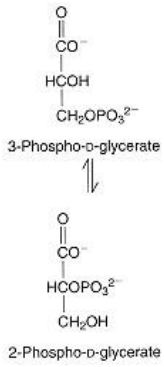
The reaction catalyzed by glyceraldehyde-3-phosphate dehydrogenase requires NAD^+ and produces NADH. Since the cytosol has only a limited amount of NAD^+, it is imperative for continuous glycolytic activity that the NADH be reoxidized to NAD^+, otherwise glycolysis will stop for want of NAD^+. The options that cells have for accomplishing the regeneration of NAD^+ are considered later (see p. 281).



Step 7

The next reaction, catalyzed by **phosphoglycerate kinase,** produces ATP from the high-energy compound 1,3-bisphosphoglycerate (Figure 7.6c; Step 7). This is the first site of ATP production in the glycolytic pathway. Because two ATP molecules were invested for each glucose molecule in the priming stage, and because two molecules of 1,3-bisphosphoglycerate are produced from each glucose, all of the ATP "invested" in the priming stage is recovered in this step of glycolysis. Since ATP production occurs in the forward direction and ATP utilization in the reverse direction, it may seem surprising that the reaction is freely reversible and can be used in both the glycolytic and gluconeogenic pathways. The reaction provides a means for the generation of ATP in the glycolytic pathway but, when needed for glucose synthesis, can also be used in the reverse direction for the synthesis of 1,3-bisphosphoglycerate at the expense of ATP. The glyceraldehyde-3-phosphate dehydrogenase-phosphoglycerate kinase system is an example of **substrate-level phosphory-**

lation, a term used for a process in which a substrate participates in an enzyme-catalyzed reaction that yields ATP or GTP. Substrate-level phosphorylation stands in contrast to mitochondrial oxidative phosphorylation (see Chapter 6). Note, however, that the combination of the reactions catalyzed by glyceraldehyde-3-phosphate dehydrogenase and phosphoglycerate kinase accomplishes the coupling of an oxidation (an aldehyde goes to a carboxylic acid) to a phosphorylation.



Step 8

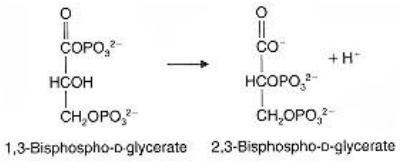
Phosphoglycerate mutase converts 3-phosphoglycerate to 2-phosphoglycerate (Step 8). This is a freely reversible reaction in which 2,3-bisphosphoglycerate (or 2,3-diphosphoglycerate) functions as an obligatory intermediate at the active site of the enzyme (E):

E-phosphate + 3-phosphoglycerate ⇌ E + 2,3-bisphosphoglycerate

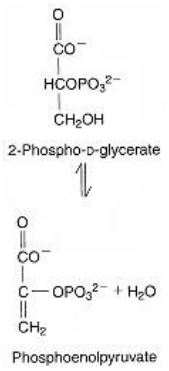
E + 2,3-bisphosphoglycerate ⇌ E-phosphate + 2-phosphoglycerate

Sum: 3-phosphoglycerate ⇌ 2-phosphoglycerate

The involvement of 2,3-bisphosphoglycerate as an intermediate creates an absolute requirement for the presence of a catalytic amount of this compound in cells. This can be appreciated by noting that E-phosphate in this reaction cannot be generated without 2,3-bisphosphoglycerate. Cells synthesize 2,3-bisphosphoglycerate, independent of the reaction catalyzed by phosphoglycerate mutase, by a reaction catalyzed by 2,3-bisphosphoglycerate mutase:



The mutase is unusual in that it is a bifunctional enzyme, serving also as a phosphatase that converts 2,3-bisphosphoglycerate to 3-phosphoglycerate and P_i. All cells contain at least minute quantities of 2,3-bisphosphoglycerate for no apparent purpose other than to produce the phosphorylated form of newly synthesized phosphoglycerate mutase. The amounts needed are small because phosphoglycerate mutase has to be phosphorylated only once, the phosphorylated enzyme being regenerated during each reaction cycle. Red blood cells contain very high 2,3-bisphosphoglycerate concentrations because it serves as an important allosteric effector of the association of oxygen with the hemoglobin (see Chapter 25). From 15% to 25% of the glucose converted to lactate in red blood cells goes by way of the **"BPG shunt"** (Figure 7.8). Catabolism of glucose by the BPG shunt generates no net ATP since the reaction catalyzed by the phosphoglycerate kinase is bypassed.



Step 9

Enolase catalyzes elimination of water from 2-phosphoglycerate to form phosphoenolpyruvate (PEP) in the next reaction (Step 9; Figure 7.6*c*). This is a remarkable reaction from the standpoint that a high-energy phosphate compound is generated from one of markedly lower energy level. The standard free-energy change (ΔG°) for the hydrolysis of phosphoenolpyruvate is –14.8 kcal mol^{-1}, a much greater value than the standard free energy for 2-phosphoglycerate hydrolysis (–4.2 kcal mol^{-1}). Although the reaction catalyzed by enolase is freely reversible, a large change in the distribution of energy occurs as a consequence of its action on 2-phosphoglycerate. The free-energy levels of PEP and 2-phosphoglycerate are not markedly different; however, the free-energy levels of their products of hydrolysis (pyruvate and glycerate, respectively) are quite different. Since $\Delta G^{\circ\prime} = \Delta G^{\circ\prime}_{\text{products}} - G^{\circ\prime}_{\text{substrates}}$, this accounts for the marked differences in the standard free energy of hydrolysis of these two compounds.

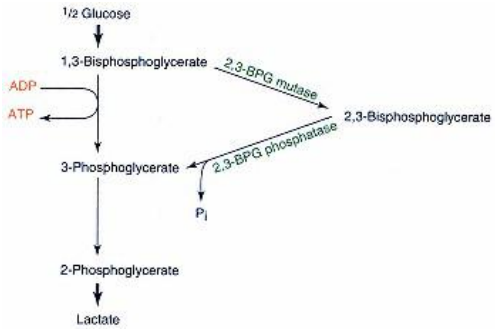
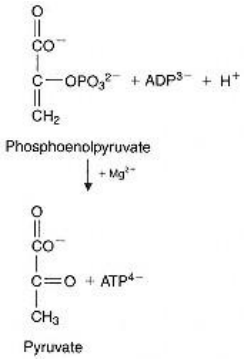


Figure 7.8
The 2,3-bisphosphoglycerate (2,3-BPG) shunt consists of reactions catalyzed by the bifunctional enzyme, 2,3-BPG mutase/phosphatase.

Pyruvate kinase (Step 10; Figure 7.6c) accomplishes another **substrate-level phosphorylation:** that is, the synthesis of ATP with the conversion of the high-energy compound PEP into pyruvate. It constitutes a way to synthesize ATP but, in contrast to the phosphoglycerate kinase reaction, is not reversible under conditions that exist in cells and cannot be used for the synthesis of PEP when needed for glucose synthesis.

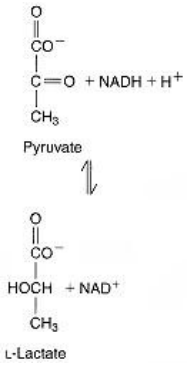


Step 10

The last step of the glycolytic pathway is an oxidoreduction reaction catalyzed by **lactate dehydrogenase** (Step 11; Figure 7.6c). Pyruvate is reduced to give L-lactate and NADH is oxidized to NAD^+. This is a freely reversible reaction and the only reaction that can result in L-lactate formation or L-lactate utilization.

A Balance of Reduction of NAD^+ and Reoxidation of NADH Is Required: Role of Lactate Dehydrogenase

There is a perfect coupling between the generation of NADH and its utilization in glycolysis (Figure 7.6*c*). Two molecules of NADH are generated at the level of glyceraldehyde-3-phosphate dehydrogenase and two molecules of NADH are utilized by lactate dehydrogenase in the conversion of one molecule of glucose into two molecules of lactate. NAD^+ a, soluble molecule present in the cytosol, is available in only limited amounts and must be regenerated from NADH for glycolysis to continue unabated. The overall reaction catalyzed by the combined actions of glyceraldehyde-3-phosphate dehydrogenase and lactate dehydrogenase is the conversion of pyruvate, glyceraldehyde 3-phosphate, and P_i into lactate and 1,3-bisphosphoglycerate. The two reactions are



Step 11

D-Glyceraldehyde 3-phosphate + NAD^+ + P_i → 1,3-bisphospho-D-glycerate + NADH + H^+

Pyruvate + NADH + H^+ → L-lactate + NAD^+

Sum: D-Glyceraldehyde 3-phosphate + pyruvate + P_i → 1,3-bisphosphoglycerate + L-lactate

This perfect coupling of reducing equivalents in the glycolytic pathway has to occur under conditions of anaerobiosis or in cells that lack mitochondria. With the availability of oxygen and mitochondria, reducing equivalents in the form of NADH generated at the level of glyceraldehyde-3-phosphate dehydrogenase can be shuttled into the mitochondria for the synthesis of ATP, leaving pyruvate rather than lactate as the end product of glycolysis. Two shuttle systems are

known to exist for the transport of reducing equivalents from the cytosolic space to the mitochondrial matrix space (mitosol). The mitochondrial inner membrane is not permeable to NADH.

NADH Generated during Glycolysis Can Be Reoxidized Via Substrate Shuttle Systems

The **glycerol phosphate shuttle** is shown in Figure 7.9*a* and the **malate–aspartate shuttle** in Figure 7.9*b*. Tissues with cells that contain mitochondria have the capability of shuttling reducing equivalents from the cytosol to the mitosol. The relative proportion of the activities of the two shuttles varies from tissue to tissue, with liver making greater use of the malate–aspartate shuttle, whereas some muscle cells may be more dependent on the glycerol phosphate shuttle. The shuttle systems are irreversible; that is, they represent mechanisms for moving reducing equivalents into the mitosol, but not mechanisms for moving mitochondrial reducing equivalents into the cytosol.

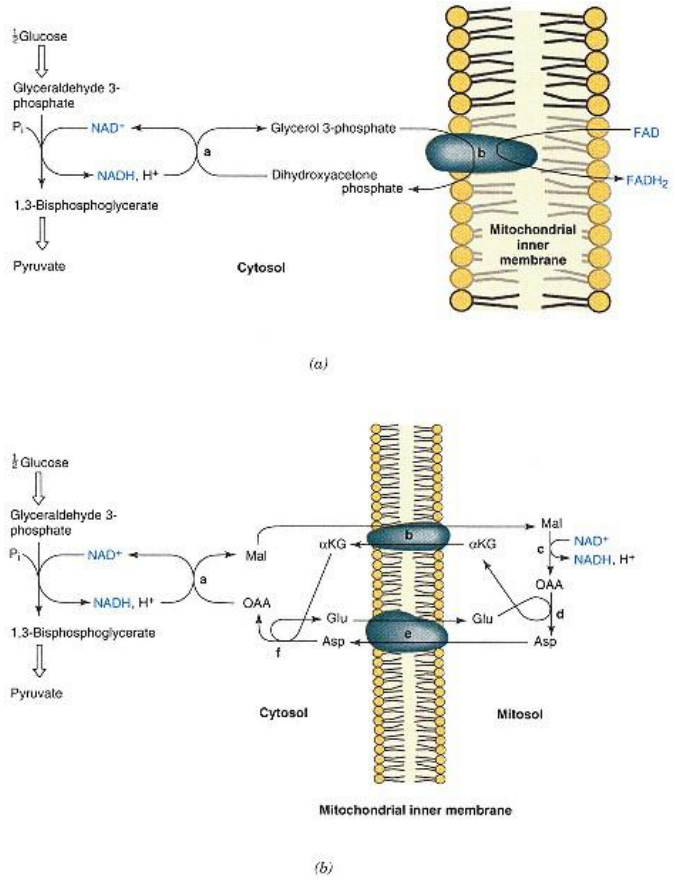


Figure 7.9
Shuttles for the transport of reducing equivalents from the cytosol to the mitochondrial electron-transfer chain.
(a) Glycerol phosphate shuttle: a, cytosolic glycerol 3-phosphate dehydrogenase oxidizes NADH; b, mitochondrial glycerol-3-phosphate dehydrogenase of the outer surface of the inner membrane reduces FAD.
(b) Malate–aspartate shuttle: a, cytosolic malate dehydrogenase reduces oxaloacetate (OAA) to malate; b, dicarboxylic acid antiport of the mitochondrial inner membrane catalyzes electrically silent exchange of malate for -ketoglutarate (-KG); c, mitochondrial malate dehydrogenase produces intramitochondrial NADH; d, mitochondrial aspartate aminotransferase transaminates glutamate and oxaloacetate; e, glutamate–aspartate antiport of the mitochondrial inner membrane catalyzes electrogenic exchange of glutamate for aspartate; f, cytosolic aspartate aminotransferase transaminates aspartate and -ketoglutarate.

The transport of aspartate out of mitochondria in exchange for glutamate is the irreversible step in the malate–aspartate shuttle. The mitochondrial inner membrane has a large number of transport systems (see Chapter 6) but lacks one that is effective for oxaloacetate. For this reason oxaloacetate transaminates with glutamate to produce aspartate, which then exits irreversibly from the mitochondrion in exchange for glutamate. The aspartate entering the cytosol transaminates with α-ketoglutarate to give oxaloacetate and glutamate. The oxaloacetate accepts the reducing equivalents of NADH and becomes malate. Malate then penetrates the mitochondrial inner membrane, where it is oxidized by the mitochondrial **malate dehydrogenase.** This produces NADH within the mitosol and regenerates oxaloacetate to complete the cycle. The overall balanced equation for the sum of all the reactions of the malate–aspartate shuttle is simply

$$NADH_{cytosol} + H^+_{cytosol} + NAD^+_{mitosol} \rightarrow NAD^+_{cytosol} + NADH_{mitosol} + H^+_{mitosol}$$

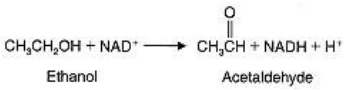
The glycerol phosphate shuttle is simpler, in the sense that fewer reactions are involved, but $FADH_2$ is generated within the mitochondrial inner membrane rather than NADH within the mitosolic compartment. The irreversible step of the shuttle is catalyzed by the mitochondrial **glycerol-3-phosphate dehydrogenase.** The active site of this enzyme is exposed on the cytosolic surface of the mitochondrial inner membrane, making it unnecessary for glycerol 3-phosphate to penetrate into the mitosol for oxidation. The overall balanced equation for the sum of the reactions of the glycerol phosphate shuttle is

$$NADH_{cytosol} + H^+_{cytosol} + FAD_{\text{inner membrane}} \rightarrow NAD^+_{cytosol} + FADH_{2\ \text{inner membrane}}$$

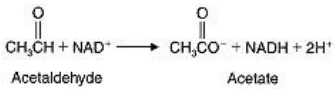
Shuttles Are Important in Other Oxidoreductive Pathways

Alcohol Oxidation

The first step of **alcohol** (i.e., ethanol) metabolism is its oxidation to **acetaldehyde** with production of NADH by **alcohol dehydrogenase.**



This enzyme is located almost exclusively in the cytosol of liver parenchymal cells. The acetaldehyde generated traverses the mitochondrial inner membrane for oxidation by a mitosolic **aldehyde dehydrogenase.**



The NADH generated by the last step can be used directly by the mitochondrial electron-transfer chain. However, NADH generated by cytosolic alcohol dehydrogenase must be oxidized back to NAD^+ by one of the shuttles. Thus the capacity of a human being to oxidize alcohol is dependent on the ability of the liver to transport reducing equivalents from the cytosol to the mitosol by these shuttle systems.

Glucuronide Formation

The shuttles play an important role in the formation of water-soluble **glucuronides** of bilirubin and various drugs (see p. 1018) so that these compounds can be eliminated from the body in the urine and bile. In this process **UDP-glucose** (structure on p. 343) is oxidized to **UDP-glucuronic acid** (structure on p. 344).

UDP-D-glucose + 2 NAD^+ + H_2O → UDP-D-glucuronic acid + 2 NADH + 2 H^+

CLINICAL CORRELATION 7.1

Alcohol and Barbiturates

Acute alcohol intoxication causes increased sensitivity of an individual to the general depressant effects of barbiturates. Barbiturates and alcohol both interact with the γ-aminobutyrate (GABA)-activated chloride channel. Activation of the chloride channel inhibits neuronal firing, which may explain the depressant effects of both compounds. This drug combination is very dangerous and normal prescription doses of barbiturates have potentially lethal consequences in the presence of ethanol. In addition to the depressant effects of both ethanol and barbiturates on the central nervous system (CNS), ethanol inhibits the metabolism of barbiturates, thereby prolonging the time barbiturates remain effective in the body. Hydroxylation of barbiturates by the endoplasmic reticulum of the liver is inhibited by ethanol. This reaction, catalyzed by the NADPH-dependent cytochrome system, forms water-soluble derivatives of the barbiturates that are eliminated readily from the circulation by the kidneys. Blood levels of barbiturates remain high when ethanol is present, causing increased CNS depression.

Surprisingly, the alcoholic when sober is less sensitive to barbiturates. Chronic ethanol consumption apparently causes adaptive changes in the sensitivity of the CNS to barbiturates (cross-tolerance). It also results in the induction of the enzymes of liver endoplasmic reticulum involved in drug hydroxylation reactions. Consequently, the sober alcoholic is able to metabolize barbiturates more rapidly. This sets up the following scenario. A sober alcoholic has trouble falling asleep, even after taking several sleeping pills, because his/her liver has increased capacity to hydroxylate the barbiturate contained in the pills. In frustration he/she consumes more pills and then alcohol. Sleep results, but may be followed by respiratory depression and death because the alcoholic, although less sensitive to barbiturates when sober, remains sensitive to the synergistic effect of alcohol.

Misra, P. S., Lefevre, A., Ishii, H., Rubin, E., and Lieber, C. S. Increase of ethanol, meprobamate and pentobarbital metabolism after chronic ethanol administration in man and in rats. *Am. J. Med.* 51:346, 1971.

In a reaction that occurs primarily in the liver, the "activated" glucuronic acid molecule is then transferred to a nonpolar acceptor molecule, such as **bilirubin** or a compound foreign to the body:

$$\text{UDP-D-glucuronic acid} + \text{R}{-}\text{OH} \rightarrow \text{R}{-}\text{O}{-}\text{glucuronic acid} + \text{UDP}$$

Excess NADH generated by the first reaction has to be reoxidized by the shuttles for this process to continue. Since ethanol oxidation and drug conjugation are properties of the liver, the two of them occurring together may overwhelm the combined capacity of the shuttles. A good thing to tell patients is not to mix the intake of pharmacologically active compounds and alcohol (see Clin. Corr. 7.1).

Two Shuttle Pathways Yield Different Amounts of ATP

The mitosolic NADH formed by the malate–aspartate shuttle activity can be used by the mitochondrial respiratory chain for the production of three molecules of ATP by oxidative phosphorylation:

$$\text{NADH}_{\text{mitosol}} + \text{H}^+ + \tfrac{1}{2}\,\text{O}_2 + 3\ \text{ADP} + 3\ \text{P}_\text{i} \rightarrow \text{NAD}^+_{\text{mitosol}} + 3\ \text{ATP} + \text{H}_2\text{O}$$

In contrast, the $FADH_2$ obtained by the glycerol phosphate shuttle yields only two ATP molecules:

$$\text{FADH}_{2\ \text{inner membrane}} + \tfrac{1}{2}\,\text{O}_2 + 2\ \text{ADP} + 2\ \text{P}_\text{i} \rightarrow \text{FAD}_{\text{inner membrane}} + 2\ \text{ATP} + \text{H}_2\text{O}$$

Without the intervention of the shuttle systems, conversion of one molecule of glucose to two molecules of lactate by glycolysis results in the net formation of two molecules of ATP. Two molecules of ATP are used in the priming stage to set glucose up so that it can be cleaved. However, subsequent steps then yield four molecules of ATP so that the overall net production of ATP by the glycolytic pathway is two molecules of ATP. Biological cells have only a limited amount of ADP and P_i. Therefore flux through the glycolytic pathway is also dependent on an adequate supply of these substrates. If the ATP is not utilized for performance of work, glycolysis will stop for want of ADP and/or P_i. Consequently, the ATP generated has to be used, that is, turned over, in normal work-related processes in order for glycolysis to occur. The equation for the use of ATP for any work-related process is simply

$$\text{ATP}^{4-} \rightarrow \text{ADP}^{3-} + \text{P}_\text{i}^{2-} + \text{H}^+ + \text{“work”}$$

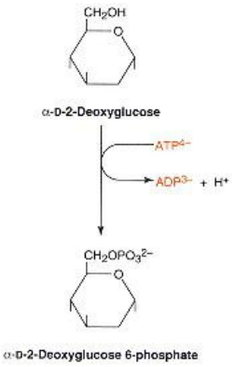


Figure 7.10
Hexokinase catalyzes the phosphorylation of 2-deoxyglucose.

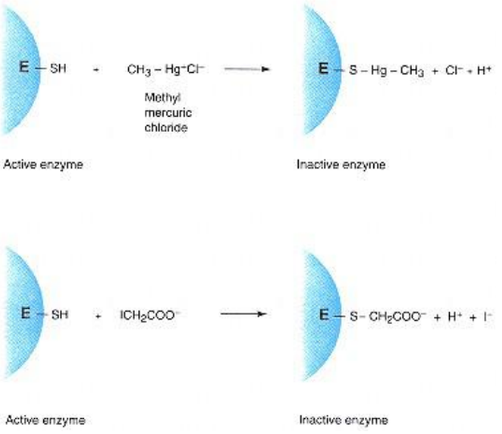


Figure 7.11
Mechanism responsible for inactivation of glyceraldehyde-3-phosphate dehydrogenase by sulfhydryl reagents.

When this equation is added to that given above for glycolysis, excluding the work accomplished, the overall balanced equation becomes

$$\text{D-Glucose} \rightarrow 2 \text{ lactate}^- + 2 \text{ H}^+$$

Glycolysis Can Be Inhibited at Different Stages

The best known inhibitors of the glycolytic pathway are **2-deoxyglucose,** sulfhydryl reagents, and **fluoride.** 2-Deoxyglucose is a substrate for hexokinase, being converted to its 6-phosphate ester (Figure 7.10). Like glucose 6-phosphate, 2-deoxyglucose 6-phosphate is an effective inhibitor of the reaction catalyzed by hexokinase but, unlike glucose 6-phosphate, will not function as a substrate for the reaction catalyzed by phosphoglucose isomerase. Thus it will accumulate in cells.

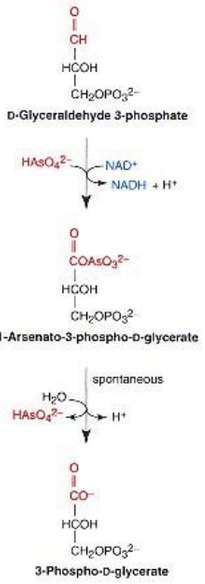


Figure 7.12
Arsenate uncouples oxidation from phosphorylation at the step catalyzed by glyceraldehyde-3-phosphate dehydrogenase.

Sulfhydryl reagents inhibit **glyceraldehyde-3-phosphate dehydrogenase.** This enzyme has a cysteine residue at the active site. The sulfhydryl group combines with glyceraldehyde 3-phosphate to give a thiohemiacetal (Figure 7.7). Sulfhydryl reagents are usually mercury-containing compounds or alkylating compounds, such as **iodoacetate,** which readily react with the sulfhydryl group of glyceraldehyde-3-phosphate dehydrogenase to prevent the formation of the thiohemiacetal (Figure 7.11).

Fluoride is a potent inhibitor of enolase. Mg^{2+} and P_i form an ionic complex with fluoride ion, which is responsible for inhibition of enolase by interfering with binding of its substrate (Mg^{2+} 2-phosphoglycerate).

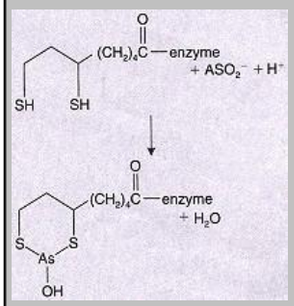
Pentavalent arsenic or arsenate is special with respect to its effects on glycolysis. It is not an inhibitor of the process, and under some conditions can even stimulate glycolytic flux. Arsenate prevents net synthesis of ATP by causing arsenolysis in the glyceraldehyde-3-phosphate dehydrogenase reaction. Arsenate looks like P_i and is able to substitute for P_i in enzyme-catalyzed reactions. The result is the formation of a mixed anhydride of arsenic acid and the carboxyl group of 3-phosphoglycerate during the reaction catalyzed by glyceraldehyde-3-phosphate dehydrogenase (Figure 7.12). 1-Arsenato 3-phosphoglycerate is

unstable, undergoing spontaneous hydrolysis to give 3-phosphoglycerate and inorganic arsenate. Hence glycolysis continues unabated in the presence of arsenate, but 1,3-bisphosphoglycerate is not formed, resulting in the loss of the capacity to synthesize ATP at the step catalyzed by phosphoglycerate kinase. Thus net ATP synthesis does not occur when glycolysis is carried out in the presence of arsenate, the ATP invested in the priming stage being balanced by the ATP generated in the pyruvate kinase step. This, along with the fact that **arsenolysis** also interferes with ATP formation by oxidative phosphorylation, makes arsenate a toxic compound (see Clin. Corr. 7.2).

CLINICAL CORRELATION 7.2

Arsenic Poisoning

Most forms of arsenic are toxic, but the trivalent form (arsenite as AsO_2^-) is much more toxic than the pentavalent form (arsenate or $HAsO_4^{2-}$). Less ATP is produced whenever arsenate substitutes for P_i in biological reactions. Arsenate competes for P_i-binding sites on enzymes, resulting in the formation of arsenate esters that are unstable. Arsenite works by a completely different mechanism, involving the formation of a stable complex with enzyme-bound lipoic acid:



For the most part arsenic poisoning is explained by inhibition of those enzymes that require lipoic acid as a coenzyme. These include pyruvate dehydrogenase, α-ketoglutarate dehydrogenase, and branched-chain α-keto acid dehydrogenase. Chronic arsenic poisoning from well water contaminated with arsenical pesticides or through the efforts of a murderer is best diagnosed by determining the concentration of arsenic in the hair or fingernails of the victim. About 0.5 mg of arsenic would be found in a kilogram of hair from a normal individual. The hair of a person chronically exposed to arsenic could have 100 times as much.

Hindmarsh, J. T., and McCurdy, R. F. Clinical and environmental aspects of arsenic toxicity. *CRC Crit. Rev. Clin. Lab. Sci.* 23:315, 1986.

7.4— Regulation of the Glycolytic Pathway

The regulatory enzymes of the glycolytic pathway are **hexokinase, 6-phosphofructo-1-kinase,** and **pyruvate kinase.** A summary of the important regulatory features of these enzymes is presented in Figure 7.13. A regulatory enzyme is controlled by either allosteric effectors or covalent modification (see p. 151). Both mechanisms are used by cells to control the most important of the regulatory enzymes. A regulatory enzyme can often be identified by determining whether the concentrations of the substrates and products within a cell indicate that the reaction catalyzed by the enzyme is close to equilibrium. An enzyme

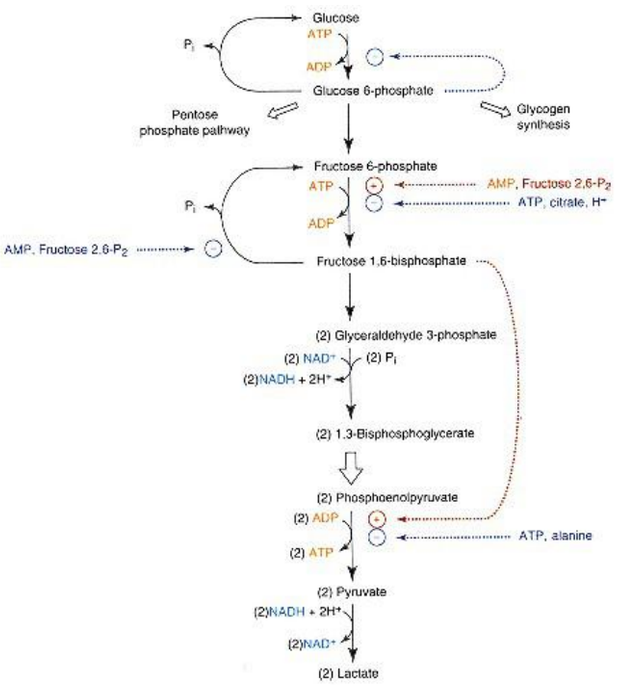


Figure 7.13
Important regulatory features of the glycolytic pathway.
Because of differences in isoenzyme distribution, not all tissues of the body have all of the regulatory mechanisms shown here.

that is not subject to regulation will catalyze a **"near-equilibrium reaction,"** whereas a regulatory enzyme will catalyze a **"nonequilibrium reaction"** under intracellular conditions. This makes sense because flux through the regulated enzyme is restricted by controls imposed on that enzyme. A nonregulatory enzyme is so active that it readily brings its substrates and products to equilibrium concentrations. Whether an enzyme-catalyzed reaction is near equilibrium or nonequilibrium can be determined by comparing the established equilibrium constant for the reaction with the mass–action ratio as it exists within a cell. The **equilibrium constant** for the reaction $A + B \rightleftharpoons C + D$ is defined as

$$K_{eq} = \frac{[C][D]}{[A][B]}$$

where the brackets indicate the concentrations at equilibrium. The **mass–action ratio** is calculated in a similar manner, except that the steady-state (ss) concentrations of reactants and products within the cell are used in the equation:

$$\text{Mass–action ratio} = \frac{[C]_{ss}[D]_{ss}}{[A]_{ss}[B]_{ss}}$$

If the mass–action ratio is approximately equal to the K_{eq}, the enzyme is said to be active enough to catalyze a near-equilibrium reaction and the enzyme is not considered subject to regulation. When the mass–action ratio is considerably different from the K_{eq}, the enzyme is said to catalyze a nonequilibrium reaction and usually will be found subject to regulation by one or more mechanisms. Mass–action ratios and equilibrium constants are compared for the glycolytic enzymes of liver in Table 7.1. The reactions catalyzed by **glucokinase** (liver isoenzyme of hexokinase), 6-phosphofructo-1-kinase, and pyruvate kinase in the intact liver are considered far enough from equilibrium to indicate that these enzymes are "regulatory" in this tissue.

Hexokinase and Glucokinase Have Different Properties

Different isoenzymes of **hexokinase** occur in different tissues. The hexokinase isoenzymes found in most tissues have a low K_m for glucose (<0.1 mM) relative

TABLE 7.1 Apparent Equilibrium Constants and Mass–Action Ratios for the Reactions of Glycolysis and Gluconeogenesis in Liver

Reaction Catalyzed by	*Reaction in the Pathway of*		*Apparent Equilibrium Constant* (K'_{eq})	*Mass–Action Ratios*	*Considered Near-Equilibrium Reaction?*
	Glycolysis	*Gluconeogenesis*			
Glucokinase	Yes	No	2×10^3	0.02	No
Glucose 6-phosphatase	No	Yes	850 M	120 M	No
Phosphoglucoisomerase	Yes	Yes	0.36	0.31	Yes
6-Phosphofructo-1-kinase	Yes	No	1×10^3	0.09	No
Fructose 1,6-bisphosphatase	No	Yes	530 M	19 M	No
Aldolase	Yes	Yes	13×10^{-5} M	12×10^{-7} M	Yes[a]
Glyceraldehyde-3-phosphate dehydrogenase + phosphoglycerate kinase	Yes	Yes	2×10^3 M^{-1}	0.6×10^3 M^{-1}	Yes
Phosphoglycerate mutase	Yes	Yes	0.1	0.1	Yes
Enolase	Yes	Yes	3.0	2.9	Yes
Pyruvate kinase	Yes	No	2×10^4	0.7	No
Pyruvate carboxylase + phosphoenolpyruvate carboxykinase	No	Yes	7.0 M	1×10^{-3} M	No

[a] Reaction catalyzed by aldolase appears to be out of equilibrium by two orders of magnitude. However, *in vivo* concentrations of fructose 1,6-micromolar bisophosphate and glyceraldehyde 3-phosphate are so low (micromolar concentration range) that significant enzyme binding of both metabolites is believed to occur. Although only the total concentration of any metabolite of a tissue can be measured, only that portion of the metabolite that is not bound should be used in the calculations of mass–action ratios. This is usually not possible, introducing uncertainty in the comparison of *in vitro* equilibrium constants to *in vivo* mass–action ratios.

CLINICAL CORRELATION 7.3

Fructose Intolerance

Patients with hereditary fructose intolerance are deficient in the liver aldolase responsible for splitting fructose 1-phosphate into dihydroxyacetone phosphate and glyceraldehyde. Consumption of fructose by these patients results in the accumulation of fructose 1-phosphate and depletion of P_i and ATP in the liver. The reactions involved are those catalyzed by fructokinase and the enzymes of oxidative phosphorylation:

Fructose + ATP → fructose 1-phosphate + ADP

ADP + P_i + "energy provided by electron transport chain" → ATP

Net: P_i + fructose → fructose 1-phosphate

Tying up P_i in the form of fructose 1-phosphate makes it impossible for liver mitochondria to generate ATP by oxidative phosphorylation. The ATP levels fall precipitously, making it also impossible for the liver to carry out its normal work functions. Damage results to the cells in large part because they are unable to maintain normal ion gradients by means of the ATP-dependent cation pumps. The cells swell and eventually lose their internal contents by osmotic lysis (see Clin. Corr. 6.6).

Although patients with fructose intolerance are particularly sensitive to fructose, humans in general have a limited capacity to handle this sugar. The capacity of the normal liver to phosphorylate fructose greatly exceeds its capacity to split fructose 1-phosphate. This means that fructose use by the liver is poorly controlled and that excessive fructose could deplete the liver of P_i and ATP. Fructose was actually tried briefly in hospitals as a substitute for glucose in patients being maintained by parenteral nutrition. The rationale was that fructose would be a better source of calories than glucose because fructose utilization is relatively independent of the insulin status of a patient. Delivery of large amounts of fructose by intravenous feeding was soon found to result in severe liver damage. Similar attempts have been made to substitute sorbitol and xylitol for glucose. These sugars also tend to deplete the liver of ATP and, like fructose, should not be used for parenteral nutrition.

Gitzelmann, R., Steinmann, B., and Van den Berghe, G. Disorders of fructose metabolism. In: C. R. Scriver, A. L. Beaudet, W. S. Sly, and D. Valle (Eds.), *The Metabolic and Molecular Bases of Inherited Disease*, 7th ed. New York: McGraw-Hill, 1995, pp. 905–934.

to its concentration in blood (~5 mM) and are strongly inhibited by the product of the reaction, glucose 6-phosphate. The latter is an important regulatory feature because it prevents hexokinase from tying up all of the P_i of a cell in the form of phosphorylated hexoses (see Clin. Corr. 7.3). Thus the reaction catalyzed by hexokinase may not be at equilibrium within cells that contain this enzyme because of the inhibition imposed by G6P. Liver parenchymal cells are unique in that they contain glucokinase, an isoenzyme of hexokinase with strikingly different kinetic properties from the other hexokinases. This isoenzyme catalyzes an ATP-dependent phosphorylation of glucose but has a much higher K_m for glucose and is not subject to product inhibition by G6P. It is, however, inhibited by fructose 6-phosphate and activated by fructose 1-phosphate. These effects depend on an inhibitory protein that inhibits by binding tightly to glucokinase. Fructose 6-phosphate promotes but fructose 1-phosphate inhibits binding of the inhibitory protein to glucokinase. The high K_m of glucokinase for glucose contributes to the capacity of the liver to "buffer" blood glucose levels. Glucose equilibrates readily across the plasma membrane of the liver on the glucose transport protein GLUT-2, the concentration of glucose within the liver reflecting that of the blood. Since the K_m of glucokinase for glucose (~ mM) is considerably greater than normal blood glucose concentrations (~5 mM), any increase in glucose concentration leads to a proportional increase in the rate of glucose phosphorylation by glucokinase (Figure 7.14). Likewise, any decrease in glucose concentration leads to a proportional decrease in the rate of glucose phosphorylation. Thus liver uses glucose at a significant rate only when blood glucose levels are greatly elevated. This buffering effect of liver glucokinase on blood glucose levels would not occur if glucokinase had the low K_m for glucose characteristic of other hexokinases and was therefore completely saturated at physiological concentrations of glucose (Figure 7.14). On the other hand, a low K_m form of hexokinase is a good choice for tissues such as the brain in that it allows phosphorylation of glucose even when blood and tissue glucose concentrations are dangerously low.

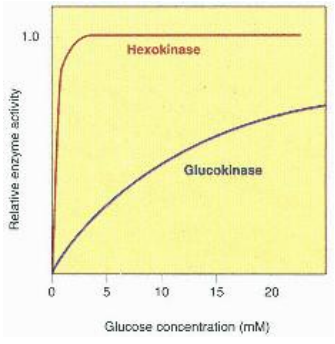


Figure 7.14
Comparison of the substrate saturation curves for hexokinase and glucokinase.

The reaction catalyzed by glucokinase is not at equilibrium under the intracellular conditions of liver cells (Table 7.1). Part of the explanation lies in the rate restriction imposed by the high K_m of glucokinase for glucose and part

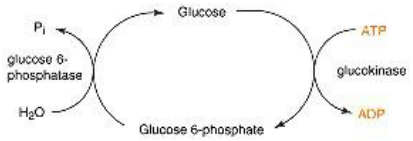


Figure 7.15
Phosphorylation of glucose followed by dephosphorylation constitutes a futile cycle in parenchymal cells of the liver.

is due to the inhibitory protein mentioned above. Yet another important factor is that the activity of glucokinase is opposed in liver by that of **glucose 6-phosphatase.** Like glucokinase, this enzyme has a high K_m (3 mM) with respect to the normal intracellular concentration (~0.2 mM) of its primary substrate, glucose 6-phosphate. Thus the flux through this step is almost directly proportional to the intracellular concentration of glucose 6-phosphate. As shown in Figure 7.15, the combined action of glucokinase and glucose 6-phosphatase constitutes a futile cycle; that is, the sum of their reactions is hydrolysis of ATP to give ADP and P_i without the performance of any work. When blood glucose concentrations are about 5 mM, the activity of glucokinase is almost exactly balanced by the opposing activity of glucose 6-phosphatase. The result is that no net flux occurs in either direction. This futile cycling between glucose and glucose 6-phosphate is wasteful of ATP but, combined with the process of gluconeogenesis, contributes significantly to the "buffering" action of the liver on blood glucose levels. Furthermore, it provides a mechanism for preventing glucokinase from tying up all of the P_i of the liver (see Clin. Corr. 7.3).

Fructose, a component of many vegetables, fruits, and sweeteners, promotes hepatic glucose utilization by an indirect mechanism. It is converted in liver to fructose 1-phosphate (see Clin. Corr. 7.3), which activates glucokinase activity by promoting dissociation of the inhibitory protein. This may be a factor in the adverse effects (e.g., **hypertriglyceridemia**) sometimes associated with excessive dietary fructose consumption.

Glucokinase is an inducible enzyme. Under various physiological conditions the amount of the enzyme protein increases or decreases. Induction of synthesis and repression of synthesis of an enzyme are relatively slow processes, usually requiring several hours before significant changes occur. **Insulin** increases the amount of glucokinase by promoting transcription of the glucokinase gene. An increase in blood glucose levels signals an increase in insulin release from the β cells of the pancreas. This results in an increase in blood insulin levels, which promotes transcription of the glucokinase gene and increases the amount of liver glucokinase enzyme protein. Thus the amount of glucokinase in liver reflects how much glucose is being delivered to the liver via the portal vein. In other words, a person consuming large meals rich in carbohydrate will have greater amounts of glucokinase in the liver than one who is not. The liver in which glucokinase has been induced can make a greater contribution to the lowering of elevated blood glucose levels. The absence of insulin makes the liver of the diabetic patient deficient in glucokinase, in spite of high blood glucose levels, and this is one of the reasons why the liver of the diabetic has less blood glucose "buffering" action (see Clin. Corr. 7.4).

6-Phosphofructo-1-kinase Is the Major Regulatory Site

Evidence suggests that **6-phosphofructo-1-kinase** is the rate-limiting enzyme and most important regulatory site of glycolysis in most tissues. Usually we think of the first step of a pathway as the most logical choice for the rate-limiting step. However, the first committed step of a pathway is most appropriate for the site of the greatest degree of control, and 6-phosphofructo-1-kinase catalyzes the first committed step of the glycolytic pathway. The phosphoglucose isomerase catalyzed reaction is reversible, and most cells can use glucose

CLINICAL CORRELATION 7.4

Diabetes Mellitus

Diabetes mellitus is a chronic disease characterized by derangements in carbohydrate, fat, and protein metabolism. Two major types are recognized clinically—the juvenile-onset or insulin-dependent type (see Clin. Corr. 14.7) and the maturity-onset or insulin-independent type (see Clin. Corr. 14.8).

In patients who do not have fasting hyperglycemia, the oral glucose tolerance test can be used for the diagnosis of diabetes. It consists of determining the blood glucose level in the fasting state and at intervals of 30–60 min for 2 h or more after consuming a 100-g carbohydrate meal. In a normal individual blood glucose returns to normal levels within 2 h after ingestion of the carbohydrate meal. In the diabetic patient, blood glucose will reach a higher level and remain elevated for longer periods of time, depending on the severity of the disease. However, many factors may contribute to an abnormal glucose tolerance test. The patient must have consumed a high carbohydrate diet for the preceding 3 days, presumably to allow for induction of enzymes of glucose-utilizing pathways, for example, glucokinase, fatty acid synthase, and acetyl-CoA carboxylase. In addition, almost any infection (even a cold) and less well-defined "stress" (presumably by effects on the sympathetic nervous system) can result in (transient) abnormalities of the glucose tolerance test. Because of problems with the glucose tolerance test, elevation of the fasting glucose level should probably be the *sine qua non* for the diagnosis of diabetes. Glucose uptake by cells of insulin-sensitive tissues, that is, muscle and adipose, is decreased in the diabetic state. Insulin is required for glucose uptake by these tissues, and the diabetic patient either lacks insulin or has developed "insulin resistance" in these tissues. Resistance to insulin is an abnormality of the insulin receptor or in subsequent steps mediating the metabolic effects of insulin. Parenchymal cells of the liver do not require insulin for glucose uptake. Without insulin, however, the liver has diminished enzymatic capacity to remove glucose from the blood. This is explained in part by decreased glucokinase activity plus the loss of insulin's action on key enzymes of glycogenesis and the glycolytic pathway.

Taylor, S. I. Diabetes mellitus. In: C. R. Scriver, A. L. Beaudet, W. S. Sly, and D. Valle (Eds), *The Metabolic and Molecular Bases of Inherited Disease*, 7th ed. New York: McGraw-Hill, 1995, pp. 843–896.

6-phosphate for glycogen synthesis and in the pentose phosphate pathway. The reaction catalyzed by 6-phosphofructo-1-kinase commits the cell to the metabolism of glucose by glycolysis and is therefore a logical site for the step of the pathway that is rate limiting and subject to the greatest degree of regulation by allosteric effectors. Citrate, ATP, and hydrogen ions (low pH) are the most important negative allosteric effectors, whereas **AMP** and **fructose 2,6-bis-phosphate** are the most important positive allosteric effectors (Figure 7.13). Through their actions as strong inhibitors or activators of 6-phosphofructo-1-kinase, these compounds signal different rates of glycolysis in response to changes in (1) energy state of the cell (ATP and AMP), (2) internal environment of the cell (hydrogen ions), (3) availability of alternate fuels such as fatty acids and ketone bodies (citrate), and (4) insulin/glucagon ratio in the blood (fructose 2,6-bisphosphate). Evidence for the physiological importance of these effectors comes in part from application of the crossover theorem to the glycolytic pathway.

Crossover Theorem Explains Regulation of 6-Phosphofructo-1-kinase by ATP and AMP

For the hypothetical pathway A B C D E F , the **crossover theorem** proposes that an inhibitor that partially inhibits conversion of C to D will cause a "crossover" in the metabolite profile between C and D. Thus when the steady-state concentrations of intermediates in the presence and absence of an inhibitor are compared, the concentrations of intermediates before the site of inhibition should increase in response to the inhibitor, whereas those after the site should decrease. Crossover plots are constructed by setting the concentrations of all intermediates without some effector of the pathway equal to 100%. Concentrations of intermediates observed in the presence of the effector are then expressed as percentages of these values. The expected result with a negative effector is shown in Figure 7.16*a*. The effect of returning the perfused rat heart from an anoxic condition to a well-oxygenated state is also shown (Figure 7.16*b*). This transition with the perfused rat heart is known to

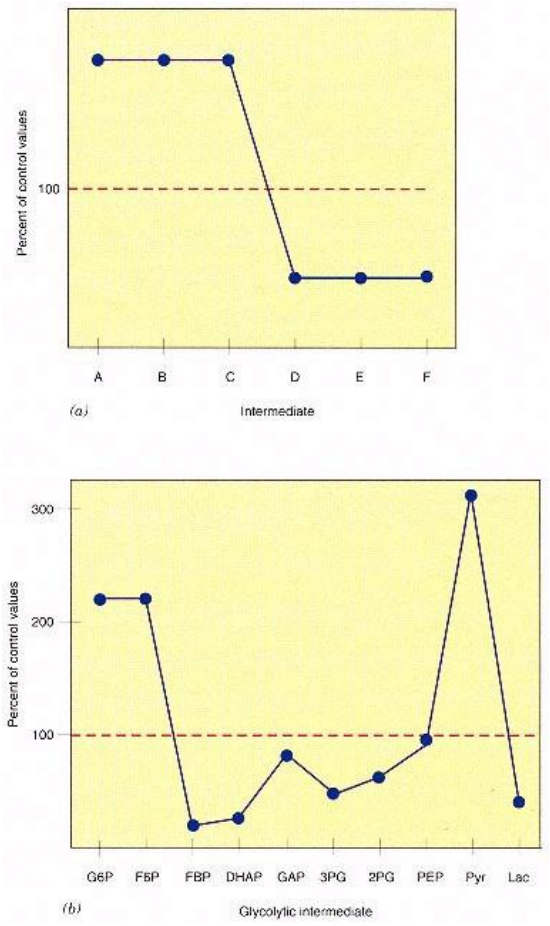


Figure 7.16
Crossover analysis is used to locate sites of regulation of a metabolic pathway.
(a) Theoretical effect of an inhibitor of the C to D step in the pathway of A B C D E F. Steady-state concentrations of all intermediates of the pathway without the inhibitor present are arbitrarily set equal to 100%. Steady-state concentrations of all intermediates when the inhibitor is present are then expressed as percentages of the control values. (b) Effect of oxygen on the relative steady-state concentrations of the intermediates of the glycolytic pathway in the perfused rat heart. The changes in concentrations of metabolites caused by perfusion with oxygen are recorded as percentages of anoxic values. Oxygen strongly inhibits glucose utilization and lactate production under such conditions. The dramatic increase in pyruvate concentration occurs as a consequence of greatly increased utilization of cytosolic NADH by the shuttle systems. Abbreviations: G6P, glucose 6-phosphate; F6P, fructose 6-phosphate; FBP, fructose 1,6-bisphosphate; DHAP, dihydroxyacetone phosphate; GAP, glyceraldehyde 3-phosphate; 3PG, 3-phosphoglycerate; 2PG, 2-phosphoglycerate; PEP, phosphoenolpyruvate; Pyr, pyruvate; Lac, lactate. Redrawn with permission from Williamson, J. R. *J. Biol. Chem.* 241:5026, 1966. © The American Society of Biological Chemists, Inc.

establish new steady-state concentrations of glycolytic intermediates, the flux being much greater through the glycolytic pathway in the absence of oxygen. Under the experimental conditions used, perfused hearts consumed glucose at rates some 20 times greater in the absence than in the presence of oxygen. This illustrates what is known as the **Pasteur effect,** defined as the inhibition of glucose utilization and lactate accumulation by the initiation of respiration (oxygen consumption). This is readily understandable on a thermodynamic basis, the complete oxidation of glucose to CO_2 and H_2O yielding much more ATP than anaerobic glycolysis:

Glycolysis: $\text{D-Glucose} + 2\,ADP^{3-} + 2\,P_i^{2-} \rightarrow 2\,\text{L-lactate}^- + 2\,ATP^{4-}$

Complete oxidation: $\text{D-Glucose} + 6\,O_2 + 38\,ADP^{3-} + 38\,P_i^{2-} + 38\,H^+ \rightarrow 6\,CO_2 + 6\,H_2O + 38\,ATP^{4-}$

ATP is used by a cell only to meet its metabolic demand, that is, to provide the necessary energy for work processes inherent to that cell. Since so much more ATP is produced from glucose in the presence of oxygen, much less glucose is consumed to meet the metabolic demand of the cell. The "crossover" at the conversion of fructose 6-phosphate to fructose 1,6-bisphosphate argues that oxygen imposes an inhibition at the level of 6-phosphofructo-1-kinase. This can readily be rationalized since **ATP** is a well-recognized inhibitor of 6-phosphofructo-1-kinase, and more ATP is generated in the presence than in the absence of oxygen. However, ATP levels do not change greatly between these two conditions (in the experiment of Figure 7.16*b*, ATP increased from 4.7 μmol/g of wet weight in the absence of oxygen to 5.6 μmol/g of wet weight in the presence of oxygen). Since 6-phosphofructo-1-kinase is severely inhibited at concentrations of ATP (2.5–6 mM) normally present in cells, such a small difference in ATP concentration cannot account completely for the change in flux through 6-phosphofructo-1-kinase. However, much greater changes, percentage wise, occur in the concentrations of **AMP,** a positive allosteric effector of 6-phosphofructo-1-kinase. The change that occurs in steady-state concentrations of AMP when oxygen is introduced into the system is exactly what might have been predicted; that is, the level goes down dramatically. This results in less 6-phosphofructo-1-kinase activity. This greatly suppresses glycolysis and accounts in part for the Pasteur effect. Levels of AMP automatically go down in a cell when ATP levels increase. The reason is simple. The sum of the adenine nucleotides in a cell, that is, ATP + ADP + AMP, is nearly constant under most physiological conditions, but the relative concentrations are such that the ATP concentration is always much greater than the AMP concentration. Furthermore, adenine nucleotides are maintained in equilibrium in the cytosol through action of **adenylate kinase** (also referred to as **myokinase**), which catalyzes the reaction (K'_{eq}) for this reaction is given by

$$K'_{eq} = \frac{[ATP][AMP]}{[ADP]^2}$$

Since this reaction is "near equilibrium" under intracellular conditions, the concentration of AMP is given by

$$[AMP] = \frac{K'_{eq}[ADP]^2}{[ATP]}$$

Because intracellular $[ATP] \gg [ADP] \gg [AMP]$, a small decrease in [ATP] causes a substantially greater percentage increase in [ADP]; and, since [AMP] is related to the square of [ADP], an even greater percentage increase in [AMP]. Because of this relationship, a small decrease in ATP concentration leads to a greater percent increase in [AMP] than in the percent decrease in [ATP]. This makes the [AMP] an excellent signal of the energy status of the cell and allows it to function as an important allosteric effector of 6-phosphofructo-1-kinase activity. Furthermore, [AMP] influences in yet another way the effectiveness of 6-phosphofructo-1-kinase. The enzyme **fructose 1,6-bisphosphatase** catalyzes an irreversible reaction, which opposes that of 6-phosphofructo-1-kinase:

$$\text{Fructose 1,6-bisphosphate} + H_2O \rightarrow \text{fructose 6-phosphate} + P_i$$

This enzyme sits "cheek by jowl" with 6-phosphofructo-1-kinase in the cytosol of many cells. Together they catalyze a futile cycle (ATP ADP + P_i + "heat"), and, at the very least, they decrease "effectiveness" of one another. AMP concentration is a perfect signal of the energy status of the cell— not only because AMP activates 6-phosphofructo-1-kinase but also because AMP inhibits fructose 1,6-bisphosphatase. Thus a small decrease in ATP concentration trig-

gers, via the increase in AMP concentration, a large increase in net conversion of fructose 6-phosphate into fructose 1,6-bisphosphate. This increases glycolytic flux by increasing the amount of substrate available for the splitting stage. In cells containing hexokinase, it also results in greater phosphorylation of glucose because a decrease in fructose 6-phosphate automatically causes a decrease in glucose 6-phosphate, which in turn results in less inhibition of hexokinase.

The decrease in lactate production in response to onset of respiration is another feature of the Pasteur effect that can readily be explained. The most important factor is decreased glycolytic flux caused by oxygen. Other factors include competition between lactate dehydrogenase and mitochondrial pyruvate dehydrogenase complex for pyruvate, as well as competition between lactate dehydrogenase and shuttle systems for NADH. For the most part, lactate dehydrogenase loses the competition in the presence of oxygen.

Intracellular pH Can Regulate 6-Phosphofructo-1-kinase

It would make sense that lactate, as the end product of glycolysis, should inhibit the rate-limiting enzyme of glycolysis. It does not. However, **hydrogen ions,** the other glycolytic end product, do inhibit 6-phosphofructo-1-kinase. As shown in Figure 7.17, glycolysis in effect generates **lactic acid,** and the cell must dispose of it as such. This explains why excessive glycolysis in the body lowers blood pH and leads to an emergency medical situation termed **lactic acidosis** (see Clin. Corr. 7.5). Plasma membranes of cells contain a symport for lactate and hydrogen ions. That allows release of lactic acid into the bloodstream. This is a defense mechanism, preventing pH from getting so low that everything becomes pickled (see Clin. Corr. 7.6). The sensitivity of 6-phosphofructo-1-kinase to hydrogen ions is also part of this mechanism. Hydrogen ions are able to shut off glycolysis, the process responsible for decreasing pH. Transport of lactic acid out of a cell requires that blood be available to carry it away. When

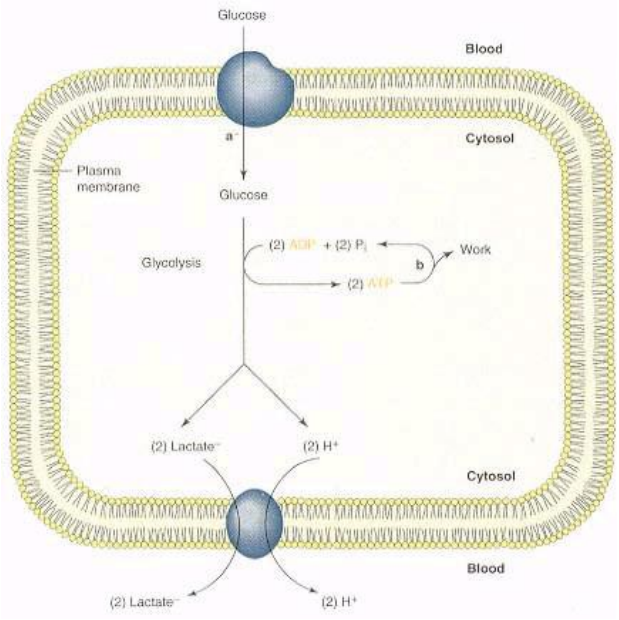


Figure 7.17
Unless lactate formed by glycolysis is transported out of the cell, the intracellular pH will be decreased by the accumulation of intracellular lactic acid.
The low pH decreases 6-phosphofructo-1-kinase activity so that further lactic acid production by glycolysis is shut off.
(a) Glucose transport into the cell.
(b) All work performances that convert ATP back to ADP and P_i.
(c) Lactate–hydrogen ion symport (actual stoichiometry of one $lactate^-$ and one H^+ transported by the symport).

CLINICAL CORRELATION 7.5

Lactic Acidosis

This problem is characterized by elevated blood lactate levels, usually greater than 5 mM, along with decreased blood pH and bicarbonate concentrations. Lactic acidosis is the most commonly encountered form of metabolic acidosis and can be the consequence of overproduction of lactate, underutilization of lactate, or both. Lactate production is normally balanced by lactate utilization, with the result that lactate is usually not present in the blood at concentrations greater than 1.2 mM. All tissues of the body have the capacity to produce lactate by anaerobic glycolysis, but most tissues do not produce large quantities because much more ATP can be gained by the complete oxidation of the pyruvate produced by glycolysis. However, all tissues respond with an increase in lactate generation when oxygenation is inadequate. A decrease in ATP resulting from reduced oxidative phosphorylation allows the activity of 6-phosphofructo-1-kinase to increase. These tissues have to rely on anaerobic glycolysis for ATP production under such conditions and this results in lactic acid production. A good example is muscle exercise, which can deplete the tissue of oxygen and cause an overproduction of lactic acid. Tissue hypoxia occurs, however, in all forms of shock, during convulsions, and in diseases involving circulatory and pulmonary failure.

The major fate of lactate in the body is either complete combustion to CO_2 and H_2O or conversion back to glucose by the process of gluconeogenesis. Both require oxygen. Decreased oxygen availability therefore increases lactate production and decreases lactate utilization. The latter can also be decreased by liver diseases, ethanol, and a number of other drugs. Phenformin, a drug that was once used to treat the hyperglycemia of insulin-independent diabetes, was well-documented to induce lactic acidosis in certain patients.

Bicarbonate is usually administered in an attempt to control the acidosis associated with lactic acid accumulation. The key to successful treatment, however, is to find and eliminate the cause of the overproduction and/or underutilization of lactic acid and most often involves the restoration of circulation of oxygenated blood.

Newsholme, E. A., and Leech, A. R. *Biochemistry for the Medical Sciences*. New York: Wiley, 1983; and Kruse, J. A., and Carlson, R. W. Lactate metabolism. *Crit. Care Clin.* 3:725, 1985.

blood flow is inadequate, for example, in heavy exercise of a skeletal muscle or an attack of **angina pectoris** in the case of the heart, hydrogen ions cannot escape from cells fast enough. Yet, the need for ATP within such cells, because of lack of oxygen, may partially override inhibition of 6-phosphofructo-l-kinase by hydrogen ions. Unabated accumulation of hydrogen ions then results in pain, which, in the case of skeletal muscle, can be relieved by simply terminating

CLINICAL CORRELATION 7.6

Pickled Pigs and Malignant Hyperthermia

In patients with malignant hyperthermia, a variety of agents, especially the widely used general anesthetic halothane, will produce a dramatic rise in body temperature, metabolic and respiratory acidosis, hyperkalemia, and muscle rigidity. This genetic abnormality occurs in about 1 in 15,000 children and 1 in 50,000–100,000 adults. It is dominantly inherited. Death may result the first time a susceptible person is anesthetized. Onset occurs within minutes of drug exposure and the hyperthermia must be recognized immediately. Packing the patient in ice is effective and should be accompanied by measures to combat acidosis. The drug dantrolene is also effective.

A phenomenon similar, if not identical, to malignant hyperthermia is known to occur in pigs. Pigs with this problem, called porcine stress syndrome, respond poorly to stress. This genetic disease usually manifests itself as the pig is being shipped to market. Pigs with the syndrome can be identified by exposure to halothane, which triggers the same response seen in patients with malignant hyperthermia. The meat of pigs that have died as a result of the syndrome is pale, watery, and of very low pH (i.e., nearly pickled).

Muscle is the site of the primary lesion in both malignant hyperthermia and porcine stress syndrome. In response to halothane the skeletal muscles become rigid and generate heat and lactic acid. The sarcoplasmic reticulum of such pigs and patients have a genetic abnormality in the ryanodine receptor, a Ca^{2+} release channel, that plays an important function in excitation–contraction coupling in muscle. Because of a defect in this protein, the anesthetic triggers inappropriate release of Ca^{2+} from the sarcoplasmic reticulum. This results in uncontrolled stimulation of a number of heat-producing processes, including myosin ATPase, glycogenolysis, glycolysis, and cyclic uptake and release of Ca^{2+} by mitochondria and sarcoplasmic reticulum. Muscle cells become irreversibly damaged as consequence of excessive heat production, lactic acidosis, and ATP loss.

Kalow, W., and Grant, D. M. Pharmacogenetics. In: C. R. Scriver, A. L. Beaudet, W. S. Sly, and D. Valle (Eds.), *The Metabolic and Molecular Bases of Inherited Disease*, 7th ed. New York: McGraw-Hill, 1995, pp. 293–326.

CLINICAL CORRELATION 7.7

Angina Pectoris and Myocardial Infarction

Chest pain associated with reversible myocardial ischemia is termed angina pectoris (literally, strangling pain in the chest). The pain is the result of an imbalance between demand for and supply of blood flow to cardiac muscles and is most commonly caused by narrowing of the coronary arteries. The patient experiences a heavy squeezing pressure or ache substernally, often radiating to either the shoulder and arm or occasionally to the jaw or neck. Attacks occur with exertion, last from 1 to 15 min, and are relieved by rest. The coronary arteries involved are obstructed by atherosclerosis (i.e., lined with characteristic fatty deposits) or less commonly narrowed by spasm. Myocardial infarction occurs if the ischemia persists long enough to cause severe damage (necrosis) to the heart muscle. Commonly, a blood clot forms at the site of narrowing and completely obstructs the vessel. In myocardial infarction, tissue death occurs and the characteristic pain is longer lasting, and often more severe.

Nitroglycerin and other nitrates are frequently prescribed to relieve the pain caused by the myocardial ischemia of angina pectoris. These drugs can be used prophylactically, enabling patients to participate in activities that would otherwise precipitate an attack of angina. Nitroglycerin may work in part by causing dilation of the coronary arteries, improving oxygen delivery to the heart and washing out lactic acid. Probably more important is the effect of nitroglycerin on the peripheral circulation. Breakdown of nitroglycerin produces nitric oxide (NO), a compound that relaxes smooth muscle, causing venodilation throughout the body. This reduces arterial pressure and allows blood to accumulate in the veins. The result is decreased return of blood to the heart, and a reduced volume of blood the heart has to pump, which reduces the energy requirement of the heart. In addition, the heart empties itself against less pressure, which also spares energy. The overall effect is a lowering of the oxygen requirement of the heart, bringing it in line with the oxygen supply via the diseased coronary arteries. Other useful agents are calcium channel blockers, which are coronary vasodilators, and β-adrenergic blockers. The β-blockers prevent the increase in myocardial oxygen consumption induced by sympathetic nervous system stimulation of the heart, as occurs with physical exertion.

The coronary artery bypass operation is used in severe cases of angina that cannot be controlled by medication. In this operation veins are removed from the leg and interposed between the aorta and coronary arteries of the heart. The purpose is to bypass the portion of the artery diseased by atherosclerosis and provide the affected tissue with a greater blood supply. Remarkable relief from angina can be achieved by this operation, with the patient being able to return to normal productive life in some cases.

Hugenholtz, P. G. Calcium antagonists for angina pectoris. *Ann. N. Y. Acad. Sci.* 522:565, 1988; Feelishch, M., and Noack, E. A. Correlation between nitric oxide formation during degradation of organic nitrates and activation of guanylate cyclase. *Eur. J. Pharmacol.* 139:19, 1987; and Ignarro, L. J. Biological actions and properties of endothelium-derived nitric oxide formed and released from artery and vein. *Circ. Res.* 65:1, 1989.

the exercise. In the case of the heart, rest or pharmacologic agents that increase blood flow or decrease the need for ATP within myocytes may be effective (see Clin. Corr. 7.7).

Intracellular Citrate Levels Regulate 6-Phosphofructo-1-kinase

Many tissues prefer to use fatty acids and ketone bodies as oxidizable fuels in place of glucose. Most of these tissues can use glucose but actually prefer to oxidize fatty acids and ketone bodies. This helps preserve glucose for tissues, such as brain, that are absolutely dependent on glucose as an energy source. Oxidation of both fatty acids and ketone bodies elevates levels of cytosolic **citrate,** which inhibits 6-phosphofructo-1-kinase. The result is decreased glucose utilization by the tissue when fatty acids or ketone bodies are available.

Hormonal Control of 6-Phosphofructo-1-kinase by cAMP and Fructose 2,6-bisphosphate

Fructose 2,6-bisphosphate (Figure 7.18), like AMP, functions as a positive allosteric effector of 6-phosphofructo-1-kinase and as a negative allosteric effector of fructose 1,6-bisphosphatase. Indeed, without the presence of this compound, glycolysis could not occur in liver because 6-phosphofructo-1-kinase would have insufficient activity and fructose 1,6-bisphosphatase would have too much activity for net conversion of fructose 6-phosphate to fructose 1,6-bisphosphate.

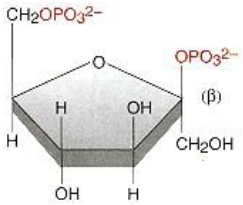


Figure 7.18
Structure of fructose 2,6-bisphosphate.

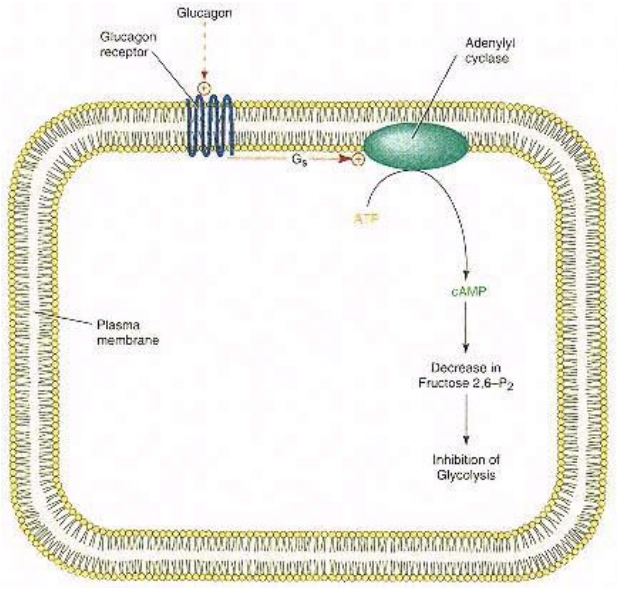
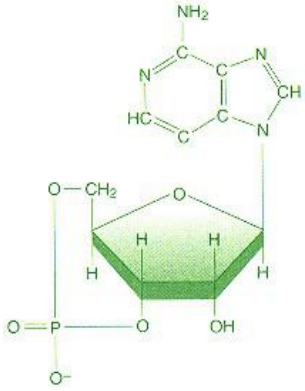


Figure 7.19
Overview of the mechanism responsible for glucagon inhibition of hepatic glycolysis.
Binding of glucagon to its receptor (a protein that spans the membrane seven times) activates adenylate cyclase (an intrinsic membrane protein) activity through the action of a stimulatory G-protein (G_s). The (+) symbol indicates activation.

Figure 7.19 gives a brief overview of the role of fructose 2,6-bisphosphate in hormonal control of hepatic glycolysis. Understanding this mechanism requires an appreciation of the role of **cAMP** (Figure 7.20) as the "second messenger" of hormone action. As discussed in more detail in Chapters 14 and 20, **glucagon** is released from α cells of pancreas and circulates in blood until it comes in contact with glucagon receptors located on the outer surface of liver plasma membrane (Figure 7.19). Binding of glucagon to these receptors is sensed by **adenylate** (adenylyl) **cyclase,** an enzyme located on the inner surface of the plasma membrane, stimulating it to convert ATP into cAMP. Cyclic AMP triggers a series of intracellular events that result ultimately in a decrease in fructose 2,6-bisphosphate levels. A decrease in this compound makes 6-phosphofructo-1-kinase less effective but makes fructose 1,6-bisphosphatase more effective, thereby severely restricting flux from fructose 6-phosphate to fructose 1,6-bisphosphate in glycolysis.

Fructose 2,6-bisphosphate is not an intermediate of glycolysis. As shown in Figure 7.21, fructose 2,6-bisphosphate is produced from F6P by the enzyme **6-phosphofructo-2-kinase.** We now have two "phosphofructokinases" to contend with: one produces an intermediate (FBP) of glycolysis and the other



Cyclic AMP

Figure 7.20
Structure of cAMP.

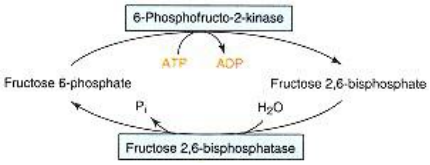


Figure 7.21
Reactions involved in the formation and degradation of fructose 2,6-bisphosphate.

produces a positive allosteric effector (fructose 2,6-bisphosphate) of the first enzyme. Fructose 2,6-bisphosphate can be destroyed by being converted back to F6P by fructose 2,6-bisphosphatase (Figure 7.21). This is a simple hydrolysis, with no ATP or ADP being involved. Synthesis and degradation of fructose 2,6-bisphosphate are catalyzed by a **bifunctional enzyme;** that is, 6-phosphofructo-2-kinase and fructose 2,6-bisphosphatase are part of the same protein. Because of its bifunctional nature, the combined name of 6-phosphofructo-2-kinase/fructose 2,6-bisphosphatase is used to refer to this enzyme that makes and degrades fructose 2,6-bisphosphate. cAMP regulates fructose 2,6-bisphosphate levels in liver. How is this possible when the same enzyme carries out both synthesis and degradation of the molecule? The answer is that a mechanism exists whereby cAMP inactivates the kinase function and, at the same time, activates the phosphatase function of this bifunctional enzyme.

cAMP Activates Protein Kinase A

Cyclic AMP activates **protein kinase A** (also called **cAMP-dependent protein kinase**). In its inactive state, this enzyme consists of two regulatory subunits plus two catalytic subunits. Binding of cAMP to regulatory subunits causes conformational changes with release of catalytic subunits, which are active only when dissociated from regulatory subunits. Liberated protein kinase then catalyzes phosphorylation of specific serine residues of several different enzymes (Figure 7.22).

Phosphorylation of an enzyme can conveniently be abbreviated as

$$\boxdot + \text{ATP} \rightarrow \odot\text{-P} + \text{ADP}$$

where $\odot$-P are used to indicate dephosphorylated and phosphorylated enzymes, respectively. Circle and square symbols are used because **phosphorylation** of enzymes subject to regulation by covalent modification causes a change in their conformation, which affects the active site. The change in conformation due to phosphorylation increases catalytic activity of some enzymes but decreases catalytic activity of others. Direction of change in activity depends on the enzyme involved. Many enzymes are subject to this type of regulation, an important type of **covalent modification.** Regardless of whether phosphorylation or dephosphorylation activates the enzyme, the active form of the enzyme is called the *a* form and the inactive form the *b* form. Likewise, regardless of the effect of phosphorylation on catalytic activity, the action of a protein kinase is always opposed by that of a phosphoprotein phosphatase, which catalyzes the reaction of

$$\odot\text{-P} + \text{H}_2\text{O} \rightarrow \boxdot + \text{P}_i$$

Putting these together creates a **cyclic control system** (see Figure 7.23), such that the ratio of phosphorylated enzyme to dephosphorylated enzyme is a function of the relative activities of protein kinase and phosphoprotein phosphatase. If the kinase has greater activity than the phosphatase, more enzyme will be in the phosphorylated mode, and vice versa. Since activity of an interconvertible enzyme (i.e., an enzyme subject to covalent modification) is determined by whether it is in the phosphorylated or dephosphorylated mode, the relative

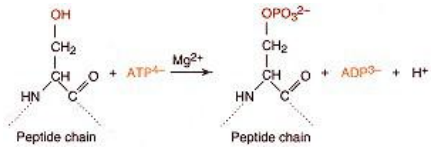


Figure 7.22
Enzymes subject to covalent modification are usually phosphorylated on specific serine residues.
Tyrosine and threonine residues are also important sites of covalent modification by phosphorylation.

activities of kinase and phosphatase determine the amount of a particular enzyme that is in the catalytically active state.

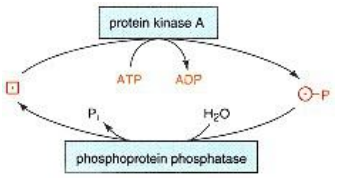


Figure 7.23
General model of the mechanism responsible for regulation of enzymes by phosphorylation–dephosphorylation.
The symbols ⊙-P indicate that different conformational and activity states of the enzyme are produced as a result of phosphorylation–dephosphorylation.

6-Phosphofructo-2-kinase and Fructose 2,6-bisphosphatase Are Domains of a Bifunctional Polypeptide Regulated by Phosphorylation—Dephosphorylation

Most enzymes are either turned on or off by phosphorylation but with **6-phosphofructo-2-kinase** and **fructose 2,6-bisphosphatase,** advantage is taken of the bifunctional nature of the enzyme. In the case of the isoenzyme present in liver, phosphorylation causes inactivation of the active site responsible for synthesis of fructose 2,6-bisphosphate but activation of the active site responsible for hydrolysis of fructose 2,6-bisphosphate (Figure 7.24). Dephosphorylation of the enzyme has the opposite effects. A sensitive mechanism has therefore evolved to set the intracellular concentration of **fructose 2,6-bisphosphate** in liver cells in response to changes in blood levels of **glucagon** or **epinephrine** (Figure 7.25). Increased levels of glucagon or epinephrine, acting through plasma membrane glucagon receptors and β-adrenergic receptors, respectively, have the common effect of inducing an increase in intracellular levels of **cAMP.** This second messenger activates **protein kinase A,** which phosphorylates a single serine residue of 6-phosphofructo-2-kinase/fructose 2,6-bisphosphatase (Figure 7.26). This inhibits fructose 2,6-bisphosphate synthesis and promotes its degradation. The resulting decrease in fructose 2,6-bisphosphate makes 6-phosphofructo-1-kinase less effective and fructose 1,6-bisphosphatase more effective. The result is inhibition of glycolysis at the level of the conversion of fructose 6-phosphate to fructose 1,6-bisphosphate. Decreased levels of either glucagon or epinephrine in blood result in less cAMP in liver because adenylate cyclase is less active and cAMP that had accumulated is converted to AMP by the action of **cAMP phosphodiesterase.** Loss of the cAMP signal results in inactivation of protein kinase A and a corresponding decrease in phosphorylation of 6-phosphofructo-2-kinase/fructose 2,6-bisphosphatase by protein kinase A. A **phosphoprotein phosphatase** removes phosphate from the bifunctional enzyme to produce active 6-phosphofructo-2-kinase and inactive fructose 2,6-bisphosphatase. Fructose 2,6-bisphosphate can now accumulate to a higher steady-state concentration and, by activating 6-phosphofructo-1-kinase and inhibiting fructose 1,6-bisphosphatase, greatly increases glycolysis. Thus glucagon and epinephrine are extracellular signals that stop liver from using glucose, whereas fructose 2,6-bisphosphate is an intracellular signal that promotes glucose utilization by this tissue.

Insulin opposes the actions of glucagon and epinephrine, but exactly how insulin works after binding to the plasma membrane remains a subject of intense investigation (see Chapter 20). There is evidence that insulin promotes formation of a second messenger, much as glucagon promotes formation of cAMP. Obvious enzyme targets that a second messenger might influence include cAMP phosphodiesterase, protein kinase A, and phosphoprotein phosphatase (Figure 7.27). There also is evidence, however, that insulin signals a cascade of events that depends upon activation of a number of protein kinases (see Chapter 20).

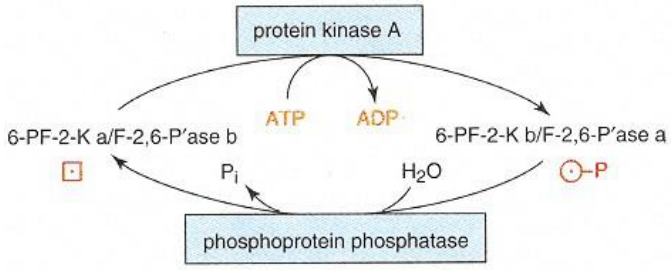


Figure 7.24
Mechanism responsible for covalent modification of the bifunctional enzyme 6-phosphofructo-2-kinase/fructose 2,6-bisphosphatase.
Name of the enzyme is abbreviated as 6-PF-2-K/F-2,6-P'ase. Letters *a* and *b* indicate the active and inactive forms of the enzymes, respectively.

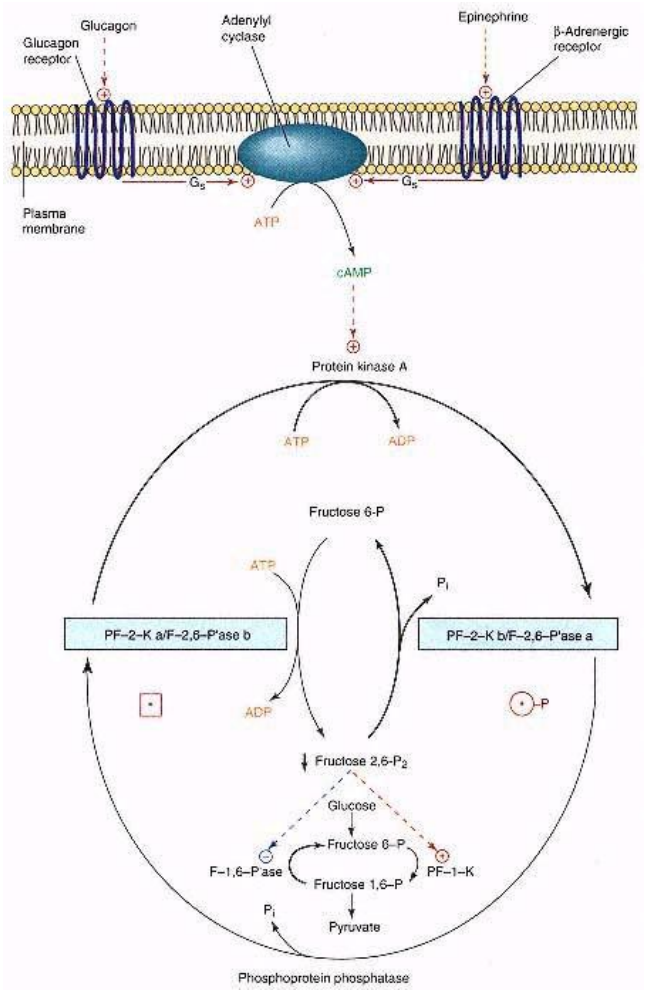


Figure 7.25
Mechanism of glucagon and epinephrine inhibition of hepatic glycolysis via cAMP-mediated decrease in fructose 2,6-bisphosphate concentration.
See legend for Figure 7.19. The heavy arrows indicate the reactions that predominate in the presence of glucagon. Small arrow before fructose 2,6-bisphosphate indicates a decrease in concentration of this compound.

Regardless of its exact mechanism, insulin acts in the opposite direction from that of glucagon and epinephrine in determining the levels of fructose 2,6-bisphosphate in liver cells and, therefore, the rate of glycolysis.

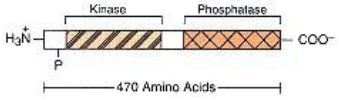


Figure 7.26
Schematic diagram of the primary structure of the liver isoenzyme of 6-phosphofructo-2-kinase/fructose-2,6-bisphosphatase.
NH_2 and CO_2H designate the N-terminal and C-terminal ends of the enzyme, respectively. Domain with kinase activity is located in the N-terminal half of the enzyme; domain with phosphatase activity in the C-terminal half of the enzyme. The letter P indicates the site (serine 32) phosphorylated by protein kinase A.

Heart Contains a Different Isoenzyme of the Bifunctional Enzyme

An increase in blood level of **epinephrine** has a markedly different effect on glycolysis in **heart** from that in liver. Glycolysis is inhibited in liver to conserve glucose for use by other tissues. Epinephrine stimulates glycolysis in heart as part of a mechanism to meet the increased demand for ATP caused by an epinephrine-signaled increase in work load. As in liver, epinephrine acts on

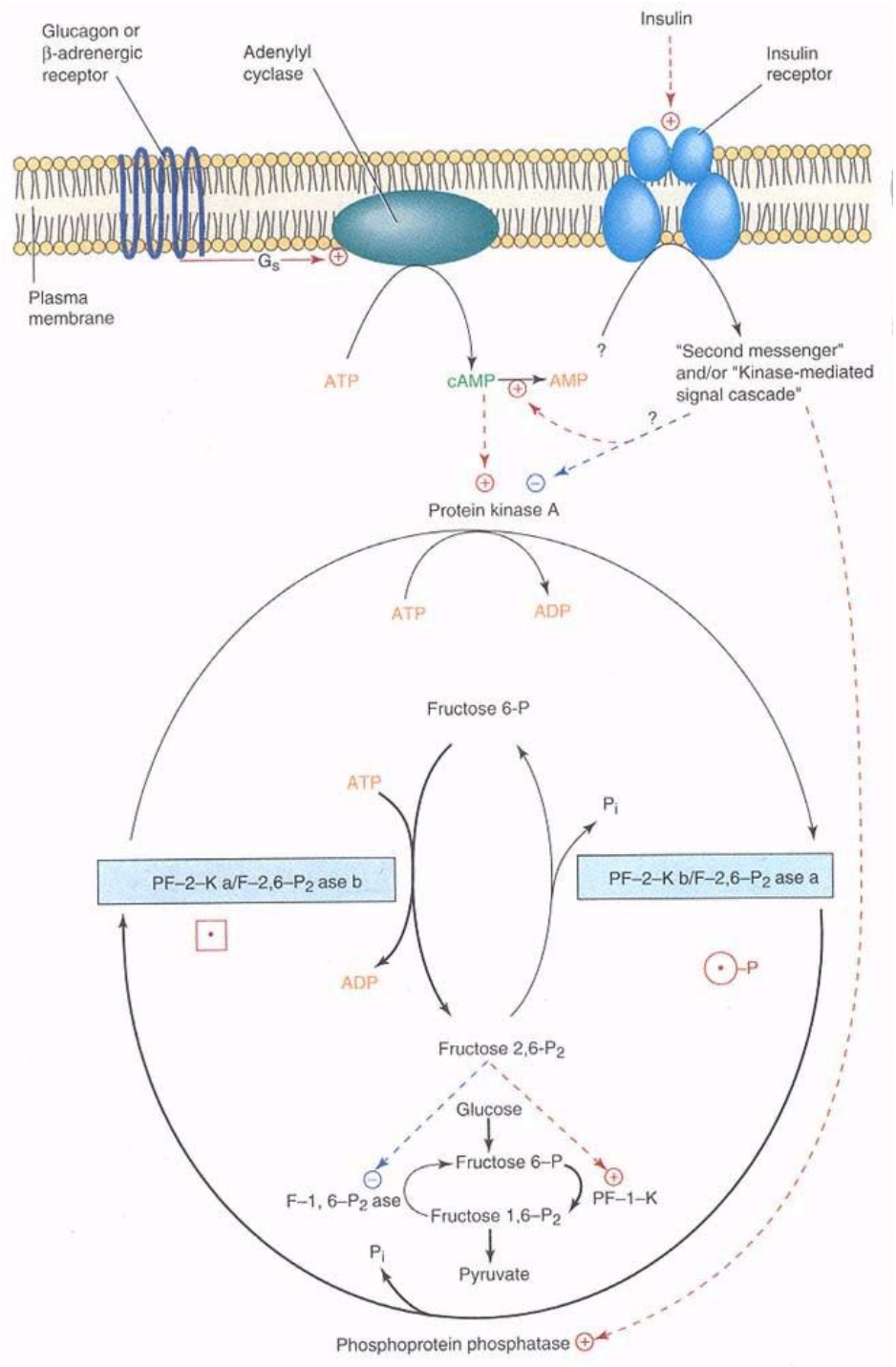


Figure 7.27
Mechanism responsible for accelerated rates of hepatic glycolysis when the concentration of glucagon and epinephrine are low and that of insulin is high in the blood.
See legends for Figures 7.19 and 7.25. The insulin receptor is an intrinsic component of the plasma membrane. Small arrow before fructose 2,6-bisphosphate indicates an increase in concentration. The question marks indicate that the details of the mechanism of action of insulin are unknown at this time.

the heart by way of a β-adrenergic receptor on the plasma membrane, promoting formation of cAMP by adenylate cyclase (Figure 7.28). This results in the activation of protein kinase A, which in turn phosphorylates 6-phosphofructo-2-kinase/fructose 2,6-bisphosphatase. In contrast, however, to what happens in liver, phosphorylation of the bifunctional enzyme in heart produces an increase rather than a decrease in fructose 2,6-bisphosphate levels. This is because heart expresses a different isoenzyme of the bifunctional enzyme. Although still a bifunctional enzyme that carries out exactly the same reactions as the liver enzyme, the amino acid sequence of the heart isoenzyme is different, and phosphorylation by protein kinase A occurs at a site that activates rather than inhibits 6-phosphofructo-2-kinase (Figure 7.29). Increased fructose 2,6-bisphos-

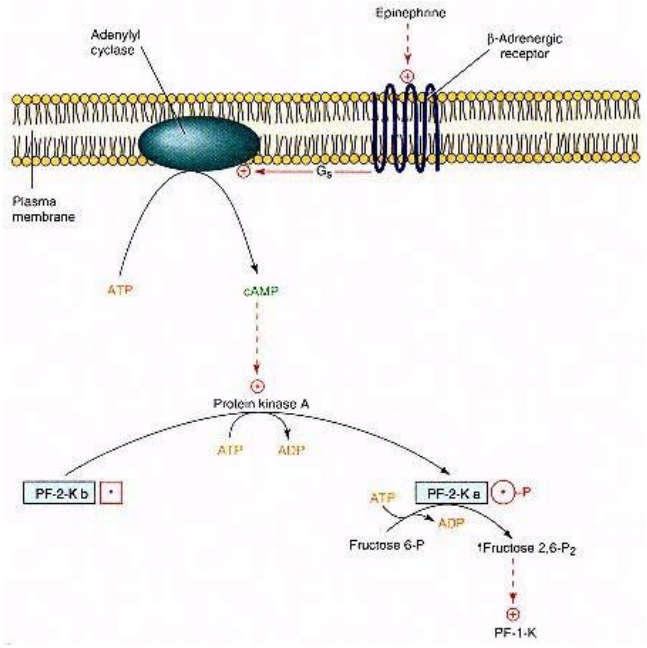


Figure 7.28
Mechanism respnsible for accelerated rates of glycolysis in the heart in response to epinephrine.
See legends for Figures 7.19 and 7.27.

phate results in increased 6-phosphofructo-1-kinase activity and increased glycolytic flux in response to epinephrine in heart.

Pyruvate Kinase Is a Regulated Enzyme of Glycolysis

Pyruvate kinase is another regulatory enzyme of glycolysis (see Clin. Corr. 7.8). This enzyme is drastically inhibited by physiological concentrations of ATP, so much so that its potential activity is never fully realized under physiological conditions. The isoenzyme found in liver is greatly activated by fructose 1,6-bisphosphate, thereby linking regulation of pyruvate kinase to what is happening to 6-phosphofructo-1-kinase. Thus, if conditions favor increased flux through 6-phosphofructo-1-kinase, the level of FBP increases and acts as a feed-forward activator of pyruvate kinase. The liver enzyme is also subject to covalent modification, being active in the dephosphorylated state and inactive in the phosphorylated state (Figure 7.30); phosphorylation is catalyzed by protein kinase A in liver. Thus glucagon inhibition of hepatic glycolysis and stimulation

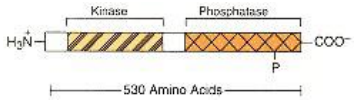


Figure 7.29
Schematic diagram of the primary structure of the heart isoenzyme of 6-phosphofructo-2-kinase/fructose 2,6-bisphosphatase that is present in the heart.
See legend for Figure 7.26. The letter P indicates the site (serine 466) phosphorylated by protein kinase A.

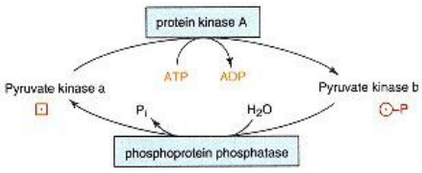


Figure 7.30
Glucagon acts via cAMP-mediated activation of protein kinase A to cause the phosphorylation and inactivation of hepatic pyruvate kinase.

of hepatic gluconeogenesis are explained in part by elevation of cAMP levels caused by this hormone. This aspect is explored more thoroughly in Section 7.5 in the discussion of gluconeogenesis.

Pyruvate kinase, like glucokinase, is induced to higher steady-state concentrations in liver by combination of high carbohydrate intake and high insulin levels. This increase in enzyme concentration is a major reason why liver of the well-fed individual has much greater capacity for utilizing carbohydrate than a fasting or diabetic person (see Clin. Corr. 7.4).

CLINICAL CORRELATION 7.8

Pyruvate Kinase Deficiency and Hemolytic Anemia

Mature erythrocytes are absolutely dependent on glycolytic activity for ATP production. ATP is needed for the ion pumps, especially the Na^+, K^+–ATPase, which maintain the biconcave disk shape of erythrocytes, a characteristic that helps erythrocytes slip through the capillaries as they deliver oxygen to the tissues. Without ATP the cells swell and lyse. Anemia due to excessive erythrocyte destruction is referred to as hemolytic anemia. Pyruvate kinase deficiency is rare but is by far the most common genetic defect of the glycolytic pathway known to cause hemolytic anemia. Most pyruvate kinase-deficient patients have 5–25% of normal red blood cell pyruvate kinase levels and flux through the glycolytic pathway is restricted severely, resulting in markedly lower ATP concentrations. The expected crossover of the glycolytic intermediates is observed; that is, those intermediates proximal to the pyruvate kinase-catalyzed step accumulate, whereas pyruvate and lactate concentrations decrease. Normal ATP levels are observed in reticulocytes of patients with this disease. Although deficient in pyruvate kinase, these "immature" red blood cells have mitochondria and can generate ATP by oxidative phosphorylation. Maturation of reticulocytes into red blood cells results in the loss of mitochondria and complete dependence on glycolysis for ATP production. Since glycolysis is defective, the mature cells are lost rapidly from the circulation. Anemia results because the cells cannot be replaced rapidly enough by erythropoiesis.

Valentine, W. N. The Stratton lecture: hemolytic anemia and inborn errors of metabolism. *Blood* 54:549, 1979; and Tanaka, K. R., and Paglia, D. E. Pyruvate kinase and other enzymopathies of the erythrocyte. In: C. R. Scriver, A. L. Beaudet, W. S. Sly, and D. Valle (Eds.), *The Metabolic and Molecular Bases of Inherited Disease*, 7th ed. New York: McGraw-Hill, 1995, pp. 3485–3511.

7.5— Gluconeogenesis

Glucose Synthesis Is Required for Survival

Net synthesis or formation of glucose from various substrates is termed **gluconeogenesis.** This includes use of various amino acids, lactate, pyruvate, propionate, and glycerol, as sources of carbon for the pathway (see Figure 7.31). Glucose is also synthesized from galactose and fructose. **Glycogenolysis,** that is, formation of glucose or glucose 6-phosphate from glycogen, should be differentiated from gluconeogenesis; glycogenolysis refers to

$$\text{Glycogen or (glucose)}_n \rightarrow \text{molecules of glucose}$$

and thus does not correspond to *de novo* synthesis of glucose, the hallmark of the process of gluconeogenesis.

The capacity to synthesize glucose is crucial for survival of humans and other animals. Blood glucose levels have to be maintained to support metabolism of tissues that use glucose as their primary substrate (see Clin. Corr. 7.9). These include brain, red blood cells, kidney medulla, lens, cornea, testis, and a number of other tissues. Gluconeogenesis enables the maintenance of blood glucose levels long after all dietary glucose has been absorbed and completely oxidized.

The Cori and Alanine Cycles

Two important cycles between tissues that involve gluconeogenesis are recognized. The **Cori cycle** and the alanine cycle (Figure 7.32) depend on gluconeo-

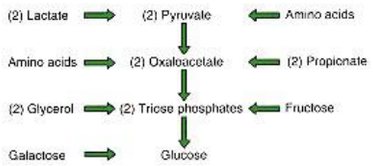


Figure 7.31
Abbreviated pathway of gluconeogenesis, illustrating the major substrate precursors for the process.

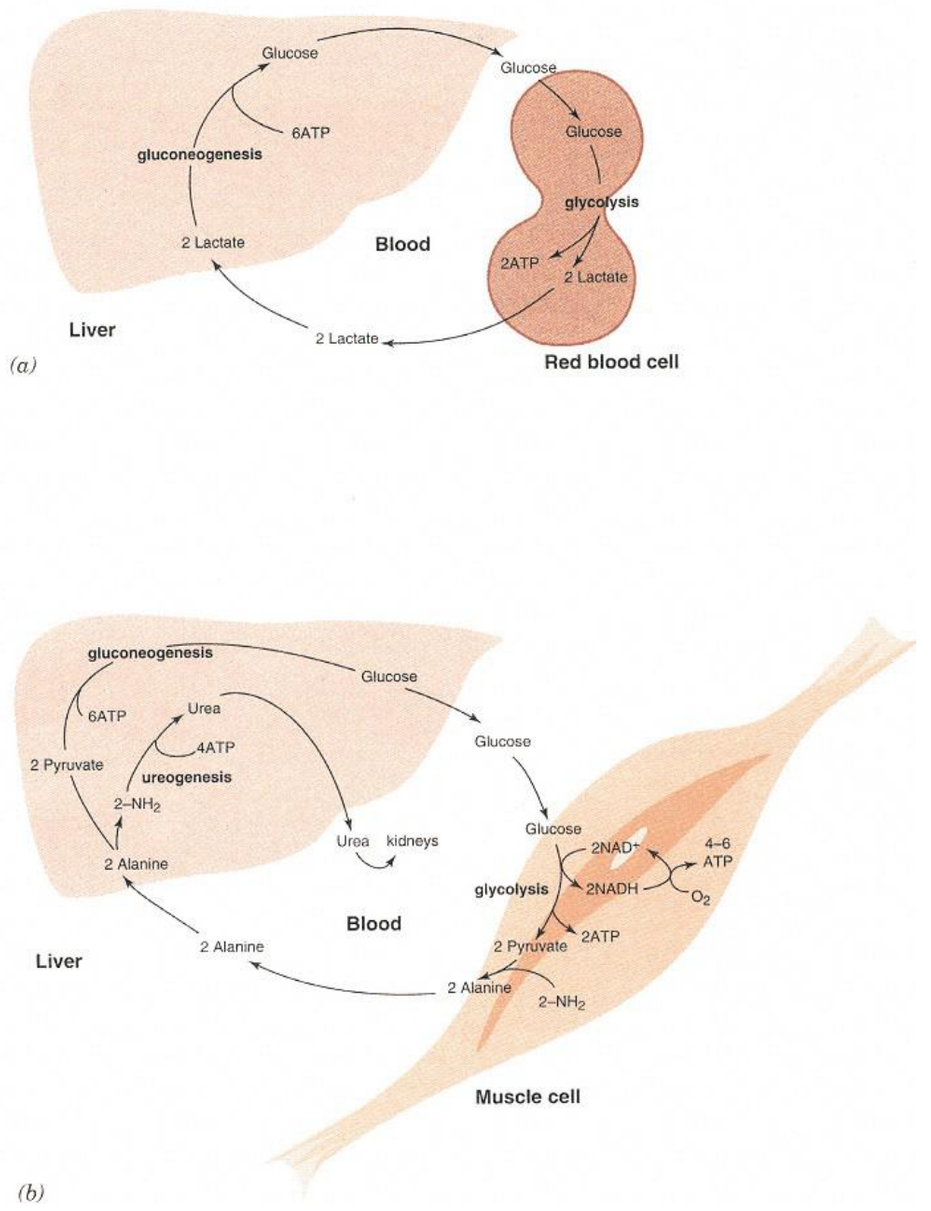


Figure 7.32
Relationship between gluconeogenesis in the liver and glycolysis in the rest of the body.
(a) Cori cycle.
(b) Alanine cycle.

CLINICAL CORRELATION 7.9

Hypoglycemia and Premature Infants

Premature and small-for-gestational-age neonates have a greater susceptibility to hypoglycemia than full-term, appropriate-for-gestational-age infants. Several factors appear to be involved. Children in general are more susceptible than adults to hypoglycemia, simply because they have larger brain/body weight ratios and the brain utilizes disproportionately greater amounts of glucose than the rest of the body. Newborn infants have a limited capacity for ketogenesis, apparently because the transport of long-chain fatty acids into liver mitochondria of the neonate is poorly developed. Since ketone body use by the brain is directly proportional to the circulating ketone body concentration, the neonate is unable to spare glucose to any significant extent by using ketone bodies. The consequence is that the neonate's brain is almost completely dependent on glucose obtained from liver glycogenolysis and gluconeogenesis.

The capacity for hepatic glucose synthesis from lactate and alanine is also limited in newborn infants. This is because the rate limiting enzyme phosphoenolpyruvate carboxykinase is present in very low amounts during the first few hours after birth. Induction of this enzyme to the level required to prevent hypoglycemia during the stress of fasting requires several hours. Premature and small-for-gestational-age infants are believed to be more susceptible to hypoglycemia than normal infants because of smaller stores of liver glycogen. Fasting depletes their glycogen stores more rapidly, making these neonates more dependent on gluconeogenesis than normal infants.

Ballard, F. J. The development of gluconeogenesis in rat liver: controlling factors in the newborn. *Biochem. J.* 124:265, 1971; and Newsholme, E. A., and Leech, A. R. *Biochemistry for the Medical Sciences*. New York: Wiley, 1983.

genesis in liver followed by delivery of glucose and its use in a peripheral tissue. Both cycles provide a mechanism for continuously supplying tissues that require glucose as their primary energy source. The cycles are only functional between liver and tissues that do not completely oxidize glucose to CO_2 and H_2O. In order to participate in these cycles, peripheral tissues must release either alanine or lactate as the end product of glucose metabolism. The type

of recycled three-carbon intermediate is the major difference between the Cori cycle (Figure 7.32*a*) and the alanine cycle (Figure 7.32*b*), carbon returning to liver as lactate in the Cori cycle but as alanine in the alanine cycle. Another major difference is that NADH generated by glycolysis in the alanine cycle cannot be used to reduce pyruvate to lactate. In tissues that have mitochondria, electrons of NADH can be transported into the mitochondria by the malate–aspartate shuttle or the glycerol phosphate shuttle for the synthesis of ATP by oxidative phosphorylation:

$$NADH + H^+ + \tfrac{1}{2} O_2 + 3\ ADP + 3\ P_i \rightarrow NAD^+ + 3\ ATP + H_2O$$

or

$$FADH_2 + \tfrac{1}{2} O_2 + 2\ ADP + 2\ P_i \rightarrow FAD + 2\ ATP + H_2O$$

The consequence is that six to eight molecules of ATP can be formed per glucose molecule in peripheral tissues that participate in the alanine cycle. This stands in contrast to the Cori cycle in which only two molecules of ATP per molecule of glucose are produced. Overall stoichiometry for the Cori cycle is

$$6\ ATP_{liver} + 2\ (ADP + P_i)_{red\ blood\ cells} \rightarrow 6\ (ADP + P_i)_{liver} + 2\ ATP_{red\ blood\ cells}$$

Six molecules of ATP are needed in liver to provide the energy necessary for glucose synthesis. The alanine cycle also transfers the energy from liver to peripheral tissues and, because of the six to eight molecules of ATP produced per molecule of glucose, is an energetically more efficient cycle. Participation of alanine in the cycle presents liver with amino nitrogen, which must be disposed of as urea (Figure 7.32*b* and p. 453). Urea synthesis is expensive since four ATP molecules are consumed per urea molecule. The concurrent need for urea synthesis results in more ATP being needed per glucose molecule synthesized in liver. Overall stoichiometry for the alanine cycle is

$$10\ ATP_{liver} + 6\text{–}8\ (ADP + P_i)_{muscle} + O_{2\ muscle} \rightarrow 10\ (ADP + P_i)_{liver} + 6\text{–}8\ ATP_{muscle}$$

In contrast to the Cori cycle, oxygen and mitochondria are required in peripheral tissue for participation in the alanine cycle.

Pathway of Glucose Synthesis from Lactate

Gluconeogenesis from lactate is an ATP-requiring process with the overall equation of

$$2\ \text{L-Lactate}^- + 6\ ATP^{4-} \rightarrow \text{glucose} + 6\ ADP^{3-} + 6\ P_i^{2-} + 4\ H^+$$

Many enzymes of glycolysis are common to the gluconeogenic pathway. Additional reactions have to be involved because glycolysis produces 2 ATPs and gluconeogenesis requires 6 ATPs per molecule of glucose. Also, certain steps of glycolysis are irreversible under intracellular conditions and are replaced by irreversible steps of the gluconeogenic pathway. The reactions of gluconeogenesis from lactate are given in Figure 7.33. The initial step is conversion of lactate to pyruvate by **lactate dehydrogenase.** NADH is generated and is also needed for a subsequent step in the pathway. Pyruvate cannot be converted to phosphoenolpyruvate (PEP) by **pyruvate kinase** because the reaction is irreversible under intracellular conditions. Pyruvate is converted into the high-energy phosphate compound PEP by coupling of two reactions requiring high-energy phosphate compounds (an ATP and a GTP). The first is catalyzed by pyruvate carboxylase and the second by PEP carboxykinase (see Figure 7.34).

Pyruvate Carboxylase and Phosphoenolpyruvate Carboxykinase

GTP, required for the **PEP carboxykinase,** is equivalent to an ATP through the action of **nucleoside diphosphate kinase** ($GDP + ATP \rightleftharpoons GTP + ADP$),

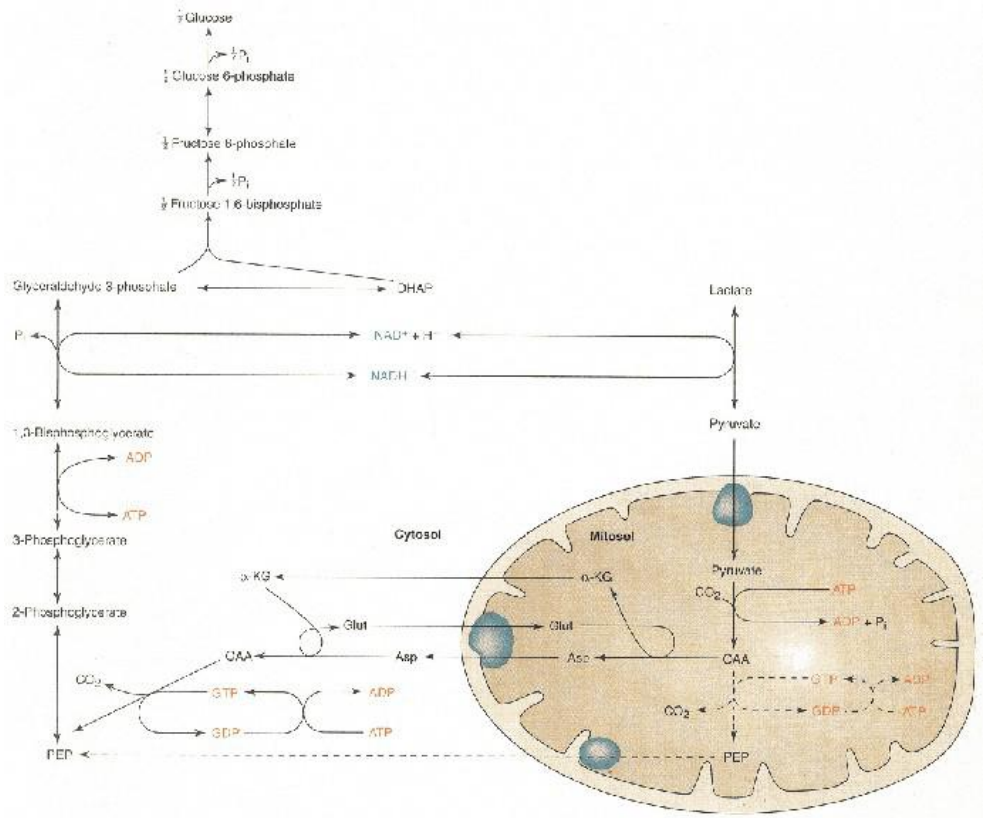


Figure 7.33
Pathway of gluconeogenesis from lactate.
The involvement of the mitochondrion in the process is indicated. Dashed arrows refer to an alternate route, which employs mitosolic PEP carboxykinase rather than the cytosolic isoenzyme. Abbreviations: OAA, oxaloacetate; -KG, -ketoglutarate; PEP, phosphoenolpyruvate; DHAP, dihydroxyacetone phosphate.

and CO_2 and HCO_{3-} readily equilibrate by action of **carbonic anhydrase** ($CO_2 + H_2O \rightleftharpoons H_2CO_3 \rightleftharpoons H+ + HCO_3^-$). Thus the sum of these reactions is

$$\text{Pyruvate}^- + 2\ ATP^{4-} \rightarrow \text{phosphoenolpyruvate}^{3-} + 2\ ADP^{3-} + Pi^{2-} + 4\ H^+$$

Thus conversion of pyruvate into PEP during gluconeogenesis costs the cell two molecules of ATP whereas conversion of PEP to pyruvate during glycolysis yields the cell one molecule of ATP.

The intracellular location of **pyruvate carboxylase** makes the mitochondrion mandatory for conversion of cytosolic pyruvate into cytosolic PEP (Figure 7.33). There are two routes that oxaloacetate can then take to glucose. This

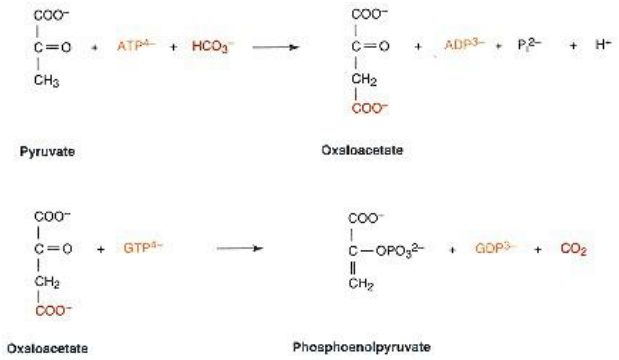


Figure 7.34
Energy-requiring steps involved in phosphoenolpyruvate formation from pyruvate.
Reactions are catalyzed by pyruvate carboxylase and PEP carboxykinase, respectively.

happens because PEP carboxykinase is present in both cytosolic and mitosolic compartments. The simplest pathway involves the mitochondrial PEP carboxykinase. Oxaloacetate is converted within the mitochondrion into PEP, which then traverses the mitochondrial inner membrane. The second pathway would be just as simple if oxaloacetate could traverse the mitochondrial inner membrane. However, oxaloacetate cannot be transported out of mitochondria for want of a transporter (Figure 7.9*b*). Thus oxaloacetate is converted into aspartate, which is transported out by the **glutamate–aspartate antiport.** In the cytosol, transamination with α-ketoglutarate converts aspartate back to oxaloacetate.

Gluconeogenesis Uses Many Glycolytic Enzymes but in the Reverse Direction

The steps from PEP to fructose 1,6-bisphosphate are steps of the glycolytic pathway in reverse. NADH generated by lactate dehydrogenase is utilized by glyceraldehyde-3-phosphate dehydrogenase, establishing an equal balance of generation and utilization of reducing equivalents.

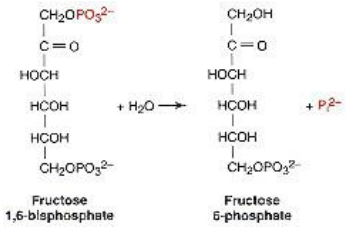


Figure 7.35
Reaction catalyzed by fructose 1,6-bisphosphatase.

6-Phosphofructo-1-kinase catalyzes an irreversible step in glycolysis and cannot be used for conversion of FBP to fructose 6-phosphate. A way around this step is provided by **fructose 1,6-bisphosphatase,** which catalyzes irreversible hydrolysis of fructose 1,6-bisphosphate (Figure 7.35). This reaction produces F6P but, since the reaction is irreversible, it cannot be used in glycolysis to produce FBP.

Phosphoglucose isomerase is freely reversible and functions in both glycolytic and gluconeogenic pathways. **Glucose 6-phosphatase,** which is used instead of glucokinase for the last step, catalyzes an irreversible hydrolytic reaction under intracellular conditions (Figure 7.36). Nucleotides have no role in this reaction; the function of this enzyme is to generate glucose, not to convert glucose into glucose 6-phosphate. Glucose 6-phosphatase is a membrane-bound enzyme, within the endoplasmic reticulum, with its active site available for G6P hydrolysis on the cisternal surface of the tubules (see Figure 7.37). A translocase for G6P is required to move G6P from the cytosol to its site of

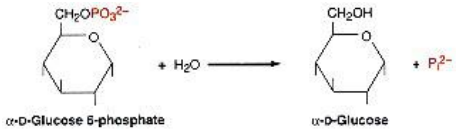


Figure 7.36
Reaction catalyzed by glucose 6-phosphatase.

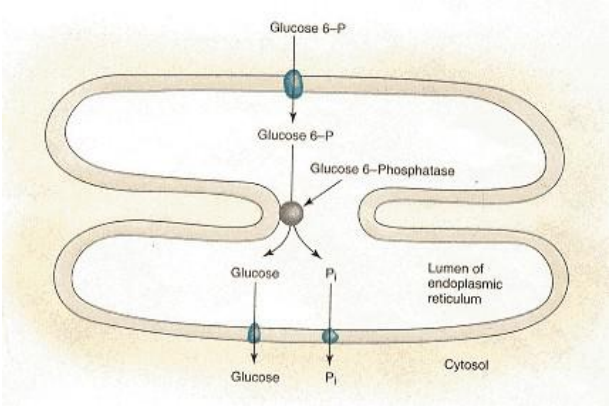


Figure 7.37
Glucose 6-phosphate is hydrolyzed by glucose 6-phosphatase located on the cisternal surface of the endoplasmic reticulum.
Three transporters are involved: one moves glucose 6-phosphate into the lumen, a second moves P_i back to the cytosol, and a third moves glucose back into the cytosol.

hydrolysis within the endoplasmic reticulum. A genetic defect in either the translocase or the phosphatase interferes with gluconeogenesis and results in accumulation of glycogen in liver, as discussed later for glycogen metabolism (Section 7.6).

Glucose Is Synthesized from the Carbon Chains of Some Amino Acids

All **amino acids** except **leucine** and **lysine** can supply carbon for net synthesis of glucose by gluconeogenesis (see Chapter 11). If catabolism of an amino acid can yield either net pyruvate or net oxaloacetate formation, then net glucose synthesis can occur from that amino acid. Oxaloacetate is an intermediate in gluconeogenesis and pyruvate is readily converted to oxaloacetate by action of pyruvate carboxylase (Figure 7.34). The abbreviated pathway given in Figure 7.31 shows where amino acid catabolism fits with the process of gluconeogenesis. Catabolism of amino acids feeds carbon into the tricarboxylic cycle at more than one point. As long as net synthesis of a TCA cycle intermediate occurs as a consequence of catabolism of a particular amino acid, net synthesis of oxaloacetate will follow. Reactions that lead to net synthesis of TCA cycle intermediates are called **anaplerotic reactions (anaplerosis)** and support gluconeogenesis because they provide for net synthesis of oxaloacetate. Reactions catalyzed by pyruvate carboxylase and glutamate dehydrogenase are good examples of anaplerotic reactions:

$$\text{Pyruvate}^- + \text{ATP}^{4-} + \text{HCO}_3^- \rightarrow \text{oxaloacetate}^{2-} + \text{ADP}^{3-} + \text{P}_i^{2-} + \text{H}^+$$

$$\text{Glutamate}^- + \text{NAD(P)}^+ \rightleftharpoons \alpha\text{-ketoglutarate}^{2-} + \text{NAD(P)H} + \text{NH}_4^+ + \text{H}^+$$

On the other hand, the reaction catalyzed by glutamate–oxaloacetate transaminase (α-ketoglutarate + aspartate $\rightleftharpoons$ glutamate + oxaloacetate) is not anaplerotic because net synthesis of a TCA cycle intermediate is not accomplished. An intermediate of the TCA cycle is utilized in the reaction.

Since gluconeogenesis from amino acids imposes a nitrogen load on liver, a close relationship exists between urea synthesis and glucose synthesis from amino acids. This relationship is illustrated in Figure 7.38 for alanine. Two alanine molecules are shown being metabolized, one yielding NH_4^+ and the other aspartate, the primary substrates for the urea cycle. Aspartate leaves the mitochondrion and becomes part of the urea cycle after reacting with citrulline. Carbon of aspartate is released from the urea cycle as fumarate, which is then converted to malate by cytosolic fumarase. Both this malate and another malate

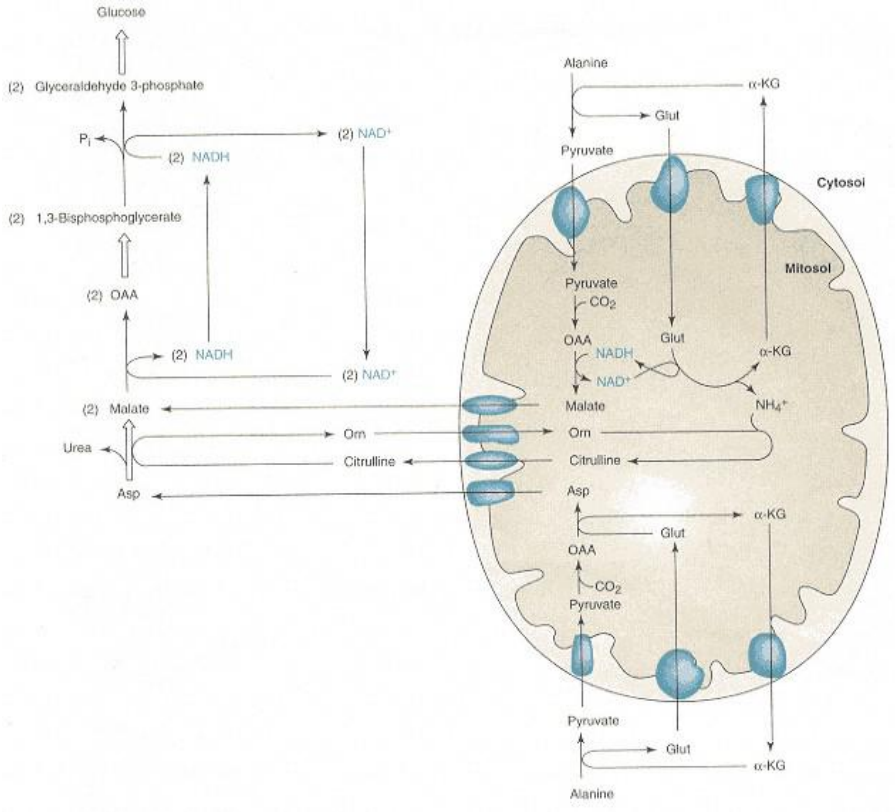


Figure 7.38
Pathway of gluconeogenesis from alanine and its relationship to urea synthesis.

exiting from the mitochondria are converted to glucose by cytosolic enzymes of gluconeogenesis. A balance is achieved between reducing equivalents (NADH) generated and those required in the cytosol and mitosol.

Leucine and **lysine** are the only amino acids that cannot function as carbon sources for net synthesis of glucose. These amino acids are **ketogenic** but not **glucogenic.** As shown in Table 7.2, all other amino acids are classified as glucogenic, or at least both glucogenic and ketogenic. Glucogenic amino acids give rise to net synthesis of either pyruvate or oxaloacetate, whereas amino acids that are both glucogenic and ketogenic also yield the ketone body acetoacetate, or acetyl CoA, which is readily converted into ketone bodies. Acetyl CoA is the end product of lysine metabolism, and acetoacetate and acetyl CoA are end products of leucine metabolism. No pathway exists for converting acetoacetate or acetyl CoA into pyruvate or oxaloacetate in humans and other animals. Acetyl CoA cannot be used for net synthesis of glucose because the reaction catalyzed by the **pyruvate dehydrogenase** complex is irreversible:

$$\text{Pyruvate} + \text{NAD}^+ + \text{CoASH} \rightarrow \text{acetyl CoA} + \text{NADH} + \text{CO}_2$$

TABLE 7.2 Glucogenic and Ketogenic Amino Acids

Glucogenic	*Ketogenic*	*Both*
Glycine	Leucine	Threonine
Serine	Lysine	Isoleucine
Valine		Phenylalanine
Histidine		Tyrosine
Arginine		Tryptophan
Cysteine		
Proline		
Hydroxyproline		
Alanine		
Glutamate		
Glutamine		
Aspartate		
Asparagine		
Methionine		

It might be argued that oxaloacetate is generated from acetyl CoA by the TCA cycle:

$$\text{Acetyl CoA} \rightarrow \text{citrate} \rightarrow\rightarrow 2\ CO_2 + \text{oxaloacetate}$$

However, this is a fallacious argument because it ignores the requirement for oxaloacetate in formation of citrate from acetyl CoA by **citrate synthase:**

$$\text{Acetyl CoA} + \text{oxaloacetate} \rightarrow \text{citrate} + \text{CoA}$$

The TCA cycle then catalyzes

$$\text{Citrate} \rightarrow\rightarrow 2\ CO_2 + \text{oxaloacetate}$$

The true sum reaction is then

$$\text{Acetyl CoA} \rightarrow 2CO_2 + \text{CoA}$$

Since net synthesis of a **TCA cycle** intermediate does not occur during oxidation of acetyl CoA, it is impossible for animals to synthesize glucose from acetyl CoA.

Glucose Can Be Synthesized from Odd-Chain Fatty Acids

Lack of an anaplerotic pathway from acetyl CoA also means that in general it is impossible to synthesize glucose from fatty acids. Most fatty acids found in humans have straight chains with an even number of carbon atoms. Their catabolism by fatty acid oxidation followed by ketogenesis or complete oxidation to CO_2 can be abbreviated as in Figure 7.39. Since acetyl CoA and other intermediates of even-numbered fatty acid oxidation cannot be converted to oxaloacetate or any other intermediate of gluconeogenesis, it is impossible to synthesize glucose from fatty acids. An exception to this general rule applies to fatty acids with methyl branches (e.g., **phytanic acid,** a breakdown product of chlorophyll; see discussion of **Refsum's disease,** Clin. Corr. 9.6) and fatty acids with an odd number of carbon atoms. Catabolism of such compounds yields propionyl CoA:

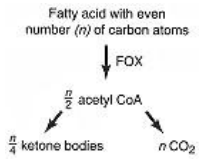


Figure 7.39
Overview of the catabolism of fatty acids to ketone bodies and CO_2.

$$\text{Fatty acid with an odd number } (n) \text{ of carbon atoms} \rightarrow \frac{(n-3)}{2}\text{ acetyl CoA} + 1\text{ propionyl CoA}$$

Propionate is a good precursor for gluconeogenesis, generating oxaloacetate by the anaplerotic pathway shown in Figure 7.40. The coenzyme A ester of

propionate is also produced in catabolism of valine and isoleucine and conversion of cholesterol into bile acids.

It is sometimes loosely stated that fat *cannot* be converted into carbohydrate (glucose) by liver. In a sense this is certainly true since catabolism of fatty acids with an even number of carbon atoms cannot give rise to net synthesis of glucose. However, the term "fat" refers to triacylglycerols, which are composed of three *O*-acyl groups combined with one glycerol molecule. Hydrolysis of a triacylglycerol yields three fatty acids and glycerol, the latter compound being an excellent substrate for gluconeogenesis (Figure 7.41). Phosphorylation of glycerol by **glycerol kinase** produces glycerol 3-phosphate, which can be converted by glycerol-3-phosphate dehydrogenase into dihydroxyacetone phosphate, an intermediate of the gluconeogenic pathway (see Figure 7.33). The last stage of glycolysis can compete with the gluconeogenic pathway and convert dihydroxyacetone phosphate into lactate (or into pyruvate for subsequent complete oxidation to CO_2 and H_2O).

Glucose Is Synthesized from Other Sugars

Fructose

Humans consume considerable quantities of fructose in the form of sucrose hydrolyzed in the small bowel. In the liver, fructose is phosphorylated by a special ATP-linked kinase (Figure 7.42), yielding fructose 1-phosphate (see Clin. Corr. 7.3). A special aldolase then cleaves **fructose 1-phosphate** to yield one molecule of dihydroxyacetone phosphate and one of glyceraldehyde. The latter is reduced to glycerol and used by the same pathway given in the previous figure. Two molecules of dihydroxyacetone phosphate obtainable from one molecule of fructose can be converted to glucose by enzymes of gluconeogenesis or, alternatively, into pyruvate or lactate by the last stage of glycolysis. In analogy to glycolysis, conversion of fructose into lactate is called **fructolysis.**

The major energy source of spermatozoa is fructose, formed from glucose by cells of seminal vesicles as shown in Figure 7.43. An NADPH-dependent reduction of glucose to sorbitol is followed by an NAD^+-dependent oxidation of sorbitol to fructose. Fructose is secreted from seminal vesicles in a fluid that becomes part of semen. Although the fructose concentration in human semen can exceed 10 mM, tissues that come in contact with semen utilize fructose poorly, allowing this substrate to be conserved to meet the energy demands of spermatozoa in their search for ova. Spermatozoa contain mitochondria and thus can metabolize fructose completely to CO_2 and H_2O by the combination of fructolysis and TCA cycle activity.

Galactose

Milk sugar or **lactose** is an important source of **galactose** in the human diet. Glucose formation from galactose follows the pathway shown in Figure 7.44. **UDP-glucose** serves as a recycling intermediate in the overall process of converting galactose into glucose. Absence of the enzyme **galactose 1-phosphate uridylyltransferase** accounts for most cases of **galactosemia** (see Clin. Corr. 8.3).

Mannose

Mannose is found in very limited quantities in our diet. It is phosphorylated by hexokinase and then converted into fructose 6-phosphate by mannose phosphate isomerase:

D-Mannose + ATP → D-mannose 6-phosphate + ADP

D-Mannose 6-phosphate → D-fructose 6-phosphate

The latter compound can then be used in either glycolysis or gluconeogenesis.

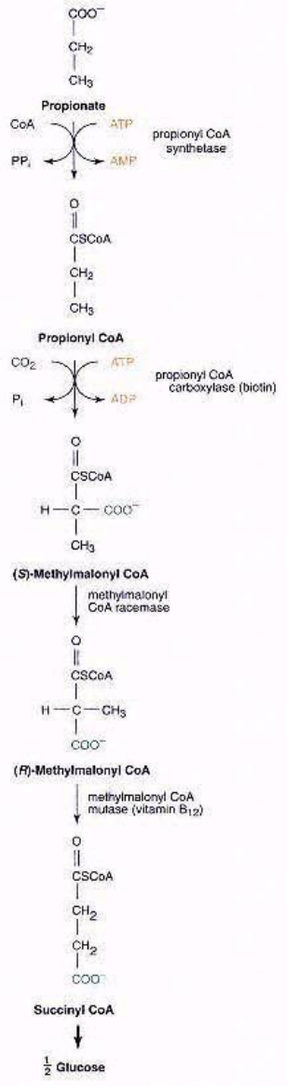


Figure 7.40
Pathway of gluconeogenesis from propionate.
The large arrow refers to steps of the tricarboxylic acid cycle plus steps of lactate gluconeogenesis (see Figure 7.33).

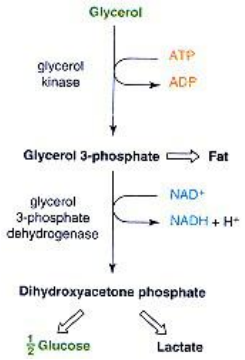


Figure 7.41
Pathway of gluconeogenesis from glycerol, along with competing pathways.
Large arrows indicate steps of the glycolytic and gluconeogenic pathways that have been given in detail in Figures 7.6 and 7.33, respectively. The large arrow pointing to fat refers to the synthesis of triacylglycerols and glycerophospholipids.

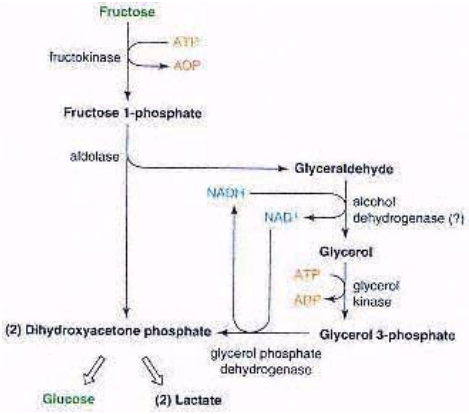


Figure 7.42
Pathway of glucose formation from fructose, along with the competing pathway of fructolysis.
Large arrows indicate steps of theglycolytic and gluconeogenic pathways that have been given in detail in Figures 7.6 and 7.33, respectively.

Gluconeogenesis Requires Expenditure of ATP

Synthesis of glucose is costly in terms of ATP. Six molecules are required for synthesis of one molecule of glucose from two molecules of lactate. ATP needed by liver cells for glucose synthesis is provided in large part by fatty acid oxidation. Metabolic conditions under which liver is required to synthesize glucose generally favor increased availability of fatty acids in blood. These fatty acids are oxidized by liver mitochondria to ketone bodies with concurrent production of large amounts of ATP. This ATP is used to support the energy requirements of gluconeogenesis, regardless of the substrate being used as carbon source for the process.

Gluconeogenesis Has Several Sites of Regulation

Sites of regulation of the gluconeogenesis pathway are apparent from the mass–action ratios and equilibrium constants in Table 7.1 and are further indicated in Figure 7.45. Those enzymes that are used to "go around" the irreversible steps of glycolysis are primarily involved in regulation of the pathway, that is, pyruvate carboxylase, PEP carboxykinase, fructose 1,6-bisphosphatase, and glucose 6-phosphatase. Regulation of hepatic gluconeogenesis is almost the

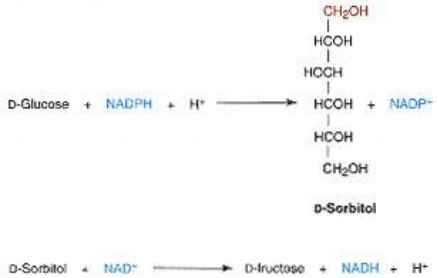


Figure 7.43
The pathway responsible for the formation of sorbitol and fructose from glucose.

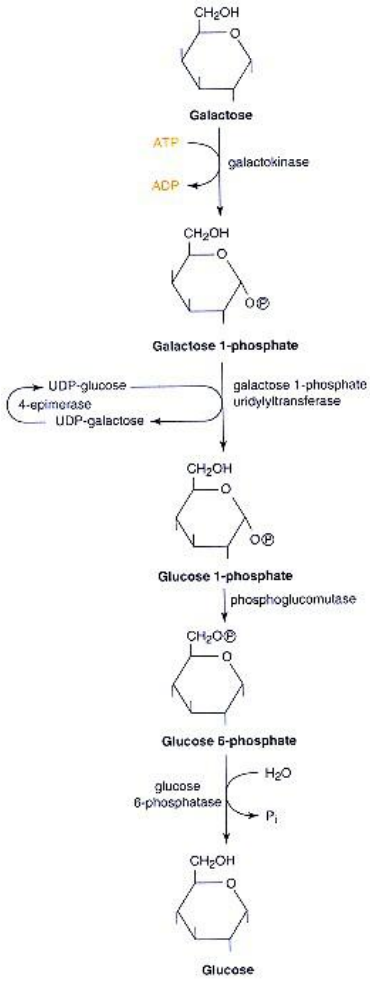


Figure 7.44
Pathway of glucose formation from galactose.

same as regulation of hepatic glycolysis. Inhibition of glycolysis at its chief regulatory sites, or repressing synthesis of enzymes involved at these sites (glucokinase, 6-phosphofructo-1-kinase, and pyruvate kinase), greatly increases effectiveness of opposing gluconeogenic enzymes. Turning on gluconeogenesis is therefore accomplished in large part by shutting off glycolysis. Fatty acid oxidation does more than just supply ATP for the process. It promotes glucose synthesis by increasing the steady-state concentration of mitochondrial acetyl CoA, a positive allosteric effector of the mitochondrial **pyruvate carboxylase.** The increase in acetyl CoA and in pyruvate carboxylase activity results in a greater synthesis of citrate, a negative effector of 6-phosphofructo-1-kinase. A secondary effect of inhibition of 6-phosphofructo-1-kinase is a decrease in fructose 1,6-bisphosphate concentration, an activator of pyruvate kinase. This

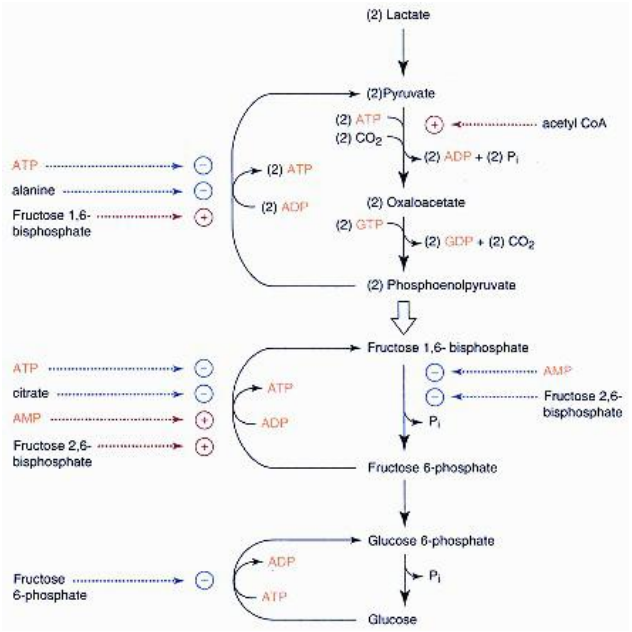


Figure 7.45
Important allosteric regulatory features of the gluconeogenic pathway.

decreases the flux of PEP to pyruvate by pyruvate kinase and increases effectiveness of the combined efforts of pyruvate carboxylase and PEP carboxykinase in conversion of pyruvate to PEP. An increase in ATP levels with the consequential decrease in AMP levels would favor gluconeogenesis by way of inhibition of 6-phosphofructo-1-kinase and pyruvate kinase and activation of fructose 1,6-bisphosphatase (see Figure 7.45 and the discussion of regulation of glycolysis, p. 283). A shortage of oxygen for respiration, a shortage of fatty acids for oxidation, or any inhibition or uncoupling of oxidative phosphorylation would be expected to cause liver to turn from gluconeogenesis to glycolysis.

Hormonal Control of Gluconeogenesis Is Critical for Homeostasis

Hormonal control of gluconeogenesis is a matter of regulating the supply of fatty acids to liver and the enzymes of both the glycolytic and gluconeogenic pathways. Glucagon increases plasma fatty acids by promoting lipolysis in adipose tissue, an action opposed by insulin. The greater availability of fatty acids results in more fatty acid oxidation by liver, which promotes glucose synthesis. Insulin has the opposite effect. Glucagon and insulin also regulate gluconeogenesis by influencing the state of phosphorylation of hepatic enzymes subject to covalent modification. As discussed previously (Figure 7.30), pyruvate kinase is active in the dephosphorylated mode and inactive in the phosphorylated mode. Glucagon activates adenylate cyclase to produce cAMP, which activates protein kinase A, which, in turn, phosphorylates and inactivates pyruvate kinase. Inactivation of this glycolytic enzyme stimulates the opposing pathway gluconeogenesis, by blocking the futile conversion of PEP to pyruvate. Glucagon also stimulates gluconeogenesis at the conversion of fructose 1,6-bisphosphate to fructose 6-phosphate by decreasing the concentration of fructose 2,6-bisphosphate in liver. **Fructose 2,6-bisphosphate** is an allosteric

activator of 6-phosphofructo-1-kinase and an allosteric inhibitor of fructose 1,6-bisphosphatase. Glucagon, again working via its second messenger cAMP, lowers fructose 2,6-bisphosphate levels by stimulating the phosphorylation of the bifunctional enzyme 6-phosphofructo-2-kinase/fructose 2,6-bisphosphatase. Phosphorylation of this enzyme inactivates the kinase activity that makes fructose 2,6-bisphosphate from F6P but activates the phosphatase activity that hydrolyzes fructose 2,6-bisphosphate back to F6P. The consequence is a glucagon-induced fall in fructose 2,6-bisphosphate levels, leading to a decrease in activity of 6-phosphofructo-1-kinase while fructose 1,6-bisphosphatase becomes more active (Figure 7.45). The overall effect is an increased conversion of FBP to F6P and a corresponding increase in the rate of gluconeogenesis. A resulting increase in fructose 6-phosphate may also favor gluconeogenesis by inhibition of glucokinase via an inhibitory protein (see discussion of the regulation of glycolysis, p. 283). Insulin has effects opposite to those of glucagon by mechanisms not completely defined.

Glucagon and insulin also have long-term effects on hepatic glycolysis and gluconeogenesis by induction and repression of synthesis of key enzymes of the pathways. A high glucagon/insulin ratio in blood increases the enzymatic capacity for gluconeogenesis and decreases enzymatic capacity for glycolysis in liver. A low glucagon/insulin ratio has the opposite effects. The glucagon/insulin ratio increases when gluconeogenesis is needed and decreases when glucose is plentiful from the gastrointestinal tract. Glucagon signals induction of synthesis of greater quantities of PEP carboxykinase, fructose 1,6-bisphosphatase, glucose 6-phosphatase, and various aminotransferases. A model for how this occurs is given in Figure 7.46. Binding of glucagon to its plasma membrane receptor increases cAMP, which activates protein kinase A. Protein kinase A

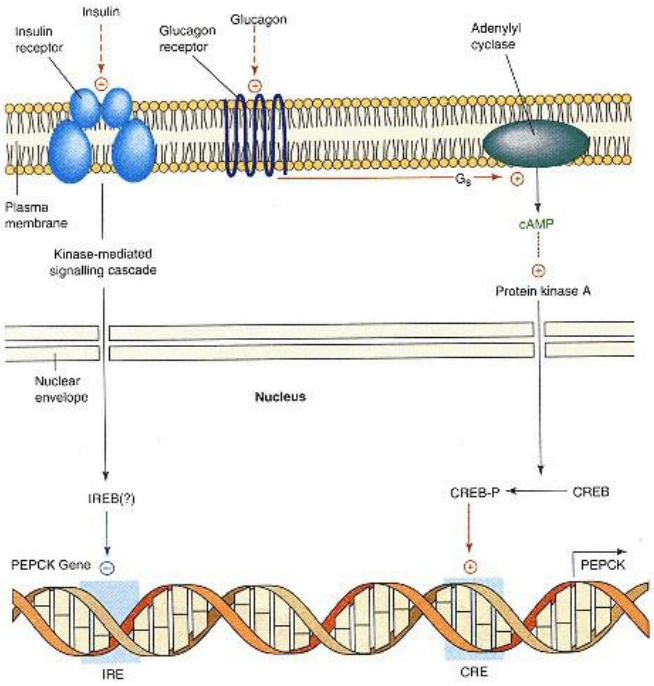


Figure 7.46
Glucagon promotes transcription of the gene that encodes PEP carboxykinase.
Abbreviations: PEPCK, PEP carboxykinase; CRE, cAMP-response element; CREB, cAMP-response element binding protein; IRE, insulin-response element; IREB, insulin-response element binding protein.

CLINICAL CORRELATION 7.10

Hypoglycemia and Alcohol Intoxication

Consumption of alcohol, especially by an undernourished person, can cause hypoglycemia. The same effect can result from drinking alcohol after strenuous exercise. In both cases the hypoglycemia results from the inhibitory effects of alcohol on hepatic gluconeogenesis and thus occurs under circumstances of hepatic glycogen depletion. The problem is caused by the NADH produced during the metabolism of alcohol. The liver simply cannot handle the reducing equivalents provided by ethanol oxidation fast enough to prevent metabolic derangements. The extra reducing equivalents block the conversion of lactate to glucose and promote the conversion of alanine into lactate, resulting in considerable lactate accumulation in the blood. Since lactate has no place to go, lactic acidosis (see Clin. Corr. 7.5) can develop, although it is usually mild.

Low doses of alcohol cause impaired motor and intellectual performance; high doses have a depressant effect that can lead to stupor and anesthesia. Low blood sugar can contribute to these undesirable effects of alcohol. What is more, a patient may be thought to be inebriated when in fact the patient is suffering from hypoglycemia that may lead to irreversible damage to the central nervous system. Children are highly dependent on gluconeogenesis while fasting, and accidental ingestion of alcohol by a child can produce severe hypoglycemia (see Clin. Corr. 7.9).

Krebs, H. A., Freedland, R. A., Hems, R., and Stubbs, M. Inhibition of hepatic gluconeogenesis by ethanol. *Biochem. J.* 112:117, 1969; and Service, F. J. Hypoglycemia. *Med. Clin. North Am.* 79:1, 1995.

then phosphorylates a protein called the **cAMP-response element binding protein (CREB),** a trans-acting factor that in its phosphorylated form can bind to a **cAMP-response element (CRE),** a cis acting element within the regulatory region of genes that respond to cAMP. This promotes transcription of genes encoding key gluconeogenic enzymes such as **PEP carboxykinase** (Figure 7.46). By a similar mechanism, but one that causes repression of **gene transcription**, glucagon acts to decrease the amounts of glucokinase, 6-phosphofructo-1-kinase, and pyruvate kinase. Insulin opposes the action of glucagon (Figure 7.46), acting through a signal cascade that results in activation of an insulin-response element binding protein (IREB), which inhibits transcription of genes encoding key gluconeogenic enzymes by binding to an **insulin-response element (IRE)** in the regulatory region of such genes. When glucose synthesis is not needed, synthesis of key gluconeogenic enzymes is turned off and synthesis of key glycolytic enzymes is turned on as a consequence of a decrease in the blood glucagon/insulin ratio.

Ethanol Ingestion Inhibits Gluconeogenesis

Ethanol inhibits gluconeogenesis by liver (see Clin. Corr. 7.10). It is oxidized primarily in liver with production of a large load of reducing equivalents that must be transported into the mitochondria by the **malate-aspartate shuttle**. This excess NADH in the cytosol creates problems for liver gluconeogenesis because it forces the equilibrium of the lactate dehydrogenase- and malate dehydrogenase-catalyzed reactions in the directions of lactate and malate formation, respectively:

Pyruvate + NADH + H^+ → lactate + NAD^+

Sum: Ethanol + pyruvate → acetaldehyde + lactate

or

Oxaloacetate + NADH + H^+ → malate + NAD^+

Sum: Ethanol + oxaloacetate → acetaldehyde + malate

Forcing these reactions in the directions shown above inhibits glucose synthesis by limiting the amounts of pyruvate and oxaloacetate available for the reactions catalyzed by pyruvate carboxylase and PEP carboxykinase, respectively.

7.6— Glycogenolysis and Glycogenesis

Glycogen, a Storage Form of Glucose, Is Required as a Ready Source of Energy

Glycogenolysis refers to breakdown of glycogen to glucose or glucose 6-phosphate; and **glycogenesis** refers to synthesis of glycogen. These processes are of some importance in almost every tissue but especially in muscle and liver. The liver has tremendous capacity for storing glycogen. In the well-fed human, liver glycogen content can account for as much as 10% of wet weight of this organ. Muscle stores less when expressed on the same basis–a maximum of only 1–2% of its wet weight. However, since the average person has more muscle than liver, there is about twice as much total muscle glycogen as liver glycogen.

Muscle and liver **glycogen stores** serve completely different roles. Glycogen serves as a fuel reserve for the synthesis of ATP within muscle, whereas liver glycogen functions as a glucose reserve for the maintenance of blood glucose concentrations. Liver glycogen levels vary greatly in response to the intake of food, accumulating to high levels shortly after a meal and then decreasing slowly as it is mobilized to help maintain a nearly constant blood glucose level (see Figure 7.47). Liver glycogen is called into play between meals and to a greater extent during the nocturnal fast. In both humans and the rat, the store of liver glycogen lasts somewhere between 12 and 24 h during fasting, depending greatly, of course, on whether the individual under consideration is caged or running wild.

Muscle glycogen is a source of ATP for increased muscular activity. Most of the glucose of glycogen is consumed within muscle cells without formation of free glucose as an intermediate. However, because of a special feature of glycogen catabolism to be discussed below, about 8% of muscle glycogen is converted into free glucose within the tissue. Some of this glucose may be released into the bloodstream, but most gets metabolized by glycolysis in muscle. Since muscle lacks glucose 6-phosphatase, and most free glucose formed during glycogen breakdown is further catabolized, muscle glycogen is not of quantitative importance in maintenance of blood glucose levels in the fasting state. Liver glycogen converted to glucose by glycogenolysis and glucose 6-phosphatase is of much greater importance as a source of blood glucose in the fasting state. Conversion of glucose to glycogen in muscle plays an important role in lowering blood glucose levels elevated by a high carbohydrate meal. Glycogenesis in liver contributes to the lowering of blood glucose but is of less importance than glycogen synthesis in muscle.

Exercise of a muscle triggers mobilization of muscle glycogen for formation of ATP. The yield of ATP and the fate of the carbon of glycogen depend on whether a "white" or "red" muscle is under consideration. Red muscle fibers are supplied with a rich blood flow, contain large amounts of myoglobin, and are packed with mitochondria. Glycogen mobilized within these cells is converted into pyruvate, which, because of the availability of O_2 and mitochondria, can be converted into CO_2 and H_2O. In contrast, white muscle fibers have a poorer blood supply and fewer mitochondria. **Glycogenolysis** within this tissue supplies substrate for glycolysis, with the end product being primarily lactate. **White muscle fibers** have enormous capacity for glycogenolysis and glycolysis, much more than **red muscle fibers**. Since their glycogen stores are limited, however, muscles of this type can only function at full capacity for relatively short periods of time. Breast muscle and the heart of chicken are good examples of white and red muscles, respectively. The heart has to beat

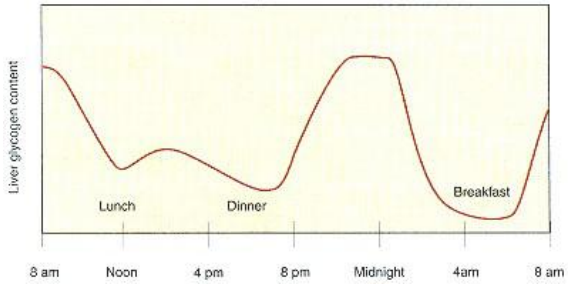


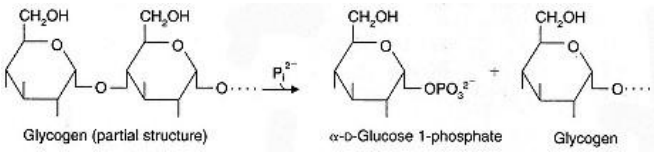
Figure 7.47
Variation of liver glycogen levels between meals and during the nocturnal fast.

continuously and has many mitochondria and a rich supply of blood via the coronary arteries. The heart stores glycogen to be used when a greater work load is imposed. Breast muscle of chicken is not continuously carrying out work. Its important function is to enable the chicken to fly rapidly for short distances, as in fleeing from predators (or amorous roosters). Because glycogen can be mobilized so rapidly, breast muscle is designed for maximal activity for a relatively short period of time. Although it was easy to point out readily recognizable white and red muscles in the chicken, most skeletal muscles of the human body are composed of a mixture of red and white fibers in order to provide for both rapid and sustained muscle activity. The distribution of white and red muscle fibers in cross sections of a human skeletal muscle can be shown by using special staining procedures (see Figure 7.48).

Glycogen granules are abundant in **liver** of the well-fed animal but are virtually absent from liver of the 24-h-fasted animal (Figure 7.49). Heavy exercise causes the same loss of glycogen granules in muscle fibers. These granules of glycogen correspond to clusters of glycogen molecules, the molecular weights of which can approach 2×10^7 Da. Glycogen is composed entirely of glucosyl residues, the majority of which are linked together by α-1,4-glycosidic linkages (Figure 7.50). Branches also occur in the glycogen molecule, however, because of frequent α-1,6-glycosidic linkages (Figure 7.50). A limb of the glycogen "tree" (see Figure 7.51) is characterized by branches at every fourth glucosyl residue within the more central core of the molecule. These branches occur much less frequently in outer regions of the molecule. An interesting question, which we shall attempt to answer below, is why this polymer is constructed with so many intricate branches and loose ends. Glycogen certainly stands in contrast to proteins and nucleic acids in this regard but, of course, it is a storage form of fuel and never has to catalyze a reaction or convey information within a cell.

Glycogen Phosphorylase Catalyzes the First Step in Glycogen Degradation

Glycogen phosphorylase catalyzes **phosphorolysis** of glycogen, a reaction in which P_i is used in the cleavage of an α1,4-glycosidic linkage to yield glucose 1-phosphate (Figure 7.52). This always occurs at a terminal, nonreducing end of a glycogen molecule:



The reaction catalyzed by glycogen phosphorylase should be distinguished from that catalyzed by α-amylase, which degrades glycogen and starch in the gut (see Chapter 26). ***α*-Amylase** acts by simple hydrolysis, using water rather than inorganic phosphate to cleave α-1,4-glycosidic bonds. Glycogen may contain up to 100,000 glucose residues; its structure is usually abbreviated $(glucose)_n$. The reaction catalyzed by glycogen phosphorylase is written as

$$(Glucose)_n + P_i^{2-} \rightarrow (glucose)_{n-1} + \alpha\text{-D-glucose 1-phosphate}^{2-}$$

The next step of glycogen degradation is catalyzed by **phosphoglucomutase:**

$$\text{Glucose 1-phosphate} \rightarrow \text{glucose 6-phosphate}$$

This is a near-equilibrium reaction under intracellular conditions, allowing it to function in both glycogen degradation and synthesis. Like phosphoglycerate mutase (see p. 277), a bisphosphate compound is an obligatory interme-

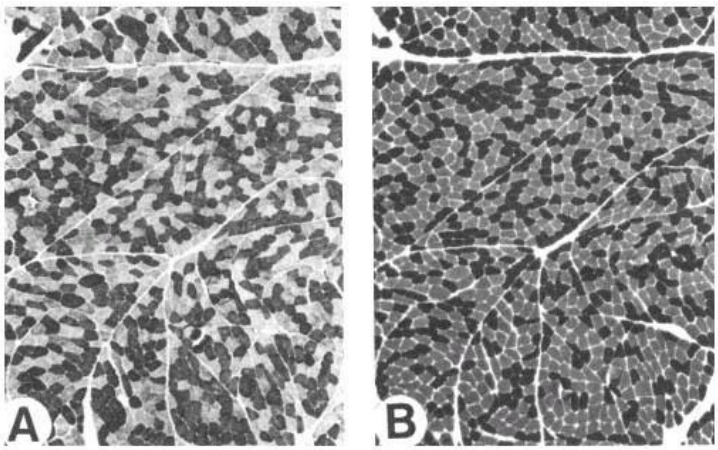


Figure 7.48
Cross section of human skeletal muscle showing red and white muscle fibers.
Sections were stained for NADH diaphorase activity in (*a*) for ATPase activity in (*b*). The red fibers are dark and the white fibers are light in (*a*); vice versa in (*b*).
Pictures generously provided by Dr. Michael H. Brooke of the Jerry Lewis Neuromuscular Research Center, St. Louis, Missouri.

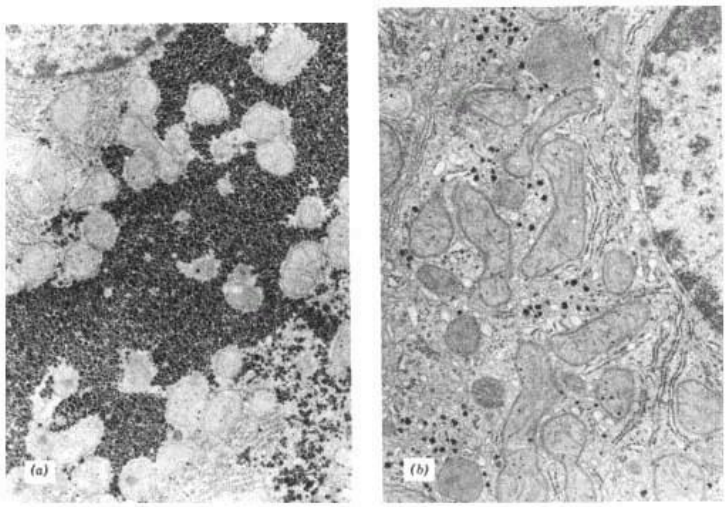


Figure 7.49
Electron micrographs showing glycogen granules (darkly stained material) in the liver of a well-fed rat (*a*) and the relative absence of such granules in the liver of a rat starved for 24 h (*b*).
Micrographs generously provided by Dr. Robert R. Cardell of the Department of Anatomy at the University of Cincinnati.

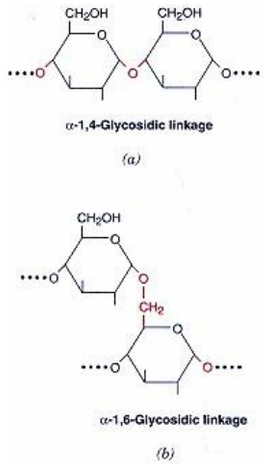


Figure 7.50
Two types of linkage between glucose molecules are present in glycogen.

diate:

E-P + glucose 1-phosphate ⇌ E + glucose 1,6-bisphosphate

E + glucose 1,6-bisphosphate ⇌ E-P + glucose 6-phosphate

Sum: Glucose 1-phosphate ⇌ glucose 6-phosphate

A catalytic amount of glucose 1,6-bisphosphate must be present for the reaction to occur. It is produced in small quantities for this specific purpose by an enzyme called **phosphoglucokinase:**

Glucose 6-phosphate + ATP → glucose 1,6-bisphosphate + ADP

The next enzyme involved in glycogenolysis depends on the tissue under consideration (Figure 7.52). In liver, glucose 6-phosphate produced by glycogenolysis is hydrolyzed by **glucose 6-phosphatase** to give free glucose:

$$\text{Glucose 6-phosphate}^{2-} + H_2O \rightarrow \text{glucose} + P_i^{2-}$$

Lack of this enzyme or of the translocase that transports G6P into the endoplasmic reticulum (Figure 7.37) results in type 1 **glycogen storage disease** (see Clin. Corr. 7.11). The overall balanced equation for removal of one glucosyl residue from glycogen in liver by glycogenolysis is then

$$(\text{Glucose})_n + H_2O \rightarrow (\text{glucose})_{n-1} + \text{glucose}$$

In other words, glycogenolysis in liver involves phosphorolysis but, because the phosphate ester is cleaved by a phosphatase, the overall reaction adds up to hydrolysis of glycogen. No ATP is used or formed in glycogenolysis.

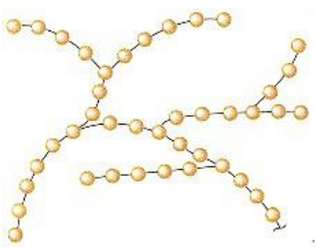


Figure 7.51
The branched structure of glycogen.

In peripheral tissues the G6P generated by glycogenolysis is used by glycolysis, leading primarily to the generation of lactate in white muscle fibers and primarily to complete oxidation to CO_2 in red muscle fibers. Since no ATP had to be invested to produce G6P obtained from glycogen, the overall equation

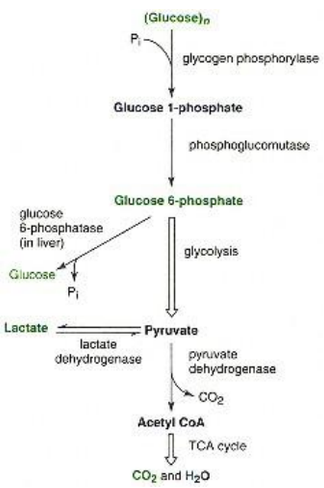


Figure 7.52
Glycogenolysis and the fate of glycogen degraded in liver versus its fate in peripheral tissues.

CLINICAL CORRELATION 7.11

Glycogen Storage Diseases

There are a number of well-characterized glycogen storage diseases, all due to inherited defects of one or more of the enzymes involved in the synthesis and degradation of glycogen. The liver is usually the tissue most affected, but heart and muscle glycogen metabolism can also be defective.

Chen, Y. T., and Burchell, A. Glycogen storage diseases. In C. R. Scriver, A. L. Beaudet, W. S. Sly, and D. Valle (Eds.), *The Metabolic and Molecular Bases of Inherited Disease*, 7th ed. New York: McGraw-Hill, 1995, pp. 935–965.

Von Gierke's Disease

The most common glycogen storage disease, referred to as type I or von Gierke's disease, is caused by a deficiency of liver, intestinal mucosa, and kidney glucose 6-phosphatase. Thus diagnosis by small bowel biopsy is possible. Patients with this disease can be further subclassified into those lacking the glucose 6-phosphatase enzyme per se (type Ia) and those lacking the glucose 6-phosphatase translocase (type Ib) (see Figure 7.37). A genetic abnormality in glucose 6-phosphate hydrolysis occurs in only about 1 person in 200,000 and is transmitted as an autosomal recessive trait. Clinical manifestations include fasting hypoglycemia, lactic acidemia hyperlipidemia, and hyperuricemia with gouty arthritis. The fasting hypoglycemia is readily explained as a consequence of the glucose 6-phosphatase deficiency, the enzyme required to obtain glucose from liver glycogen and gluconeogenesis. The liver of these patients does release some glucose by the action of the glycogen debranching enzyme. The lactic acidemia occurs because the liver cannot use lactate effectively for glucose synthesis. In addition, the liver inappropriately produces lactic acid in response to glucagon. This hormone should trigger glucose release without lactate production; however, the opposite occurs because of the lack of glucose 6-phosphatase. Hyperuricemia results from increased purine degradation in the liver; hyperlipidemia results because of increased availability of lactic acid for lipogenesis and lipid mobilization from the adipose tissue caused by high glucagon levels in response to hypoglycemia. The manifestations of von Gierke's disease can greatly be diminished by providing carbohydrate throughout the day to prevent hypoglycemia. During sleep this can be done by infusion of carbohydrate into the gut by a nasogastric tube.

Cori, G. T., and Cori, C. F. Glucose-6-phosphatase of the liver in glycogen storage disease. *J. Biol. Chem.* 199:661, 1952.

Pompe's Disease

Type II glycogen storage disease or Pompe's disease is caused by the absence of α-1,4-glucosidase (or acid maltase), an enzyme normally found in lysosomes. The absence of this enzyme leads to the accumulation of glycogen in virtually every tissue. This is somewhat surprising, but lysosomes take up glycogen granules and become defective with respect to other functions if they lack the capacity to destroy the granules. Because other synthetic and degradative pathways of glycogen metabolism are intact, metabolic derangements such as those in von Gierke's disease are not seen. The reason for extralysosomal glycogen accumulation is unknown. Massive cardiomegaly occurs and death results at an early age from heart failure.

Hers, H. G. α-Glucosidase deficiency in generalized glycogen storage disease (Pompe's disease). *Biochem. J.* 86:11, 1963.

Cori's Disease

Also called type III glycogen storage disease, Cori's disease is caused by a deficiency of the glycogen debranching enzyme. Glycogen accumulates because only the outer branches can be removed from the molecule by phosphorylase. Hepatomegaly occurs but diminishes with age. The clinical manifestations are similar to but much milder than those seen in von Gierke's disease, because gluconeogenesis is unaffected, and hypoglycemia and its complications are less severe.

Van Hoff, F., and Hers, H. G. The subgroups of type III glycogenesis. *Eur. J. Biochem.* 2:265, 1967.

McArdle's Disease

Also called the type V glycogen storage disease, McArdle's disease is caused by an absence of muscle phosphorylase. Patients suffer from painful muscle cramps and are unable to perform strenuous exercise, presumably because muscle glycogen stores are not available to the exercising muscle. Thus the normal increase in plasma lactate (released from the muscle) following exercise is absent. The muscles are probably damaged because of inadequate energy supply and glycogen accumulation. Release of muscle enzymes creatine kinase and aldolase and of myoglobin is common; elevated levels of these substances in the blood suggests a muscle disorder.

McArdle, B. Myopathy due to a defect in muscle glycogen breakdown. *Clin. Sci.* 10:13, 1951.

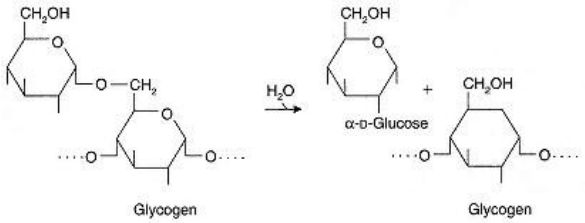
for glycogenolysis followed by glycolysis is

$$(\text{Glucose})_n + 3\ \text{ADP}^{3-} + 3\ \text{P}_i^{2-} + \text{H}^+ \rightarrow (\text{glucose})_{n-1} + 2\ \text{lactate}^- + 3\ \text{ATP}^{4-}.$$

Debranching Enzyme Is Required for Complete Hydrolysis of Glycogen

The first enzyme involved in glycogen degradation, glycogen phosphorylase, is specific for α-1,4-glycosidic linkages. However, it stops attacking α-1,4-glucosidic linkages four glucosyl residues from an α-1,6-branch point. A glycogen molecule that has been degraded by phosphorylase to the limit caused by the branches is called phosphorylase-**limit dextrin.** The action of a **debranching**

enzyme is what allows glycogen phosphorylase to continue to degrade glycogen. Debranching enzyme is a bifunctional enzyme that catalyzes two reactions necessary for debranching of glycogen. The first is a 4-α-D-glucanotransferase activity in which a strand of three glucosyl residues is removed from a four glucosyl residue branch of the glycogen molecule (Figure 7.53). The strand remains covalently attached to the enzyme until it can be transferred to a free 4-hydroxyl of a glucosyl residue at the end of the same or an adjacent glycogen molecule. The result is a longer amylose chain with only one glucosyl residue remaining in α-1,4-linkage. This linkage is broken hydrolytically by the second enzyme activity of debranching enzyme, which is its **amylo-α-1,6-glucosidase** activity:



The cooperative and repetitive action of phosphorylase and debranching enzyme results in complete phosphorolysis and hydrolysis of glycogen. **Glycogen**

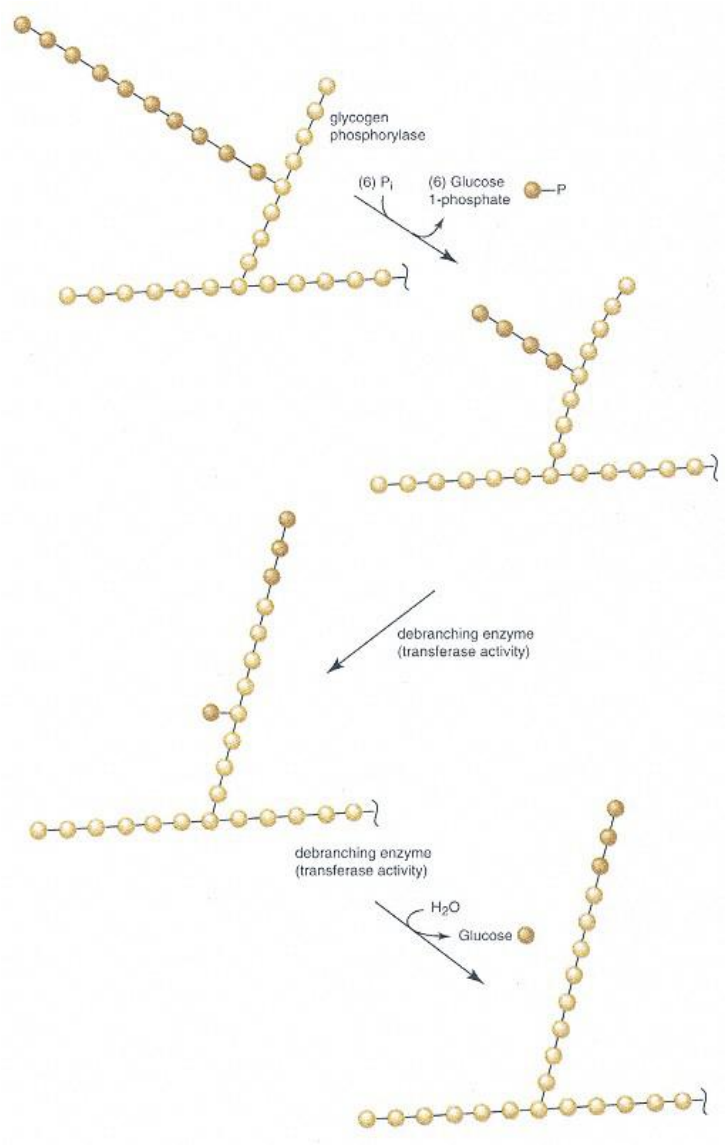


Figure 7.53
Action of the glycogen debranching enzyme.

storage diseases result when either of these enzymes is defective. The average molecule of glycogen yields about 12 molecules of glucose 1-phosphate by action of phosphorylase for every molecule of free glucose produced by action of debranching enzyme.

There is another, albeit quantitatively less important, pathway for glycogen degradation. A defect in this minor pathway, however, creates a major problem. As pointed out in Clin. Corr. 7.11, a **glucosidase** present in lysosomes degrades glycogen that enters these organelles during normal turnover of intracellular components.

Synthesis of Glycogen Requires Unique Enzymes

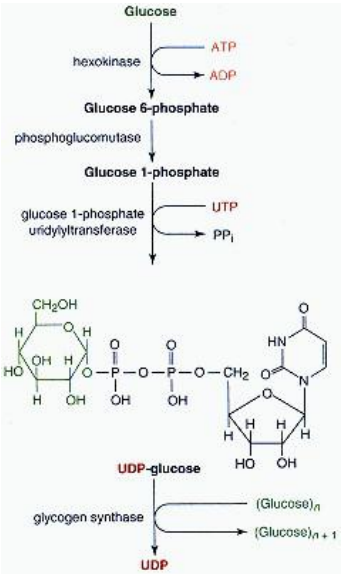


Figure 7.54
Pathway of glycogen synthesis.

The first reaction involved in **glycogen synthesis** (Figure 7.54) is already familiar, being catalyzed by glucokinase in hepatic tissue and hexokinase in peripheral tissues:

Glucose + ATP → glucose 6-phosphate + ADP

Phosphoglucomutase, discussed in relation to glycogen degradation, catalyzes a readily reversible reaction as follows:

Glucose 6-phosphate → glucose 1-phosphate

A unique reaction found at the next step involves formation of UDP-glucose by action of **glucose 1-phosphate uridylyltransferase:**

Glucose 1-phosphate + UTP → UDP-glucose + PP_i

This reaction generates UDP-glucose, sometimes called **"activated glucose"** because of its large negative free energy of hydrolysis, which is used to build the glycogen molecule. Formation of UDP-glucose is made energetically favorable and irreversible by hydrolysis of pyrophosphate by **pyrophosphatase:**

$PP_i^{4-} + H_2O \rightarrow 2\ P_i^{2-}$

Glycogen Synthase

Glycogen synthase catalyzes transfer of the activated glucosyl moiety of UDP-glucose to a glycogen molecule to form a new glycosidic bond between the hydroxyl group of C-1 of the activated sugar and C-4 of a glucosyl residue of the growing glycogen chain. The reducing end of glucose (C-1 of glucose is an aldehyde that can reduce other compounds) is always added to a nonreducing end of the glycogen chain. The glycogen molecule, regardless of its size, theoretically has only one free reducing end tucked away within its core. UDP formed as a product of glycogen synthase is converted back to UTP by action of **nucleoside diphosphate kinase:**

UDP + ATP → UTP + ADP

Glycogen synthase creates chains of glucose molecules with α-1,4-glycosidic linkages, but does not form the α-1,6-glycosidic branches found in glycogen. Its action alone would only produce α-amylose, a straight-chain polymer of glucose with α-1,4-glycosidic linkages. Once an amylose chain of at least 11 residues has been formed, a **"branching" enzyme** comes into play. Its name is **1,4-α-glucan branching enzyme** because it removes a block of about seven glucosyl residues from a growing chain and transfers it to another chain to produce an α-1,6 linkage (see Figure 7.55). The new branch has to be introduced at least four glucosyl residues from an adjacent branch point. Thus the creation of the highly branched structure of glycogen requires the concerted efforts of glycogen synthase and branching enzyme. The overall balanced equation for

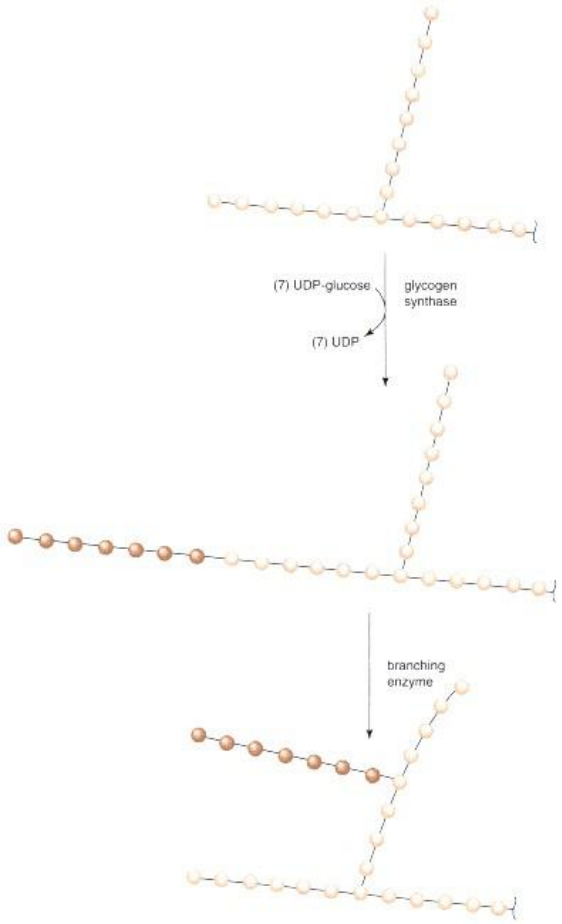


Figure 7.55
Action of the glycogen branching enzyme.

glycogen synthesis by the pathway just outlined is

$$(\text{Glucose})_n + \text{glucose} + 2\ \text{ATP} \rightarrow (\text{glucose})_{n+1} + 2\ \text{ADP} + 2\ \text{P}_i$$

As noted above, the combination of glycogenolysis and glycolysis yields only three molecules of ATP per glucosyl residue:

$$(\text{Glucose})_n + 3\ \text{ADP} + 3\ \text{P}_i \rightarrow (\text{glucose})_{n-1} + 2\ \text{lactate} + 3\ \text{ATP}$$

Thus the combination of glycogen synthesis plus glycogen degradation to lactate actually yields only one ATP. However, glycogen synthesis and degradation are normally carried out at different times in a cell. For example, white muscle fibers synthesize glycogen at rest when glucose is plentiful and less ATP is needed for muscle contraction. Glycogen is then used during periods of exertion. Although in such terms glycogen storage is not a very efficient process, it provides cells with a fuel reserve that can be very quickly and efficiently mobilized.

Special Features of Glycogen Degradation and Synthesis

Why Store Glucose As Glycogen?

Since **glycogen** is such a good fuel reserve, it is obvious why we synthesize and store glycogen in liver and muscle. But why not store our excess glucose calories entirely as fat instead of glycogen? The answer is at least threefold: (1) we do store **fat,** but fat cannot be mobilized nearly as rapidly in muscle as glycogen; (2) fat cannot be used as a source of energy in the absence of oxygen; and (3) fat cannot be converted to glucose to maintain blood glucose levels. Why not just pump glucose into cells and store it as free glucose until needed? Why waste so much ATP making a polymer out of glucose? The problem is that glucose is osmotically active. It would cost ATP to "pump" glucose into a cell against a concentration gradient, and glucose would have to reach concentrations of 400 mM in liver cells to match the "glucose reserve" provided by the usual liver glycogen levels. Unless balanced by outward movement of some other osmotically active compound, accumulation of such concentrations of glucose would cause uptake of considerable water and osmotic lysis of the cell. Assuming the molecular mass of a glycogen molecule is of the order of 10^7 Da, 400 mM glucose is in effect stored at an intracellular glycogen concentration of 0.01 μM. Storage of glucose as glycogen therefore creates no osmotic pressure problem for the cell.

Glycogenin Is Required As a Primer for Glycogen Synthesis

Like DNA synthesis, a primer is needed for glycogen synthesis. No template, however, is required. Glycogen itself is the usual **primer,** in that glycogen synthesis can take place by addition of glucosyl units to glycogen "core" molecules, which are almost invariably present in the cell. The outer regions of the glycogen molecule get removed and resynthesized more rapidly than the inner core. Glycogen within a cell is frequently sheared by the combined actions of glycogen phosphorylase and debranching enzyme but is seldom obliterated before glycogen synthase and branching enzyme rebuild the molecule. This begs the question why glycogen is a branched molecule with only one real beginning (the reducing end) and many branches terminating with nonreducing glucosyl units. The answer is that this gives numerous sites of attack for glycogen phosphorylase on a mature glycogen molecule and the same number of sites that function as primers for the addition of glucosyl units by glycogen synthase. If cells synthesized **α-amylose,** that is, an unbranched glucose polymer, there would only be one nonreducing end per molecule. This would surely make glycogen degradation and synthesis much slower. As it is, glycogen phosphorylase and glycogen synthase are found in tight association with glycogen granules in a cell. By taking up residence in the branches of the glycogen tree, both enzymes have ready access to a multitude of nonreducing sugars at the ends of the limbs.

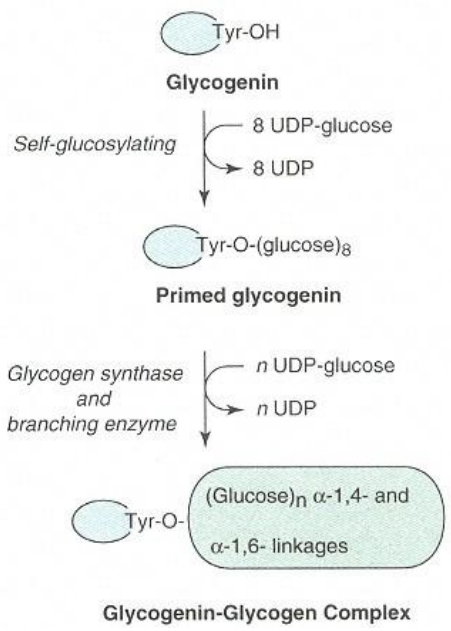


Figure 7.56
Glycogenin provides a primer for glycogen synthesis by glycogen synthase.
Tyr designates a tyrosine residue of glycogenin.

But why is a primer needed for glycogen synthesis? It turns out to be impossible to initiate glycogen synthesis with simply a glucose molecule as the acceptor of an activated glucosyl residue from UDP-glucose. Glycogen synthase has a very low K_m for very large glycogen molecules and therefore readily adds glucosyl residues to make even larger glycogen molecules. However, the K_m gets larger and larger as the glycogen molecule gets smaller and smaller. This phenomenon is so pronounced that glucose, at its physiological concentration, could never function as a primer. This led for some time to the notion that glycogen must be immortal; that is, some glycogen must be handed down from one cell generation to the next in order for glycogen to be synthesized. However, it is now known that a polypeptide of 332 amino acids called **glycogenin** functions as a primer for glycogen synthesis. Glycogenin is a self-glucosylating enzyme that uses UDP-glucose to link glucose to one of its own tyrosine residues (Figure 7.56). Glycosylated glycogenin then serves as a primer for synthesis of glycogen. Alas, glycogen is not immortal.

Glycogen Limits Its Own Synthesis

If glycogen synthase becomes more efficient as the glycogen molecule gets bigger, how is synthesis of this ball of sugar curtailed? Fat cells have an almost unlimited capacity to pack away fat— but then fat cells have nothing else to do. Muscle cells participate in mechanical activity and liver cells carry out many processes other than glycogen synthesis. Even in the face of excess glucose, there has to be a way to limit the intracellular accumulation of glycogen. Glycogen itself inhibits glycogen synthase by a mechanism discussed later (see p. 326).

Glycogen Synthesis and Degradation Are Highly Regulated Pathways

Glycogen synthase and glycogen phosphorylase are regulatory enzymes of glycogen synthesis and degradation, respectively. Both catalyze nonequilibrium reactions, and both are subject to control by allosteric effectors and covalent modification.

Regulation of Glycogen Phosphorylase

Glycogen phosphorylase is subject to allosteric activation by AMP and allosteric inhibition by glucose and ATP (Figure 7.57). Control by these effectors is integrated with a very elaborate control by covalent modification. Phosphorylase exists in an *a* form, which is active, and a *b* form, which is inactive. These forms are interconverted by the actions of **phosphorylase kinase** and **phosphoprotein phosphatase** (Figure 7.57). A conformational change caused by phosphorylation transforms the enzyme into a more active catalytic state. Phosphorylase *b* has some catalytic activity and can be greatly activated by AMP. This allosteric effector has little activating effect, however, on the already active phosphorylase *a*. Hence the covalent modification mechanism can be bypassed by the allosteric mechanism and vice versa.

Phosphorylase kinase is responsible for phosphorylation and activation of phosphorylase (Figure 7.57). Moreover, phosphorylase kinase itself is also sub-

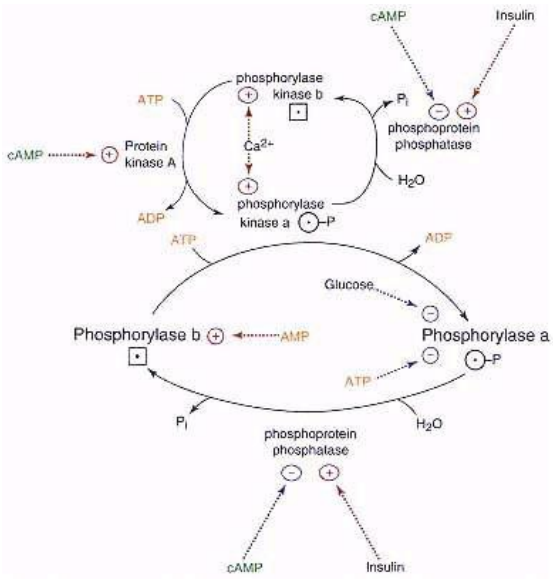


Figure 7.57
Regulation of glycogen phosphorylase by covalent modification.
Phosphorylation converts glycogen phosphorylase and phosphorylase kinase from their inactive *b* forms to their active *a* forms.

ject to regulation by a cyclic phosphorylation–dephosphorylation mechanism. Protein kinase A phosphorylates and activates phosphorylase kinase; phospho-protein phosphatase in turn dephosphorylates and inactivates phosphorylase kinase. Phosphorylase kinase is a large enzyme complex (1.3×10^6 Da), composed of four subunits with four molecules of each subunit in the complex ($\alpha_4\beta_4\gamma_4\delta_4$). Catalytic activity resides with the γ subunit; α, β, and δ subunits exert regulatory control. The α and β subunits are phosphorylated in the transition from the inactive *b* form to the active *a* form of the enzyme. Protein kinase A can only exert an effect on phosphorylase via its ability to phosphorylate and activate phosphorylase kinase. Thus a bicyclic system is required for activation of phosphorylase in response to cAMP-mediated signals.

The δ subunit of phosphorylase kinase also plays a regulatory role. It corresponds to a Ca^{2+}-binding regulatory protein, called **calmodulin.** Not unique to phosphorylase kinase, calmodulin is found in cells as the free molecule and also bound to other enzyme complexes. It functions as a **Ca^{2+}** receptor in the cell, responding to changes in intracellular Ca^{2+} concentration and affecting the relative activities of a number of enzyme systems. Binding of Ca^{2+} to the calmodulin subunit of phosphorylase kinase changes the conformation of the complex, making the enzyme more active with respect to the phosphorylation of phosphorylase. As shown in Figure 7.57, Ca^{2+} is an activator of both phosphorylase kinase *a* and phosphorylase kinase *b*. Maximum activation of phosphorylase kinase requires both phosphorylation of specific serine residues of the enzyme and interaction of Ca^{2+} with the calmodulin subunit of the enzyme. This is one mechanism by which Ca^{2+} functions as an important "second messenger" of hormone action, as will be discussed below.

Activation of phosphorylase kinase by phosphorylation and Ca^{2+} will have a substantial effect on the activity of glycogen phosphorylase. It is equally obvious, however, that turning off the phosphoprotein phosphatase that modulates the phosphorylation states of both phosphorylase kinase and glycogen phosphorylase (Figure 7.57) could achieve the same effect. Ultimate control of glycogen phosphorylase would involve the reciprocal regulation of phosphoprotein phosphatase and phosphorylase kinase activities. Although numerous details remain to be understood, there is evidence that activities of phosphoprotein phosphatase and phosphorylase kinase are controlled in a reciprocal manner. Regulation of phosphoprotein phosphatase activity is linked to cAMP (see p. 325). The important point in Figure 7.57 is that hormones that increase cAMP levels, such as **glucagon** and **epinephrine,** promote activation of glycogen phosphorylase by signaling activation of phosphorylase kinase and inactivation of phosphoprotein phosphatase. On the other hand, **insulin,** which acts either though a second messenger or a kinase-mediated signal cascade (see p. 879), exerts the opposite effect on phosphorylase by promoting activation of phosphoprotein phosphatase activity.

The Cascade that Regulates Glycogen Phosphorylase Amplifies a Small Signal into a Very Large Effect

There is a good reason for the existence of the bicyclic control system for phosphorylation of glycogen phosphorylase. It provides a tremendous amplification mechanism of a very small initial signal. Activation of **adenylate cyclase** by one molecule of epinephrine causes formation of many molecules of cAMP. Each cAMP molecule activates a protein kinase A molecule, which in turn activates many molecules of phosphorylase kinase as well as many molecules of phosphoprotein phosphatase. In turn, phosphorylase kinase phosphorylates many molecules of glycogen phosphorylase, which in turn catalyze phosphorolysis of many glycosidic bonds of glycogen. A very elaborate amplification system is therefore provided in which the signal provided by just a few molecules of hormone is amplified into production of an enormous number of glucose 1-phosphate molecules. If each step represents, for argument's sake, an amplification factor of 100, then a total of four steps would result in an amplification

of 100 million! This system is so rapid, in large part because of this **amplification mechanism,** that all of the stored glycogen of white muscle fibers could be completely mobilized within just a few seconds.

Regulation of Glycogen Synthase

Glycogen synthase has to be active for glycogen synthesis and inactive for glycogen degradation. The combination of the reactions catalyzed by glycogen synthase, glycogen phosphorylase, glucose 1-phosphate uridylyltransferase, and nucleoside diphosphate kinase adds up to a futile cycle with the overall equation ATP ADP + P_i. Hence glycogen synthase needs to be turned off when glycogen phosphorylase is turned on, and vice versa.

Activation of **glycogen synthase** by glucose 6-phosphate, an allosteric effector, is probably of physiological significance under some circumstances (Figure 7.58). However, as with glycogen phosphorylase, this mode of control is integrated with regulation by covalent modification (Figure 7.58). Glycogen synthase exists in two forms. One is designated the D form because it is dependent on the presence of G6P for activity. The other is designated the I form because its activity is independent of the presence of G6P. The D form corresponds to the *b* or inactive form of the enzyme, the I form to the *a* or active form of the enzyme. Phosphorylation of glycogen synthase is catalyzed by several different kinases, which in turn are regulated by second messengers of

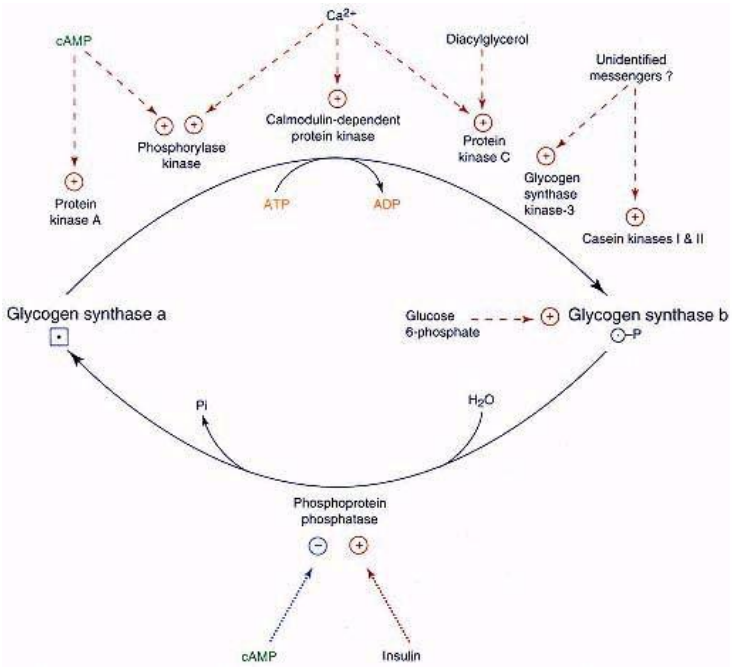


Figure 7.58
Regulation of glycogen synthase by covalent modification.
Phosphorylation converts glycogen synthase from its active *a* form to its inactive *b* form.

hormone action, including cAMP, Ca^{2+}, **diacylglycerol,** and probably yet to be identified compounds. Each of the protein kinases shown in Figure 7.58 is capable of catalyzing the phosphorylation and contributing to inactivation of glycogen synthase. Although glycogen synthase is a simple tetramer (α_4) of only one subunit type (mol wt 85,000 Da), it can be phosphorylated on at least nine different serine residues. Eleven different protein kinases have been identified that can phosphorylate glycogen synthase. This stands in striking contrast to glycogen phosphorylase, which is regulated by phosphorylation of one site by one specific kinase.

Cyclic AMP is an extremely important intracellular signal for reciprocally controlling glycogen synthase (Figure 7.58) and glycogen phosphorylase (Figure 7.57). An increase in cAMP signals activation of glycogen phosphorylase and inactivation of glycogen synthase via activation of protein kinase A and inhibition of phosphoprotein phosphatase. Ca^{2+} likewise can influence the phosphorylation state of both enzymes and reciprocally regulate their activity via its effects on phosphorylase kinase. Two cAMP-independent, Ca^{2+}-activated protein kinases have been identified that also may have physiological significance. One of these is a calmodulin-dependent protein kinase and the other a Ca^{2+}-and phospholipid-dependent protein kinase (protein kinase C). Both enzymes phosphorylate glycogen synthase, but neither can phosphorylate glycogen phosphorylase. Protein kinase C requires phospholipid, diacylglycerol, and Ca^{2+} for full activity. There is considerable interest in protein kinase C because **tumor-promoting agents** called **phorbol esters** have been found to mimic diacylglycerol as activators of this enzyme. Diacylglycerol is considered an important "second messenger" of hormone action, acting via protein kinase C to regulate numerous cellular processes (see p. 865).

Glycogen synthase is also phosphorylated by glycogen synthase kinase-3, casein kinase I, and casein kinase II. These kinases are not subject to regulation by cAMP or Ca^{2+}. It is likely, however, that special regulatory mechanisms exist to regulate these kinases. Herein may lie solutions to unsolved problems such as the mechanism of action of insulin and other hormones.

The phosphoprotein phosphatase that converts glycogen synthase *b* back to glycogen synthase *a* (Figure 7.58) is regulated in a manner analogous to that described in the discussion of glycogen phosphorylase regulation (Figure 7.57). Cyclic AMP promotes inactivation whereas insulin promotes activation of glycogen synthase through opposite effects on phosphoprotein phosphatase activity.

Regulation of Phosphoprotein Phosphatases

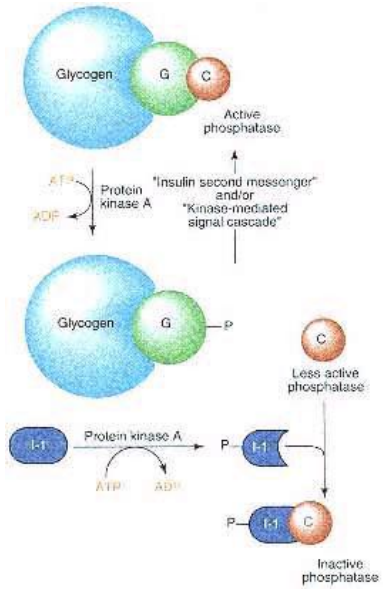


Figure 7.59
Mechanism for regulation of a phosphatase that binds to glycogen.
The glycogen-binding subunit G binds directly to glycogen; the phosphoprotein phosphatase catalytic subunit C binds to glycogen via the G subunit; and the phosphorylated inhibitor 1 (I-1) binds the free catalytic subunit.

About a dozen different **phosphoprotein phosphatases** with specificity for removal of phosphate from serine residues of proteins are currently being studied. In general, phosphoprotein phosphatases occur as catalytic subunits associated with a number of different regulatory subunits that control the activity of the catalytic subunit, determine which substrate(s) the catalytic subunit can interact with and dephosphorylate, and target the association of a catalytic subunit with a specific structure or component within a cell. One such regulatory protein important for glycogen metabolism has been given the name **G subunit,** denoting a glycogen-binding protein. G subunit binds both glycogen and a phosphatase catalytic subunit (Figure 7.59). This association makes the phosphatase ten times more active toward glycogen synthase and glycogen phosphorylase and thereby greatly promotes their dephosphorylation. However, phosphorylation of the G subunit by protein kinase A results in release of the phosphatase catalytic subunit, which is then less active. Interaction of the free catalytic subunit with yet another regulatory protein (called **inhibitor 1**) then causes further inhibition of phosphatase activity. Effective inhibition of the residual phosphatase activity of the catalytic subunit requires phosphorylation of inhibitor 1 by protein kinase A, thereby creating yet another link to hormones that increase cAMP levels. **Insulin** has effects opposite to those of cAMP;

that is, insulin promotes activation of the catalytic subunit of phosphoprotein phosphatase. This presumably involves reversal of the steps promoted by cAMP, but details of how this is accomplished remain to be established.

Effector Control of Glycogen Metabolism

Certain muscles are known to mobilize their glycogen stores rapidly in response to anaerobic conditions without marked conversion of phosphorylase *b* to phosphorylase *a* or glycogen synthase *a* to glycogen synthase *b*. Presumably this is accomplished by effector control in which ATP levels decrease, causing less inhibition of phosphorylase; glucose 6-phosphate levels decrease, causing less activation of glycogen synthase; and AMP levels increase, causing activation of phosphorylase. This enables muscle to keep working, for at least a short period of time, by using ATP produced by glycolysis of glucose 6-phosphate obtained from glycogen.

Proof that effector control can operate has also been obtained in studies of a special strain of mice that are deficient in muscle phosphorylase kinase. Phosphorylase *b* in muscle of such mice cannot be converted into phosphorylase *a*. Nevertheless, heavy exercise of these mice results in depletion of muscle glycogen, presumably because of stimulation of phosphorylase *b* by effectors.

Negative Feedback Control of Glycogen Synthesis by Glycogen

Glycogen exerts feedback control over its own formation. The portion of glycogen synthase in the active *a* form decreases as glycogen accumulates in a particular tissue. The mechanism is not well understood, but glycogen may make the *a* form a better substrate for one of the protein kinases, or, alternatively, glycogen may inhibit dephosphorylation of glycogen synthase *b* by phosphoprotein phosphatase. Either mechanism would account for the shift in the steady state in favor of glycogen synthase *b* that occurs in response to glycogen accumulation.

Phosphorylase a Functions As a "Glucose Receptor" in the Liver

Consumption of a carbohydrate-containing meal results in an increase in blood and liver glucose, which signals an increase in glycogen synthesis in the latter tissue. The mechanism involves glucose stimulation of insulin release from the pancreas and its effects on hepatic glycogen phosphorylase and glycogen synthase. However, hormone-independent mechanisms also appear to be important in liver (Figure 7.60). Direct inhibition of phosphorylase *a* by glucose is probably of importance. Binding of glucose to phosphorylase makes the *a* form of phosphorylase a better substrate for dephosphorylation by phosphoprotein phosphatase. Therefore phosphorylase *a* functions as a glucose receptor in liver. Binding of glucose to phosphorylase *a* promotes inactivation of phosphorylase *a*, with the overall result being inhibition of glycogen degradation by glucose. This "negative feedback" control of glycogenolysis by glucose would not necessarily promote glycogen synthesis. However, there also is evidence that phosphorylase *a* is an inhibitor of the dephosphorylation of glycogen synthase *b* by phosphoprotein phosphatase. This inhibition is lost once phosphorylase *a* has been converted to phosphorylase *b* (Figure 7.60). In other words, phosphoprotein phosphatase can turn its attention to glycogen synthase *b* only following dephosphorylation of phosphorylase *a*. Thus, as a result of interaction of glucose with phosphorylase *a*, phosphorylase becomes inactivated, glycogen synthase becomes activated, and glycogen is synthesized rather than degraded in liver. Phosphorylase *a* can serve this function of "glucose receptor" in liver because the concentration of glucose in liver always reflects the blood concentration of glucose. This is not true for extrahepatic tissues. Liver cells have a very high-capacity transport system for glucose and a high K_m enzyme for glucose phosphorylation (glucokinase). Cells of extrahepatic

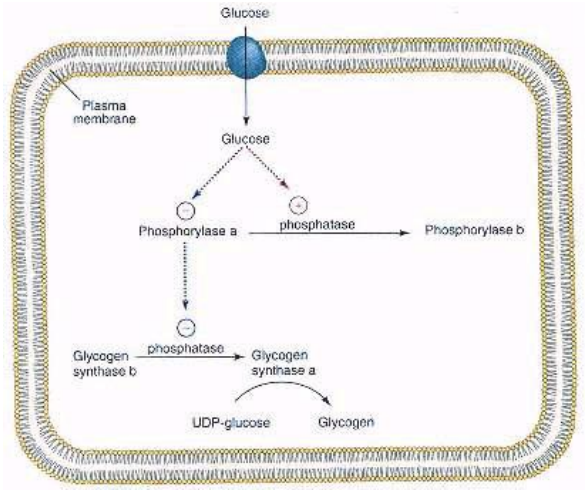


Figure 7.60
Overview of the mechanism responsible for glucose stimulation of glycogen synthesis in the liver.

tissues as a general rule have glucose transport and phosphorylation systems that maintain intracellular glucose at concentrations too low for phosphorylase *a* to function as a "glucose receptor."

Glucagon Stimulates Glycogen Degradation in the Liver

Glucagon is released from α cells of pancreas in response to low blood glucose levels. One of glucagon's primary jobs during periods of low food intake (fasting or starvation) is to mobilize **liver glycogen,** that is, stimulate glycogenolysis, in order to ensure that adequate blood glucose is available to meet the needs of glucose-dependent tissues. Glucagon circulates in blood until it interacts with glucagon receptors such as those located on the plasma membrane of liver cells (see Figure 7.61). Binding of glucagon to these receptors activates adenylate cyclase and triggers the cascades that result in activation of glycogen phosphorylase and inactivation of glycogen synthase by the mechanisms given in Figures 7.57 and 7.58, respectively. Glucagon also inhibits glycolysis at the level of 6-phosphofructo-1-kinase and pyruvate kinase by the mechanisms given in Figures 7.25 and 7.30, respectively. The net result of these effects of glucagon, all mediated by the second messenger cAMP and covalent modification, is a very rapid increase in blood glucose levels. Hyperglycemia might be expected but does not occur because less glucagon is released from the pancreas as blood glucose levels increase.

Epinephrine Stimulates Glycogen Degradation in the Liver

Epinephrine is released into blood from chromaffin cells of the adrenal medulla in response to stress. This hormone is our "fright, flight, or fight" hormone, preparing the body for either combat or escape.

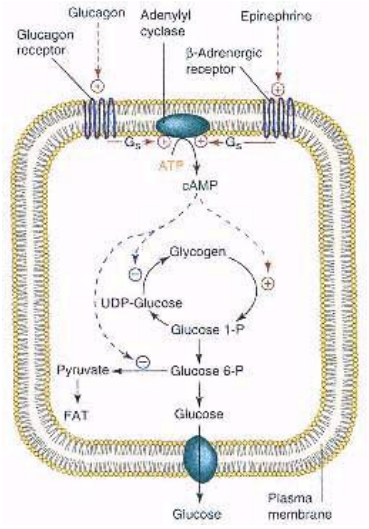


Figure 7.61
Cyclic AMP mediates the stimulation of glycogenolysis in liver by glucagon and β agonists (epinephrine).
See legends for Figures 7.19 and 7.25.

Epinephrine interacts directly with receptors in the plasma membrane of liver cells to activate **adenylate cyclase** (Figure 7.61). The resulting increase in cAMP has the same effect as that caused by glucagon, that is, activation of glycogenolysis and inhibition of glycogenesis and glycolysis to maximize the release of glucose from liver. The plasma membrane receptor for epinephrine,

which is in communication with adenylate cyclase, is the β-adrenergic receptor. The plasma membrane of liver cells also has another binding protein for epinephrine, called the **α-adrenergic receptor**. Interaction of epinephrine with α-adrenergic receptors leads to formation of **inositol 1,4,5-trisphosphate** (IP_3) and **diacylglycerol** (Figure 7.62). These compounds are second messengers, produced in the plasma membrane by the action of a phospholipase C on phosphatidylinositol 4,5-bisphosphate (Figure 7.63). Inositol 1,4,5-trisphosphate stimulates the release of Ca^{2+} from the endoplasmic reticulum (Figure 7.62). As previously discussed (Figure 7.57), the increase in Ca^{2+} activates phosphorylase kinase, which in turn activates glycogen phosphorylase. Likewise (Figure 7.58), Ca^{2+}-mediated activation of phosphorylase kinase, calmodulin-dependent protein kinase, and protein kinase C, as well as diacylglycerol-mediated activation of protein kinase C, may all be important for inactivation of glycogen synthase.

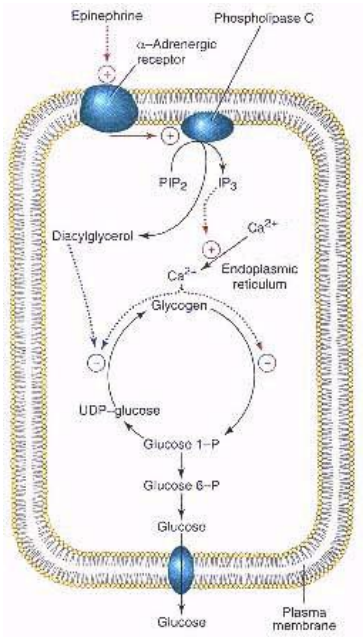


Figure 7.62
Inositol trisphosphate (IP_3) and Ca^{2+} mediate the stimulation of glycogenolysis in liver by α agonists.
The -adrenergic receptor and glucose transporter are intrinsic components of the plasma membrane. Although not indicated, phosphatidylinositol 4,5-bisphosphate (PIP_2) is also a component of the plasma membrane.

The consequences of epinephrine action is an increased release of glucose into the blood from the glycogen stored in liver. This makes more blood glucose available to tissues that are called upon to meet the challenge of the stressful situation that triggered the release of epinephrine from adrenal medulla.

Epinephrine Stimulates Glycogen Degradation in Heart and Skeletal Muscle

Epinephrine also stimulates glycogen degradation in heart and skeletal muscle. Cyclic AMP, produced in response to epinephrine stimulation of adenylate cyclase via **β-adrenergic** receptors (Figure 7.64), signals concurrent activation of glycogen phosphorylase and inactivation of glycogen synthase by mechanisms given previously in Figures 7.57 and 7.58, respectively. This does not lead, however, to glucose release into blood from these tissues. In contrast to liver, heart and skeletal muscle lack glucose 6-phosphatase, and in these tissues cAMP does not inhibit but rather stimulates glycolysis (see Figure 7.28). Thus the role of epinephrine on glycogen metabolism in heart and skeletal muscle is to make more glucose 6-phosphate available for glycolysis. ATP generated by glycolysis can then be used to meet the metabolic demand imposed on these muscles by the stress that triggered epinephrine release.

Neural Control of Glycogen Degradation in Skeletal Muscle

Nervous excitation of muscle activity is mediated via changes in intracellular Ca^{2+} concentrations (Figure 7.65). A **nerve impulse** causes **membrane depo-**

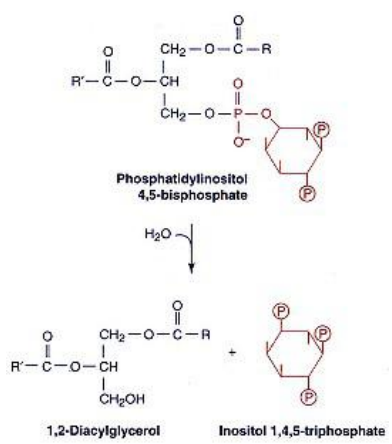


Figure 7.63
Phospholipase C cleaves phosphatidylinositol 4,5-bisphosphate to produce 1,2-diacylglycerol and inositol 1,4,5-trisphosphate.

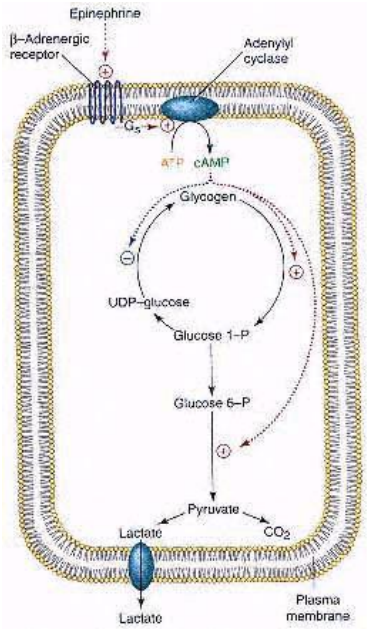


Figure 7.64
Cyclic AMP mediates the stimulation of glycogenolysis in muscle by β agonists (epinephrine).
The -adrenergic receptor is an intrinsic component of the plasma membrane that acts to stimulate adenylate cyclase via a stimulatory G-protein (G_s).

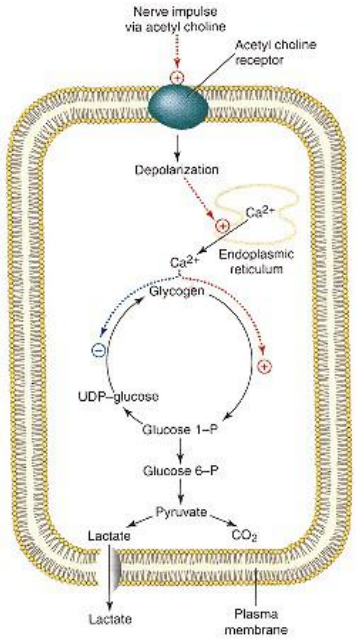


Figure 7.65
Ca^{2+} mediates the stimulation of glycogenolysis in muscle by nervous excitation.

larization, which in turn causes Ca^{2+} release from the **sarcoplasmic reticulum** into the sarcoplasm of muscle cells. This release of Ca^{2+} triggers muscle contraction, whereas reaccumulation of Ca^{2+} by the sarcoplasmic reticulum causes relaxation. The same change in Ca^{2+} concentration effective in causing muscle contraction (from 10^{-8} to 10^{-6} M) also greatly affects the activity of phosphorylase kinase. As Ca^{2+} concentrations increase there is more muscle activity and a greater need for ATP. Activation of phosphorylase kinase by Ca^{2+} leads to the subsequent activation of glycogen phosphorylase and perhaps the inactivation of glycogen synthase. The result is that more glycogen is converted to glucose 6-phosphate so that more ATP can be produced to meet the greater energy demand of muscle contraction.

Insulin Stimulates Glycogen Synthesis in Muscle and Liver

An increase in blood glucose signals release of insulin from β cells of the pancreas. Insulin circulates in blood, serving as a first messenger to inform several tissues that excess glucose is present. Insulin receptors, located on the plasma membranes of insulin-responsive cells, respond to insulin binding by either producing a second messenger of insulin action or inducing a protein kinase cascade that promotes glucose use within these tissues (Figures 7.66 and 7.67). The pancreas responds to a decrease in blood glucose with less release of insulin but greater release of glucagon. These hormones have opposite effects on glucose utilization by liver, thereby establishing the pancreas as a fine-tuning device that prevents dangerous fluctuations in blood glucose levels.

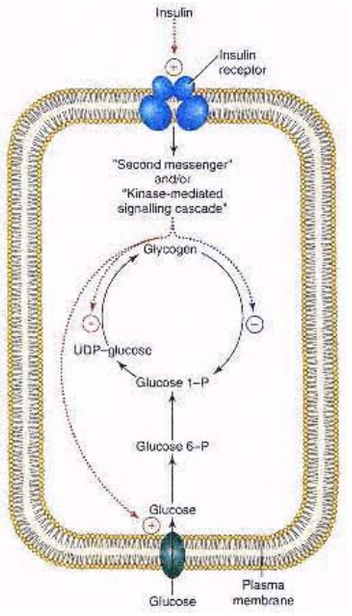


Figure 7.66
Insulin acts via a plasma membrane receptor to promote glycogen synthesis in muscle.

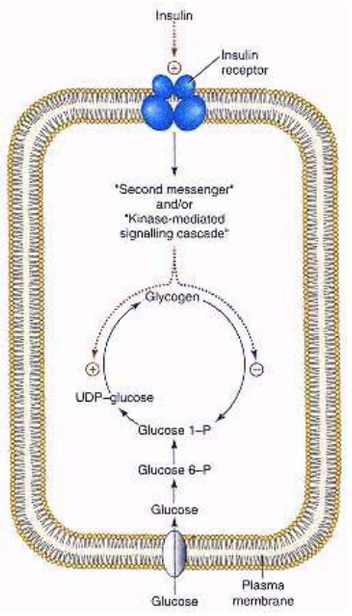


Figure 7.67
Insulin acts via a plasma membrane receptor to promote glycogen synthesis in liver.

Insulin increases glucose utilization in part by promoting glycogenesis and inhibiting glycogenolysis in muscle (Figure 7.66) and liver (Figure 7.67). Insulin stimulation of glucose transport at the plasma membrane is essential for these effects in muscle but not liver. Hepatocytes have a high-capacity, **insulin-insensitive glucose transport system** (GLUT-2), whereas muscle cells and adipocytes are equipped with a **glucose transport system** (GLUT-4) that requires insulin for maximum rates of glucose uptake. Insulin stimulates muscle and adipose tissue glucose transport by signaling an increase in the number of functional GLUT-4 proteins associated with the plasma membrane. This is accomplished by promoting translocation of GLUT-4 from an intracellular pool to the plasma membrane (see Chapter 20). Insulin further promotes glycogen accumulation in both tissues by activating glycogen synthase and inhibiting glycogen phosphorylase as discussed previously (Figures 7.57, 7.58, and 7.59).

Bibliography

Arion, W. J., Lange, A. J., Walls, H. E., and Ballas, L. M. Evidence for the participation of independent translocases for phosphate and glucose 6-phosphate in the microsomal glucose 6-phosphatase system. *J. Biol. Chem.* 255:10396, 1980.

Berridge, M. J. Review article: inositol trisphosphate and diacylglycerol as second messengers. *Biochem. J.* 220:345, 1984.

Brooke, M. H., and Kaiser, K. K. The use and abuse of muscle histochemistry. *Ann. N.Y. Acad. Sci.* 228:121, 1974.

Cheatham, B., and Kahn, C. R. Insulin action and the insulin signaling network. *Endocr. Rev.* 16:117, 1995.

Cohen, P. Dissection of the protein phosphorylation cascades involved in insulin and growth factor action. *Biochem. Soc. Trans.* 21:555, 1993.

DeFronzo, R. A., and Ferrannini, E. Regulation of hepatic glucose metabolism in humans. *Diabetes Metab. Reviews* 3:415, 1987.

DePaoli-Roach, A. A., Park, I.-K., Cerovsky, V., Csortos, C., Durbin, S. D., Kuntz, M. J., Sitikov, A., Tang, P. M., Verin, A., and Zolnierowicz, S.

Serine/threonine protein phosphatases in the control of cell function. *Adv. Enzyme Regul.* 34:199, 1994.

Exton, J. H. Mechanisms of hormonal regulation of hepatic glucose metabolism. *Diabetes Metab. Rev.* 3:163, 1987.

Garcia, C. K., Goldstein, J. L., Pathak, R. K., Anderson, R. G. W., and Brown, M. S. Molecular characterization of a membrane transporter for lactate, pyruvate, and other monocarboxylates: implications for the Cori cycle. *Cell* 76:865, 1994.

Geelen, M. J. H., Harris, R. A., Beynen, A. C., and McCune, S. A. Short-term hormonal control of hepatic lipogenesis. *Diabetes* 29:1006, 1980.

Gitzelmann, R., Steinmann, B., and Van den Berghe, G. Disorders of fructose metabolism. In: C. R. Scriver, A. L. Beaudet, W. S. Sly, and D. Valle (Eds.), *The Metabolic and Molecular Bases of Inherited Disease,* 7th ed. New York: McGraw-Hill, 1995, pp. 905–934.

Gould, G. W., and Holman, G. D. The glucose transporter family: structure, function and tissue-specific expression. *Biochem. J.* 295:329, 1993.

Greene, H. L., Slonin, A. E., and Burr, I. M. Type I glycogen storage disease: a metabolic basis for advances in treatment. In: L. A. Barness (Ed.), *Advances in Pediatrics,* Vol. 26. Chicago: Year Book Publishers, 1979, p. 63.

Gurney, A. L., Park, E. A., Liu, J., Giralt, M., McGrane, M. M., Patel, Y. M., Crawford, D. R., Nizielski, S. E., Savon, S., and Hanson, R. W. Metabolic regulation of gene transcription. *J. Nutr.* 124:1533S, 1994.

Hallfrisch, J. Metabolic effects of dietary fructose. *FASEB J.* 4:2652, 1990.

Hanson, R. W., and Mehlman, M. A. (Eds.). *Gluconeogenesis, Its Regulation in Mammalian Species*. New York: Wiley, 1976.

Hunter, T. Protein kinases and phosphatases: the yin and yang of protein phosphorylation and signaling. *Cell* 80:225, 1995.

Ingebritsen, T. S., and Cohen, P. Protein phosphatases: properties and role in cellular regulation. *Science* 221:331, 1983.

Isselbacher, K.J., Adams, R. D., Braundwald, E., Petersdorf, R. B., and Wilson, J. D. (Eds.). *Harrison's principles of internal medicine,* 9th ed. New York: McGraw-Hill, 1980.

Lalli, E., and Sassone-Corsi, P. Signal transduction and gene regulation: the nuclear response to cAMP. *J. Biol. Chem.* 269:17359, 1994.

Lieber, C. S. The metabolism of alcohol. *Sci. Am.* 234:25, 1976.

Lomako, J., Lomako, W. M., and Whelan, W. J. A self-glucosylating protein is the primer for rabbit muscle glycogen biosynthesis. *FASEB J.* 2:3097, 1988.

Metzler, D. E. *Biochemistry, The Chemical Reactions of Living Cells*. New York: Academic Press, 1977.

Newsholme, E. A., and Leech, A. R. *Biochemistry for the Medical Sciences*. New York: Wiley, 1983.

Newsholme, E. A., and Start, C. *Regulation in Metabolism*. New York: Wiley, 1973.

Pilkis, S. J., Claus, T. H., Kurland, I. J., and Lange, A. J. 6-Phosphofructo-2-kinase/fructose-2,6-bisphosphatase: a metabolic signaling enzyme. *Annu. Rev. Biochem.* 64:799, 1995.

Pilkis, S. J., and El-Maghrabi, M. R. Hormonal regulation of hepatic gluconeogenesis and glycolysis. *Annu. Rev. Biochem.* 57:755, 1988.

Roach, P. J. Hormonal control of glycogen metabolism. In: H. Rupp (Ed.), *Regulation of Heart Function: Basic Concepts and Clinical Applications*. New York: Thieme-Stratton, 1985.

Roach, P. J. Principles of the regulation of enzyme activity. In: L. Goldstein and D. M. Prescott (Eds.), *Cell Biology, A Comprehensive Treatise,* Vol. IV. New York: Academic Press, 1980.

Scriver, C. R., Beaudet, A. L., Sly, W. S., and Valle, D. (Eds.). *The Metabolic and Molecular Bases of Inherited Disease,* 7th ed. New York: McGraw-Hill, 1995.

Stanley, C. A., Anday, E. K., Baker, L., and Delivoria-Papadopolous, M. Metabolic fuel and hormone responses to fasting in newborn infants. *Pediatrics* 64:613, 1979.

Taylor, S. I. Diabetes mellitus. In: C. R. Scriver, A. L. Beaudet, W. S. Sly, and D. Valle (Eds.), *The Metabolic and Molecular Bases of Inherited Disease,* 7th ed. New York: McGraw-Hill, 1995, pp. 843–896.

van de Werve, G., and Jeanrenaud, B. Liver glycogen metabolism: an overview. *Diabetes Metab. Rev.* 3:47, 1987.

van Schaftingen, E., Vandercammen, A., Detheux, M., and Davies, D. R. The regulatory protein of liver glucokinase. *Adv. Enzyme Regul.* 32:133, 1992.

Vaulont, S., and Kahn, A. Transcriptional control of metabolic regulation genes by carbohydrates. *FASEB J.* 8:28, 1994.

Viskupic, E., Cao, Y., Zhang, W., Cheng, C., DePaoli-Roach, A. A., and Roach, P. J. Rabbit skeletal muscle glycogenin. *J. Biol. Chem.* 267:25759, 1992.

Questions

J. Baggott and C. N. Angstadt

1. In glycolysis ATP synthesis is catalyzed by:

   A. hexokinase.

   B. 6-phosphofructo-1-kinase.

   C. glyceraldehyde-3-phosphate dehydrogenase.

   D. phosphoglycerate kinase.

   E. none of the above.

2. The irreversible reactions of glycolysis include that catalyzed by:

   A. phosphoglucose isomerase.

   B. 6-phosphofructo-1-kinase.

   C. fructose-bisphosphate aldolase.

   D. glyceraldehyde-3-phosphate dehydrogenase.

   E. phosphoglycerate kinase.

3. NAD^+ can be regenerated in the cytoplasm if NADH reacts with any of the following EXCEPT:

   A. pyruvate.

   B. dihydroxyacetone phosphate.

   C. oxaloacetate.

   D. the flavin bound to NADH dehydrogenase.

4. Glucokinase:

   A. has a K_m considerably greater than the normal blood glucose concentration.

   B. is found in muscle.

   C. is inhibited by glucose 6-phosphate.

   D. is also known as the GLUT-2 protein.

   E. has glucose 6-phosphatase activity as well as kinase activity.

5. The primary short-term regulation of glucokinase activity in the liver is effected by:

   A. substrate concentration.

   B. fructose 1-phosphate concentration.

   C. induction of glucokinase synthesis by high intracellular glucose concentrations.

   D. insulin-induced increase in transcription of the glucokinase gene.

   E. allosteric activation by ADP.

6. 6-Phosphofructo-1-kinase activity can be decreased by all of the following EXCEPT:

A. ATP at high concentrations.

B. citrate.

C. AMP.

D. low pH.

E. decreased concentration of fructose 2,6-bisphosphate.

7. Which of the following supports gluconeogenesis?

A. α-ketoglutarate + aspartate = glutamate + oxaloacetate

B. pyruvate + ATP + HCO_3 $\rightleftharpoons$ oxaloacetate + ADP + P_i + H^+

C. acetyl CoA + oxaloacetate + H_2O $\rightleftharpoons$ citrate + CoA

D. leucine degradation

E. lysine degradation

8. In the Cori cycle:

A. only tissues with aerobic metabolism (i.e., mitochondria and O_2) are involved.

B. a three-carbon compound arising from glycolysis is converted to glucose at the expense of energy from fatty acid oxidation.

C. glucose is converted to pyruvate in anaerobic tissues, and this pyruvate returns to the liver, where it is converted to glucose.

D. the same amount of ATP is used in the liver to synthesize glucose as is released during glycolysis, leading to no net effect on whole-body energy balance.

E. nitrogen from alanine must be converted to urea, increasing the amount of energy required to drive the process.

9. The uncontrolled production of NADH from NAD^+ during ethanol metabolism blocks gluconeogenesis from all of the following EXCEPT:

A. galactose.

B. glycerol.

C. α-ketoglutarate.

D. oxaloacetate.

E. pyruvate.

10. Gluconeogenic enzymes include all of the following EXCEPT:

A. fructose 1,6-bisphosphatase.

B. glucose 6-phosphatase.

C. phosphoenolpyruvate carboxykinase.

D. phosphoglucomutase.

E. pyruvate carboxylase.

11. When blood glucagon rises, which of the following hepatic enzyme activities FALLS?

A. adenylate cyclase

B. protein kinase

C. 6-phosphofructo-2-kinase

D. fructose 1,6-bisphosphatase

E. hexokinase

12. Phospho–dephospho regulation of 6-phosphofructo-1-kinase, 6-phosphofructo-2-kinase, and pyruvate kinase is an important regulatory mechanism in:

A. brain.

B. erythrocytes.

C. intestine.

D. liver.

E. skeletal muscle.

Refer to the following for Questions 13–15.

A. glycogen phosphorylase

B. phosphoglucomutase

C. phosphoglucokinase

D. glucose 6-phosphatase

E. debranching enzyme

13. A bifunctional enzyme.

14. Lacking in muscle, but present in normal liver.

15. Catalyzes phosphorolysis of glycogen.

16. Phosphorylation activates all of the following EXCEPT:

A. glycogen phosphorylase.

B. inhibitor 1.

C. phosphorylase kinase.

D. protein kinase.

17. cAMP activates:

A. glycogen phosphorylase.

B. hexokinase.

C. 6-phosphofructo-1-kinase.

D. protein kinase.

E. protein kinase C.

Answers

1. D Phosphoglycerate kinase synthesizes ATP in the forward direction (p. 276). A and B use ATP. C synthesizes 1,3-bisphosphoglycerate.

2. B E: The phosphoglycerate kinase reaction is reversible because the product contains a high energy carboxylic acid–phosphoric acid anhydride link. Many other kinases, including 6-phosphofructo-1-kinase, form phosphate esters, which have low-energy bonds.

3. D The flavin is mitochondrial (p. 279). A may be converted to lactate. B and C are the cytoplasmic acceptors for shuttle systems.

4. A Blood glucose is ~5 mM. K_m of glucokinase is ~10 mM. B: Glucokinase is hepatic, and, unlike the muscle hexokinase, it is not inhibited by glucose 6-phosphate (p. 285).

5. A B, C, and D affect glucokinase activity, but they are not the primary short-term regulators (pp. 285–286). E: ADP is not a known effector of glucokinase.

6. C AMP is an allosteric regulator that relieves inhibition by ATP (p. 287). B and D are probably important physiological regulators in muscle, and E is critical in liver.

7. B This reaction is on the direct route of conversion of pyruvate to glucose. A :α-Ketoglutarate and oxaloacetate both give rise to glucose; interconversion of one to the other accomplishes nothing (p. 304). C: Citrate ultimately gives rise to oxaloacetate, losing two carbon atoms in the process; again nothing is gained (p. 306). D and E involve the two amino acids that are strictly ketogenic.

8. B The liver derives the energy required for gluconeogenesis from aerobic oxidation of fatty acids. A: The liver is an essential organ in the Cori cycle; it is aerobic. C: In anaerobic tissues the end product of glycolysis is lactate; in aerobic tissues it is pyruvate, but there the pyruvate would likely be oxidized aerobically. D: Gluconeogenesis requires six ATP molecules per glucose synthesized; glycolysis yields two ATP molecules per glucose metabolized. E: Alanine is not part of the Cori cycle. See Figure 7.32, p. 300.

9. A Gluconeogenesis from galactose is not affected by the redox state of the cell (p. 309, Figure 7.44). B is converted to α-glycerophosphate, which cannot be oxidized to dihydroxyacetone phosphate when NADH/NAD^+ is high. For the same reason, C and D will be trapped as malate, since the interconversion of malate and oxaloacetate strongly favors malate even under normal conditions. E will be converted to lactate. Clearly, very high NADH blocks gluconeogenesis at many points (p. 312). If an inebriated person has depleted glycogen stores and has not been ingesting carbohydrates, sources of blood glucose are seriously compromised, and hypoglycemia could become life-threatening (Clin. Corr 7.10). The oft-heard statement that alcohol is metabolized as a carbohydrate is false.

10. D Phosphoglucomutase is on the pathway of glycogen metabolism (p. 314). A–C and E are the so-called gluconeogenic enzymes; they get around the irreversible steps of glycolysis (p. 303).

11. C As blood glucagon rises, A is activated, producing cAMP; cAMP activates B, and B inactivates C. Low levels of fructose 2,6-bisphosphate increase the activity of D (p. 310). E is not an important hepatic enzyme; its role is filled in liver by glucokinase.

12. D Regulation of these enzymes by hormonally controlled phosphorylation and dephosphorylation is of central importance in liver. 6-Phosphofructo-2-kinase is present in other tissues but does not appear to change its activity in response to hormones except in liver. Other enzymes in extrahepatic tissues, such as those of glycogen metabolism in muscle, are under phospho–dephospho regulation.

13. E Debranching enzyme has 4-α-D-glucanotransferase activity, which moves a strand of three glucosyl units from a branch to an end of a glucosyl chain. It also has amylo-α-[1,6]-glucosidase activity, which hydrolyzes the last glucosyl residue from the branch, yielding free glucose (p. 318).

14. D This is why muscle glycogen cannot contribute directly to blood glucose (p. 316).

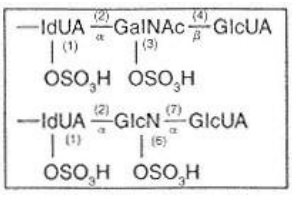
15. A Note that it is phosphorolysis, not hydrolysis; the product is glucose 1-phosphate, not glucose (p. 314).

16. D A, B, and C are activated by phosphorylation (p. 322, Figure 7.57). Protein kinase (sometimes referred to as protein kinase A) is not a phospho–dephospho enzyme (p. 323).

17. D A (p. 322) and C (p. 287) are allosterically activated by AMP. B is controlled by glucose 6-phosphate. E is activated by Ca^{2+}, phospholipid, and diacylglycerol (p. 325).

Chapter 8— Carbohydrate Metabolism II: Special Pathways

Nancy B. Schwartz



8.1— Overview

In addition to catabolism of glucose for the specific purpose of energy production in the form of ATP, several other pathways involving sugar metabolism exist in cells. One, the **pentose phosphate pathway**, known also as the **hexose monophosphate shunt** or the **6-phosphogluconate pathway**, is particularly important in animal cells. It functions side by side with glycolysis and the tricarboxylic acid cycle for production of reducing power in the form of NADPH and pentose intermediates. It has previously been mentioned that NADPH serves as a hydrogen and electron donor in reductive biosynthetic reactions, while in most biochemical reactions NADH is oxidized by the respiratory chain to produce ATP (Chapter 6). The enzymes involved in this pathway are located in the cytosol, indicating that the oxidation that occurs is not dependent on mitochondria or the tricarboxylic acid cycle. Another important function is to convert hexoses into pentoses, particularly ribose 5-phosphate. This C_5 sugar or its derivatives are components of ATP, CoA, NAD, FAD, RNA, and DNA. The pentose phosphate pathway also catalyzes the interconversion of C_3, C_4, C_6, and C_7 sugars, some of which can enter glycolysis.

There are also specific pathways for synthesis and degradation of monosaccharides, oligosaccharides, and polysaccharides (other than glycogen) and a profusion of chemical **interconversions**, whereby one sugar can be changed into another. All monosaccharides, and most oligo- and polysaccharides synthesized from the monosaccharides, can originate from glucose. The interconversion reactions by which one sugar is changed into another can occur directly or at the level of nucleotide-linked sugars. In addition to their important role in sugar transformation, nucleotide sugars are the obligatory activated form for polysaccharide synthesis. Monosaccharides are also often found as components of more complex macromolecules like oligo- and polysaccharides, glycoproteins, glycolipids, and proteoglycans. In higher animals these complex carbohydrate molecules are predominantly structural elements filling the extracellular space in tissues and associated with cell membranes. However, more dynamic functions for these complex macromolecules, such as recognition markers and determinants of biological specificity, have been discovered. The discussion of complex carbohydrates in this chapter is limited to the chemistry and biology of those complex carbohydrates found in animal tissues and fluids. The Appendix discusses the nomenclature and chemistry of the carbohydrates.

8.2— Pentose Phosphate Pathway

The Pentose Phosphate Pathway Has Two Phases

The oxidative pentose phosphate pathway provides a means for cutting the carbon chain of a sugar molecule one carbon at a time. However, in contrast to glycolysis and the tricarboxylic acid cycle, the operation of this pathway does not occur as a consecutive set of reactions leading directly from glucose 6-phosphate (G6P) to six molecules of CO_2. The pathway can be visualized as occurring in two stages. In the first stage, hexose is decarboxylated to pentose, followed by two oxidation reactions that lead to formation of NADPH. The pathway then continues and, by a series of transformations, six molecules of pentose undergo rearrangements to yield five molecules of hexose. To understand this pathway, it is necessary to examine each reaction individually.

Glucose 6-Phosphate Is Oxidized and Decarboxylated to a Pentose Phosphate

The first reaction of the pentose phosphate pathway (Figure 8.1) is **dehydrogenation** of G6P at C-1 to form **6-phosphoglucono-δ-lactone** and **NADPH**.

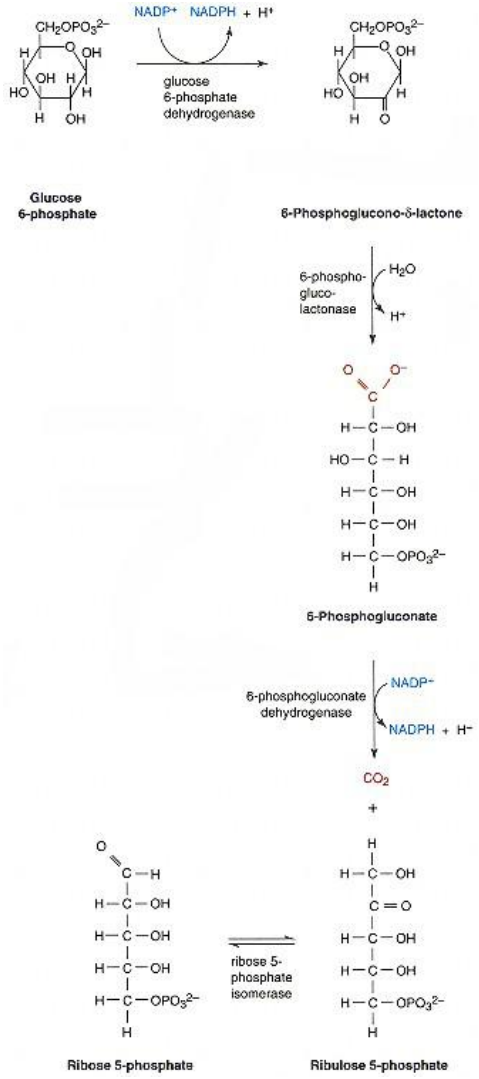


Figure 8.1
Formation of pentose phosphate.

The enzyme catalyzing this reaction is **G6P dehydrogenase,** the first enzyme found to be specific for $NADP^+$ and the major regulatory site for the pathway. Special interest in this enzyme stems from the severe anemia that may result from the absence of G6P dehydrogenase in erythrocytes or from the presence of one of several genetic variants of the enzyme (see Clin. Corr. 8.1). The intermediate product of this reaction, a lactone, is a substrate for gluconolacto-

CLINICAL CORRELATION 8.1

Glucose 6-Phosphate Dehydrogenase: Genetic Deficiency or Presence of Genetic Variants in Erythrocytes

When certain seemingly harmless drugs, such as antimalarials,antipyretics, or sulfa antibiotics, are administered to susceptible patients, an acute hemolytic anemia may result in 48–96 h. Susceptibility to drug-induced hemolytic disease may be due to a deficiency of G6P dehydrogenase activity in erythrocytes and was one of the early indications that X-linked genetic deficiencies of this enzyme exist. This enzyme, which catalyzes the oxidation of G6P to 6-phosphogluconate and the reduction of $NADP^+$, is particularly important, since the pentose phosphate pathway is the major pathway of NADPH production in the red cell. For example, red cells with the relatively mild A-type of glucose-6-phosphate dehydrogenase deficiency can oxidize glucose at a normal rate when the demand for NADPH is normal. However, if the rate of NADPH oxidation is increased, the enzyme-deficient cells cannot increase the rate of glucose oxidation and carbon dioxide production adequately. In addition, cells lacking glucose-6-phosphate dehydrogenase do not reduce enough NADP to maintain glutathione in its reduced state. Reduced glutathione is necessary for the integrity of the erythrocyte membrane, thus rendering enzyme-deficient red cells more susceptible to hemolysis by a wide range of compounds. Therefore the basic abnormality in G6P deficiency is the formation of mature red blood cells that have diminished glucose-6-phosphate dehydrogenase activity. Young red blood cells may have significantly higher enzyme activity than older cells, because of an unstable enzyme variant; following an episode of hemolysis, young red cells predominate and it may not be possible to diagnose this genetic deficiency until the red cell population ages. This enzymatic deficiency, which is usually undetected until administration of certain drugs, illustrates the interplay of heredity and environment on the production of disease. Enzyme defects are only one of several abnormalities that can affect enzyme activity, and others have been detected independent of drug administration. There are more than 300 known genetic variants of this enzyme that contains 516 amino acids, accounting for a wide range of symptoms. These variants can be distinguished from one another by clinical, biochemical, and molecular differences (see Clin. Corr. 4.5).

nase, which ensures that the reaction goes to completion. The overall equilibrium of these two reactions lies far in the direction of NADPH maintaining a high $NADPH/NADP^+$ ratio within cells. A second **dehydrogenation** and decarboxylation is catalyzed by **6-phosphogluconate dehydrogenase** and produces the pentose phosphate, **ribulose 5-phosphate**, and a second molecule of NADPH. The final step in synthesis of ribose 5-phosphate is the **isomerization**, through an enediol intermediate, of ribulose 5-phosphate by **ribose isomerase**.

These first reactions result in decarboxylation and production of NADPH and are considered to be the most important. Under certain metabolic conditions, the pentose phosphate pathway can end at this point, with utilization of NADPH for reductive biosynthetic reactions and ribose 5-phosphate as a precursor for nucleotide synthesis. The overall equation may be written as

$$\text{Glucose 6-phosphate} + 2\ NADP^+ + H_2O \rightleftharpoons \text{ribose 5-phosphate} + 2\ NADPH + 2\ H^+ + CO_2$$

Interconversions of Pentose Phosphates Lead to Glycolytic Intermediates

In certain cells more NADPH is needed for **reductive biosynthesis** than ribose 5-phosphate for incorporation into nucleotides. A **sugar rearrangement** system (Figure 8.2) forms triose, tetrose, hexose, and heptose sugars from the pentoses, thus creating a **disposal mechanism** for ribose 5-phosphate and providing a **reversible link** between the pentose phosphate pathway and glycolysis via intermediates common to both pathways. For the interconversions, another pentose phosphate, **xylulose 5-phosphate**, must first be formed through **isomerization of ribulose 5-phosphate** by the action of **phosphopentose epimerase**. As a consequence, these three pentose phosphates exist as an equilibrium mixture and can then undergo further transformations catalyzed by **transketolase** and **transaldolase**. Both enzymes catalyze chain cleavage and transfer reactions involving the same group of substrates.

Transketolase requires thiamine pyrophosphate (TPP) and Mg^{2+}, transfers a C_2 unit designated "**active glycolaldehyde**" from **xylulose 5-phosphate**

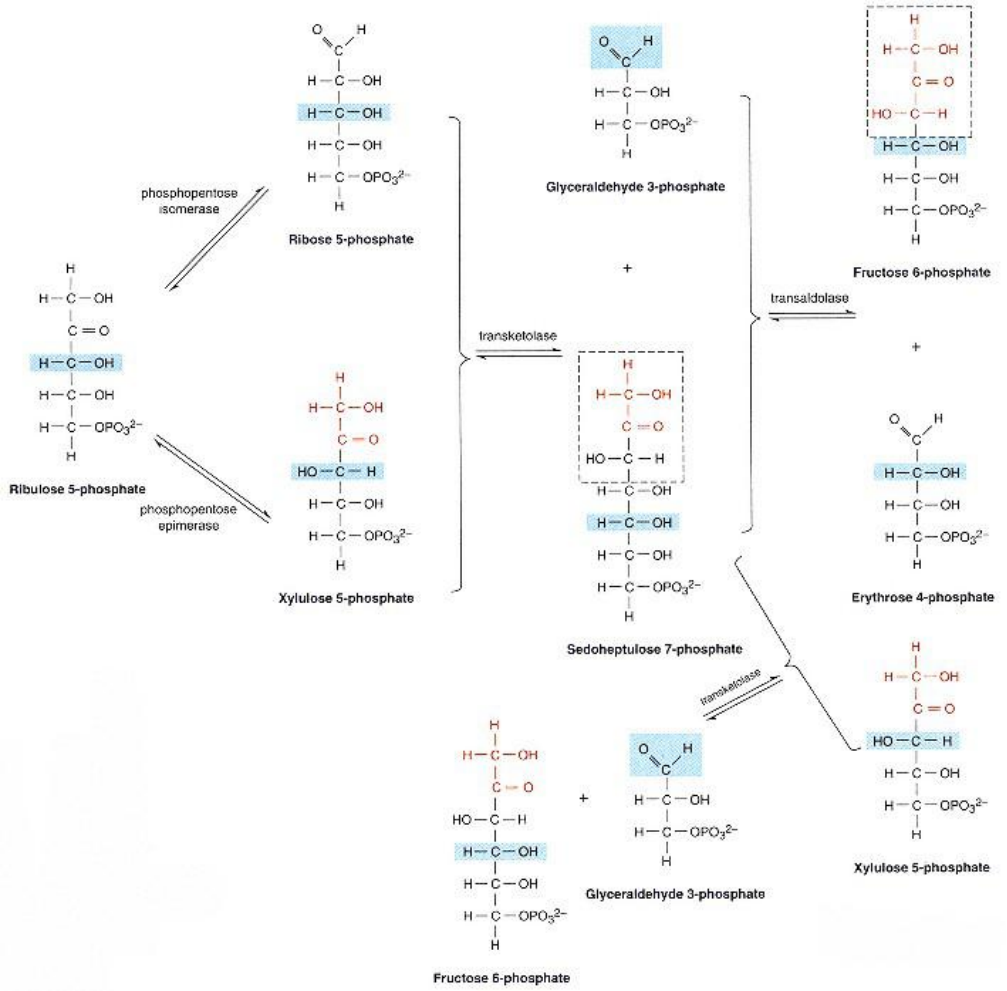


Figure 8.2
Interconversions of pentose phosphates.

to **ribose 5-phosphate,** and produces the C_7 sugar **sedoheptulose** and **glyceraldehyde 3-phosphate,** an intermediate of glycolysis. A further transfer reaction, catalyzed by transaldolase, results in the recovery of the first hexose phosphate. In this reaction a C_3 unit **(dihydroxyacetone)** from sedoheptulose 7-phosphate is transferred to glyceraldehyde 3-phosphate, forming the tetrose, **erythrose 4-phosphate,** and fructose 6-phosphate, another intermediate of glycolysis. In a third reaction, transketolase catalyzes the synthesis of fructose 6-phosphate and glyceraldehyde 3-phosphate from erythrose 4-phosphate and a second molecule of xylulose 5-phosphate. In this case, the C_2 unit is transferred

from xylulose 5-phosphate to an acceptor C_4 sugar, forming two glycolytic intermediates. The sum of these reactions is

2 Xylulose 5-phosphate + ribose 5-phosphate
⇌ 2 fructose 6-phosphate + glyceraldehyde 3-phosphate

Since xylulose 5-phosphate is derived from ribose 5-phosphate, the net reaction starting from ribose 5-phosphate is

3 Ribose 5-phosphate ⇌ 2 fructose 6-phosphate + glyceraldehyde 3-phosphate

Therefore excess ribose 5-phosphate, whether it arises from the initial oxidation of G6P or from the degradative metabolism of nucleic acids, is effectively scavenged by conversion to intermediates that can enter the carbon flow of glycolysis.

Glucose 6-Phosphate Can Be Completely Oxidized to CO_2

In certain tissues, like lactating mammary gland, a pathway for complete **oxidation** of **G6P** to CO_2, with concomitant reduction of $NADP^+$ to NADPH, also exists (Figure 8.3). By a complex sequence of reactions, ribulose 5-phosphate produced by the pentose phosphate pathway is recycled into G6P by transketolase, transaldolase, and certain enzymes of the gluconeogenic pathway. Hexose continually enters this system, and CO_2 evolves as the only carbon compound. A balanced equation for this process would involve the oxidation of six molecules of G6P to six molecules of ribulose 5-phosphate and six molecules of CO_2. This represents essentially the first part of the pentose phosphate pathway and results in transfer of 12 pairs of electrons to $NADP^+$, the requisite

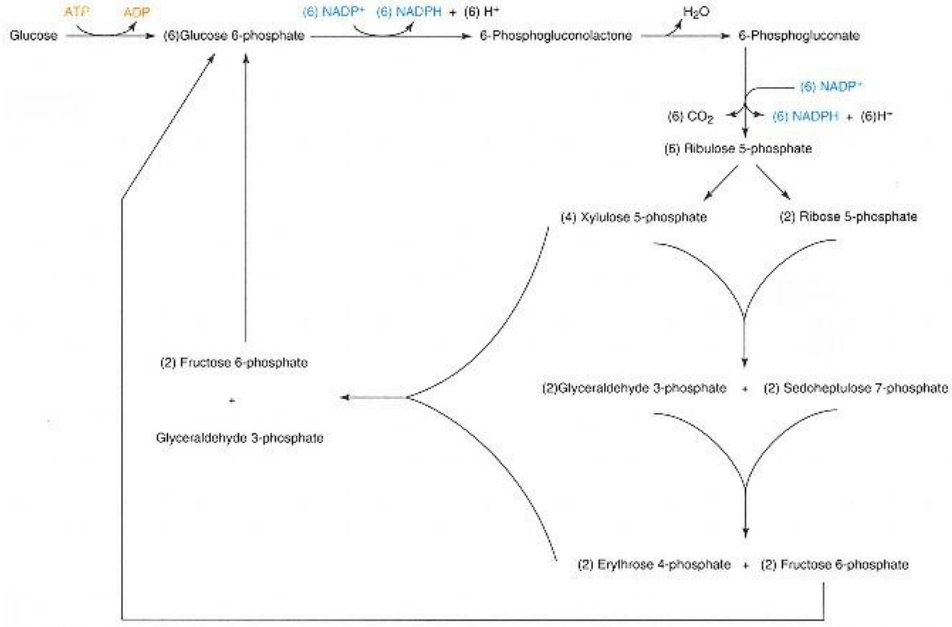


Figure 8.3
Pentose phosphate pathway.

amount for total oxidation of one glucose to six CO_2. The remaining six molecules of ribulose 5-phosphate are then rearranged by the pathway described above to regenerate five molecules of G6P. The overall equation can be written as

$$6 \text{ Glucose 6-phosphate} + 12 \text{ NADP}^+ + 7 \text{ H}_2\text{O} \rightleftharpoons 5 \text{ glucose 6-phosphate} + 6 \text{ CO}_2 + 12 \text{ NADPH} + 12 \text{ H}^+ + \text{P}_i$$

The net reaction is therefore

$$\text{Glucose 6-phosphate} + 12 \text{ NADP}^+ + 7 \text{ H}_2\text{O} \rightleftharpoons 6 \text{ CO}_2 + 12 \text{ NADPH} + 12 \text{ H}^+ + \text{P}_i$$

Pentose Phosphate Pathway Produces NADPH

The pentose phosphate pathway serves several purposes, including synthesis and degradation of sugars other than hexoses, particularly pentoses necessary for nucleotides and nucleic acids, and other glycolytic intermediates. Most important is the ability to synthesize NADPH, which has a unique role in biosynthetic reactions. The direction of flow and path taken by G6P after entry into the pathway is determined largely by the needs of the cell for NADPH or sugar intermediates. When more NADPH than ribose 5-phosphate is required, the pathway leads to complete oxidation of G6P to CO_2 and resynthesis of G6P from ribulose 5-phosphate. Alternatively, if more ribose 5-phosphate than NADPH is required, G6P is converted to fructose 6-phosphate and glyceraldehyde 3-phosphate by the glycolytic pathway. Two molecules of fructose 6-phosphate and one molecule of glyceraldehyde 3-phosphate are converted into three molecules of ribose 5-phosphate by reversal of the transaldolase and transketolase reactions.

The **distribution** of the **pentose phosphate pathway** in tissues is consistent with its functions. It is present in erythrocytes for production of NADPH, used to generate reduced glutathione, which is essential for maintenance of normal red cell structure. It is also active in liver, mammary gland, testis, and adrenal cortex, sites of fatty acid or steroid synthesis, which also require the reducing power of NADPH. In contrast, in mammalian striated muscle, which exhibits little fatty acid or steroid synthesis, all catabolism proceeds via glycolysis and the TCA cycle and no direct oxidation of glucose 6-phosphate occurs through the pentose phosphate pathway. In some other tissues like liver, 20–30% of the CO_2 produced may arise from the pentose phosphate pathway, and the balance between glycolysis and the pentose phosphate pathway depends on the metabolic requirements of the cell.

8.3— Sugar Interconversions and Nucleotide Sugar Formation

In considering the general principles of carbohydrate metabolism, certain aspects of the origin and fate of other monosaccharides, oligosaccharides, and polysaccharides should be included. Most monosaccharides found in biological compounds derive from glucose. The most common reactions for sugar transformations in mammalian systems are summarized in Figure 8.4.

Isomerization and Phosphorylation Are Common Reactions for Interconverting Carbohydrates

Formation of some sugars can occur directly, starting from glucose via modification reactions, such as the conversion of G6P to fructose 6-phosphate by phosphoglucose isomerase in the glycolytic pathway. A similar **aldose–ketose**

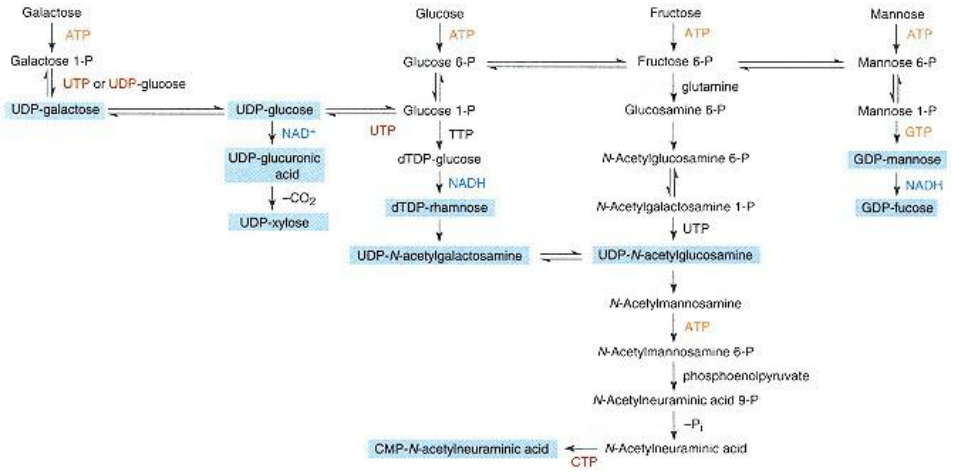


Figure 8.4
Pathways of formation of nucleotide sugars and interconversions of some hexoses.

isomerization catalyzed by **phosphomannose isomerase** results in synthesis of mannose 6-phosphate.

Internal transfer of a phosphate group on the same sugar molecule from one hydroxyl group to another is a common modification. Glucose 1-phosphate, resulting from enzymatic phosphorolysis of glycogen, is converted to G6P by phosphoglucomutase. Galactose can be phosphorylated directly to galactose 1-phosphate by a galactokinase and mannose to mannose 6-phosphate by a mannokinase. Free fructose, an important dietary constituent, can be phosphorylated in the liver to fructose 1-phosphate by a special fructokinase. However, no mutase exists to interconvert fructose 1-phosphate and fructose 6-phosphate, nor can phosphofructokinase synthesize fructose 1,6-bisphosphate from fructose 1-phosphate. Rather, a fructose 1-phosphate aldolase cleaves fructose 1-phosphate to dihydroxyacetone phosphate (DHAP), which enters the glycolytic pathway directly, and glyceraldehyde, which must first be reduced to glycerol, phosphorylated, and then reoxidized to DHAP. Lack of this aldolase leads to fructose intolerance (see Clin. Corr. 8.2).

CLINICAL CORRELATION 8.2

Essential Fructosuria and Fructose Intolerance: Deficiency of Fructokinase and Fructose 1-Phosphate Aldolase

Fructose may account for 30–60% of the total carbohydrate intake of mammals. It is predominantly metabolized by a specific fructose pathway. The first enzyme in this pathway, fructokinase, is deficient in essential fructosuria. This disorder is a benign asymptomatic metabolic anomaly, which appears to be inherited as an autosomal recessive. Following intake of fructose, blood levels and urinary fructose are unusually high; however, 90% of fructose is eventually metabolized. In contrast, hereditary fructose intolerance is characterized by severe hypoglycemia after ingestion of fructose. Prolonged ingestion in young children may lead to death. In this disorder fructose 1-phosphate aldolase is deficient, and fructose 1-phosphate accumulates intracellularly (see Clin. Corr. 7.3).

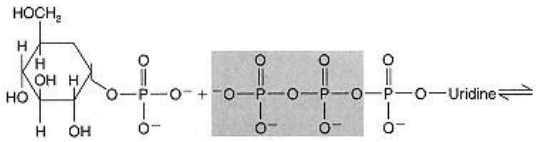
Steinitz, H., and Mizrohy, O. Essential fructosuria and hereditary fructose intolerance. *N. Eng. J. Med.* 280:222, 1969.

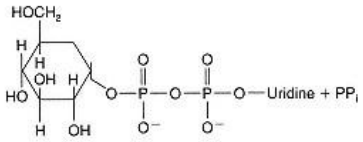
Nucleotide-Linked Sugars Are Intermediates in Many Sugar Transformations

Most other sugar transformation reactions require prior conversion into **nucleotide-linked sugars.** Formation of nucleoside diphosphate (NDP)-sugar involves the reaction of hexose 1-phosphate and nucleoside triphosphate (NTP), catalyzed by a pyrophosphorylase. While these reactions are readily reversible, *in vivo* pyrophosphate is rapidly hydrolyzed by pyrophosphatase, thereby driving the synthesis of nucleotide sugars. These reactions are summarized as follows:

$$\text{NTP} + \text{sugar 1-phosphate} + \text{H}_2\text{O} \rightleftharpoons \text{NDP-sugar} + \text{PP}_i$$
$$\underline{\text{PP}_i + \text{H}_2\text{O} \rightleftharpoons 2\,\text{P}_i}$$
$$\text{NTP} + \text{sugar 1-phosphate} + \text{H}_2\text{O} \rightleftharpoons \text{NDP-sugar} + 2\,\text{P}_i$$

UDP-glucose is a common nucleotide sugar involved in synthesis of glycogen and glycoproteins. It is synthesized from glucose 1-phosphate and UTP in a reaction catalyzed by UDP-glucose pyrophosphorylase.





UDP-Glucose

Nucleoside diphosphate-sugars contain two phosphoryl bonds, with a large negative G of hydrolysis, that contribute to the energized character of these compounds as glycosyl donors in further transformation and transfer reactions, as well as conferring specificity on the enzymes catalyzing these reactions. For instance, uridine diphosphate usually serves as the glycosyl carrier, while ADP, GDP, and CMP act as carriers in other reactions. Many sugar transformation reactions, including epimerization, oxidation, decarboxylation, reduction, and rearrangement, occur only at the level of nucleotide sugars.

CLINICAL CORRELATION 8.3

Galactosemia: Inability to Transform Galactose into Glucose

Reactions of galactose are of particular interest because in humans they are subject to genetic defects resulting in the hereditary disorder galactosemia. When a defect is present, individuals are unable to metabolize the galactose derived from lactose (milk sugar) to glucose metabolites, often with resultant cataract formation, growth failure, mental retardation, or eventual death from liver damage. The genetic disturbance is expressed as a cellular deficiency of either galactokinase, causing a relatively mild disorder characterized by early cataract formation, or of galactose 1-phosphate uridylyl-transferase, resulting in severe disease.

Galactose is reduced to galactitol in a reaction similar to the reduction of glucose to sorbitol. Galactitol is the initiator of cataract formation in the lens and may play a role in the central nervous system damage. Accumulation of galactose 1-phosphate is responsible for liver failure; the toxic effects of galactose metabolites disappear when galactose is removed from the diet.

Segal, S., Blair, A., and Roth, H. The metabolism of galactose by patients with congenital galactosemia. *Am. J. Med.* 83:62, 1965.

Epimerization Interconverts Glucose and Galactose

Epimerization is a common type of reaction in carbohydrate metabolism. Reversible conversion of glucose to galactose in animals occurs by epimerization of UDP-glucose to UDP-galactose, catalyzed by UDP-glucose epimerase. UDP-galactose is also an important intermediate in metabolism of free galactose, derived from the hydrolysis of lactose in the intestinal tract. Galactose is phosphorylated by galactokinase and ATP to yield galactose 1-phosphate. Then galactose 1-phosphate uridylyltransferase transforms galactose 1-phosphate into UDP-galactose by displacing glucose 1-phosphate from UDP-glucose. These reactions are summarized as follows:

$$\text{Galactose} + \text{ATP} \rightleftharpoons \text{galactose 1-phosphate} + \text{ADP}$$

$$\text{UDP-glucose} + \text{galactose 1-phosphate} \rightleftharpoons \text{UDP-galactose} + \text{glucose 1-phosphate}$$

A hereditary disorder, galactosemia, results from the absence of this uridylyl-transferase (see Clin. Corr. 8.3).

A combination of these reactions allows an efficient transformation of dietary galactose into glucose 1-phosphate, which can then be further metabolized by previously described pathways. Alternatively, the 4-epimerase can operate in the reverse direction when UDP-galactose is needed for biosynthesis. Epimerization reactions are not exclusively restricted to nucleotide-linked sugars but also occur at the polymer level; D-glucuronic acid is epimerized to L-iduronic acid after incorporation into heparin and dermatan sulfate (see Section 8.6).

Glucuronic Acid Is Formed by Oxidation of UDP-Glucose

Oxidation and reduction interconversions result in formation of several additional sugars. **Glucuronic acid** is formed by oxidation of UDP-glucose catalyzed by **UDP-glucose dehydrogenase** (Figure 8.5) and most likely follows the outline in Figure 8.6. In humans glucuronic acid is converted to L-xylulose, the ketopentose excreted in **essential pentosuria** (see Clin. Corr. 8.4), and participates in detoxification by production of glucuronide conjugates (see Clin. Corr. 8.5).

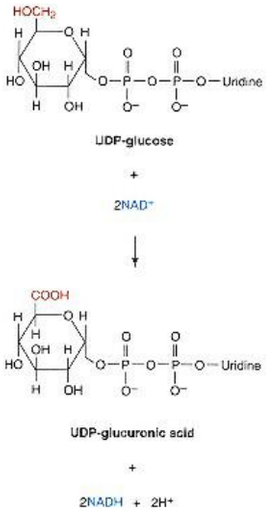


Figure 8.5
Formation of UDP-glucuronic acid from UDP-glucose.

Glucuronic acid is a precursor of **L-ascorbic acid** in those animals that synthesize vitamin C. Free glucuronic acid can be metabolized by reduction with NADPH to L-gulonic acid (Figure 8.7), which is then converted by a two-step process through L-gulonolactone to L-ascorbic acid (vitamin C) in plants and most higher animals. Humans, other primates, and the guinea pig lack the enzyme that converts L-gulonolactone to L-ascorbic acid and therefore must satisfy their needs for ascorbic acid by its ingestion. Gulonic acid can also be oxidized to 3-ketogulonic acid and decarboxylated to L-xylulose. L-Xylulose is reduced to xylitol, reoxidized to D-xylulose, and phosphorylated with ATP and an appropriate kinase to xylulose 5-phosphate. The latter compound can then reenter the pentose phosphate pathway described previously. The glucuronic acid pathway represents another pathway for oxidation of glucose. This pathway operates in adipose tissue, and its activity can be increased in tissue from starved or diabetic animals.

Decarboxylation, Oxidoreduction, and Transamination of Sugars Produce Necessary Products

Although **decarboxylation,** which degrades sugars one carbon atom at a time, has been encountered previously in the major metabolic pathways, the only known decarboxylation of a nucleotide sugar is the conversion of UDP-glucuronic acid to UDP-xylose. UDP-xylose is necessary for synthesis of proteoglycans (Section 8.6) and is a potent inhibitor of UDP-glucose dehydrogenase, the enzyme that oxidizes UDP-glucose to UDP-glucuronic acid (Figure 8.5). Thus the level of these nucleotide sugar precursors is regulated by this sensitive feedback mechanism.

Deoxyhexoses and **dideoxyhexoses** are also synthesized while the sugars are attached to nucleoside diphosphates, by a multistep process. For example, L-rhamnose is synthesized from glucose by a series of oxidation–reduction reactions starting with dTDP-glucose and yielding dTDP-rhamnose, catalyzed by oxidoreductases. Presumably, similar reactions account for synthesis of GDP-fucose from GDP-mannose and for various dideoxyhexoses.

Formation of amino sugars, major components of human oligo- and polysaccharides and as constituents of antibiotics, occurs by **transamidation.** For example, synthesis of glucosamine 6-phosphate occurs by reaction of fructose 6-phosphate with glutamine.

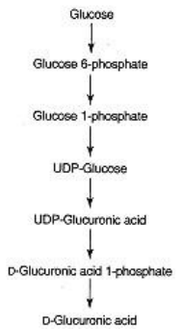
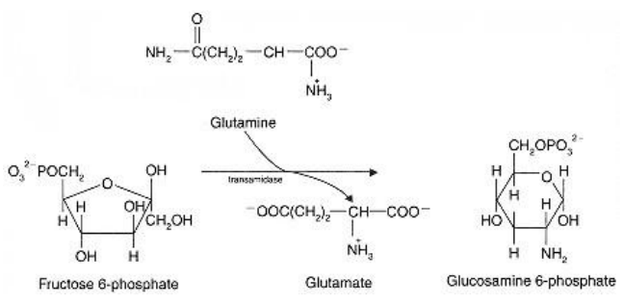


Figure 8.6
Biosynthesis of D-glucuronic acid from glucose.



Glucosamine 6-phosphate can be *N*-acetylated, forming N-acetylglucosamine 6-phosphate, followed by isomerization to *N*-acetylglucosamine 1-phosphate. This latter sugar is converted to UDP-*N*-acetylglucosamine by reactions similar to those of UDP-glucose synthesis. UDP-*N*-acetylglucosamine, a precursor of glycoprotein synthesis, can be epimerized to UDP-*N*-acetylgalactosamine, necessary for proteoglycan synthesis. The fructose 6-phosphate–glutamine transamidase reaction is under negative feedback control by UDP-*N*-acetylglucosamine; thus synthesis of both nucleotide sugars is regulated (Figure 8.4). This regulation is meaningful in certain tissues such as skin, in which this pathway can involve up to 20% of glucose flux.

CLINICAL CORRELATION 8.4

Pentosuria: Deficiency of Xylitol Dehydrogenase

The glucuronic acid oxidation pathway presumably is not essential for human carbohydrate metabolism, since individuals in whom the pathway is blocked suffer no ill effects. A metabolic variation, called idiopathic pentosuria, results from reduced activity of NADP-linked L-xylulose reductase, the enzyme that catalyzes the reduction of xylulose to xylitol. Hence affected individuals excrete large amounts of pentose into the urine especially following intake of glucuronic acid.

Wang, Y. M., and van Eys, J. The enzymatic defect in essential pentosuria. *N. Engl. J. Med.* 282:892, 1970.

Sialic Acids Are Derived from N-Acetylglucosamine

Another product derived from UDP-*N*-acetylglucosamine is **acetylneuraminic acid,** one of a family of C_9 sugars, called **sialic acids** (Figure 8.8). The first

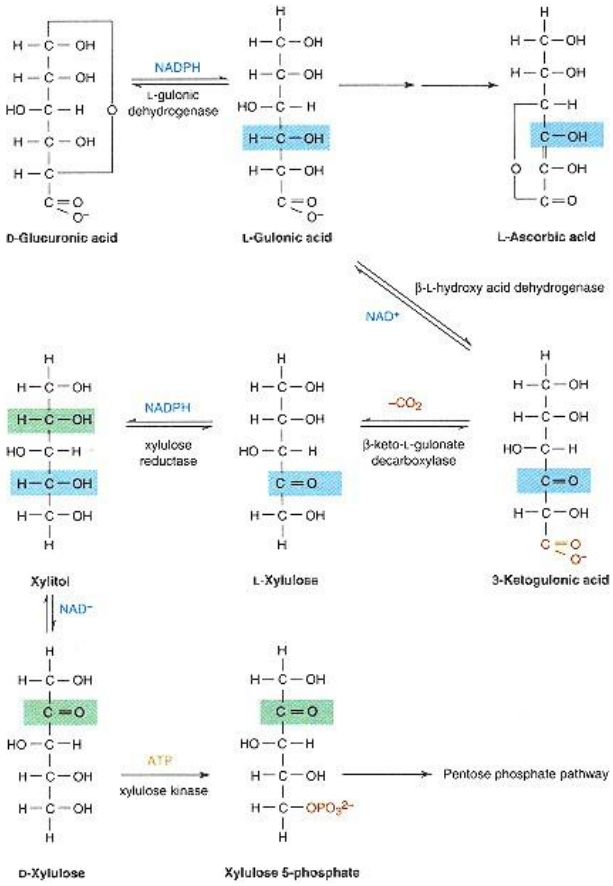
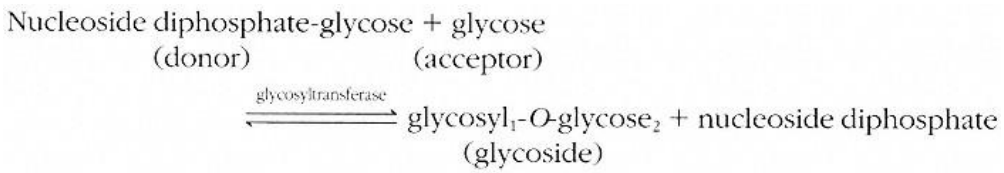


Figure 8.7
Glucuronic acid oxidation pathway.

reaction involves epimerization of UDP-*N*-acetylglucosamine by a 2-epimerase to *N*-acetylmannosamine, concomitant with elimination of UDP. Since the monosaccharide product is no longer bound to nucleotide, this epimerization is clearly different from those previously encountered. Most likely, this 2-epimerase reaction proceeds by a trans elimination of UDP, with formation of the unsaturated intermediate, 2-acetamidoglucal. In mammalian tissues *N*-acetyl-mannosamine is phosphorylated by ATP to *N*-acetylmannosamine 6-phosphate, which then condenses with phosphoenolpyruvate to form *N*-acetylneuraminic acid 9-phosphate. This product is cleaved by a phosphatase and activated by CTP to form the CMP derivative, CMP-*N*-acetylneuraminic acid. This is an unusual nucleotide sugar, containing only one phosphate group, and is formed by an irreversible reaction. *N*-Acetylneuraminic acid is a precursor of other sialic acid derivatives, some of which evolve by modification of *N*-acetyl to *N*-glycolyl or *O*-acetyl after incorporation into glycoprotein.

8.4—
Biosynthesis of Complex Carbohydrates

In complex carbohydrate-containing molecules, sugars are linked to other sugars by glycosidic bonds, formed by specific **glycosyltransferases.** Energy is required for synthesis of a glycosidic bond and is derived from nucleotide sugars as donor substrates. A glycosyltransferase reaction proceeds by donation of the glycosyl unit from the nucleotide derivative to the nonreducing end of an acceptor sugar. The nature of the bond formed is determined by the specificity of the glycosyltransferase, which is unique for the sugar acceptor, the sugar transferred, and the linkage formed. Thus polysaccharide synthesis is controlled by a nontemplate mechanism directed by specific glycosyltransferases. A glycosyltransferase reaction is summarized as follows:



At least 40 different glycosidic bonds have been identified in mammalian oligosaccharides and about 15 more in connective tissue polysaccharides. The number of possible linkages is even greater and arises both from the diversity of monosaccharides covalently bonded and from the formation of both α and β

CLINICAL CORRELATION 8.5

Glucuronic Acid: Physiological Significance of Glucuronide Formation

The biological significance of glucuronic acid extends to its ability to be conjugated with certain endogenous and exogenous substances, forming a group of compounds collectively termed glucuronides in a reaction catalyzed by UDP-glucuronyltransferase. Conjugation of a compound with glucuronic acid produces a strongly acidic compound that is more water soluble at physiological pH than its precursor and therefore may alter the metabolism, transport, or excretion properties. Glucuronide formation is important in drug detoxification, steroid excretion, and bilirubin metabolism. Bilirubin is the major metabolic breakdown product of heme, the prosthetic group of hemoglobin. The central step in excretion of bilirubin is conjugation with glucuronic acid by UDP-glucuronyltransferase. Development of this conjugating mechanism occurs gradually and may take several days to 2 weeks after birth to become fully active in humans. So-called physiological jaundice of the newborn results in most cases from the inability of the neonatal liver to form bilirubin glucuronide at a rate comparable to that of bilirubin production. A defect in glucuronide synthesis has been found in a mutant strain of Wistar ("Gunn") rats, due to a deficiency of UDP-glucuronyltransferase and results in hereditary hyperbilirubinemia. In humans a similar defect is found in congenital familial nonhemolytic jaundice (Crigler–Najjar syndrome). Patients with this condition are also unable to conjugate foreign compounds efficiently with glucuronic acid.

Crigler, J. F., and Najjar, V. A. Congenital familial non-hemolytic jaundice with kernicterus. *Pediatrics* 10:169, 1952; Gunn, C. H. Hereditary acholuric jaundice in a new mutant strain of rats. *J. Hered.* 29:137, 1938; and Ostrow, J. D. (Ed.). *Bile Pigments and Jaundice*. New York: Marcel Dekker, 1986.

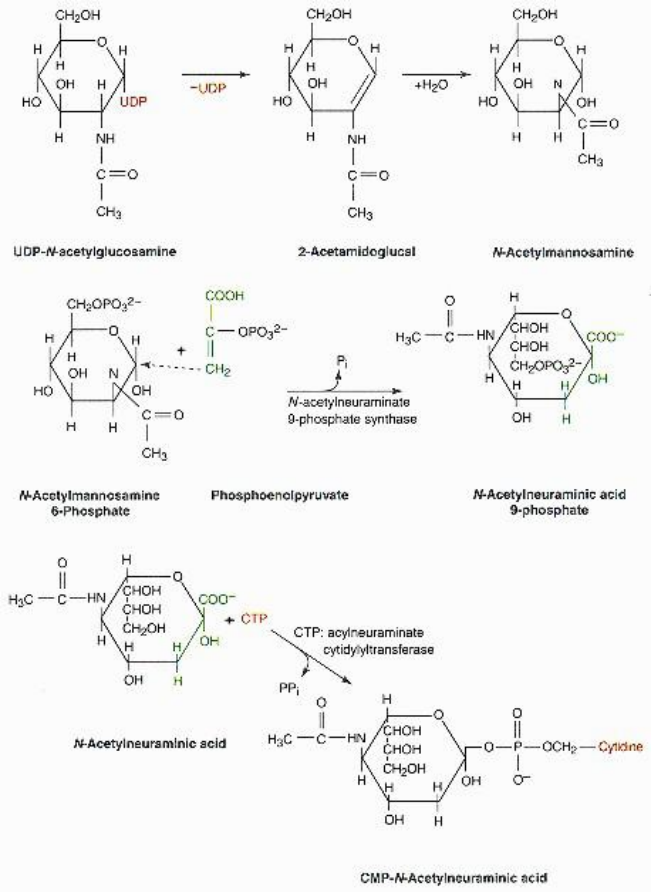


Figure 8.8
Biosynthesis of CMP-*N*-acetylneuraminic acid.

linkages, with each of the available hydroxyl groups on the acceptor saccharide. The large and diverse number of molecules that can be generated suggests that oligosaccharides have the potential for great informational content. In fact, it is known that the specificity of many biological molecules is determined by the nature of the composite sugar residues. For example, the specificity of the major blood types is determined by sugars (see Clin. Corr. 8.6). *N*-Acetylgalactosamine is the immunodeterminant of blood type A and galactose of blood type B. Removal of *N*-acetylgalactosamine from type A erythrocytes, or of galactose from type B erythrocytes, converts both to type O erythrocytes. Increasingly, other examples of sugars as determinants of specificity for cell surface receptor and lectin interactions, targeting of cells to certain tissues, and survival or clearance from the circulation of certain molecules are being recognized. All glycosidic bonds identified in biological compounds are degraded by specific hydrolytic enzymes, glycosidases. In addition to being valuable tools for the

CLINICAL CORRELATION 8.6

Blood Group Substances

The surface of human erythrocytes is covered with a complex mosaic of specific antigenic determinants, many of which are polysaccharides. There are about 100 blood group determinants, belonging to 21 independent human blood group systems. The most widely studied are the antigenic determinants of the ABO blood group system and the closely related Lewis system. From the study of these systems, a definite correlation was established between gene activity as it relates to specific glycosyltransferase synthesis and oligosaccharide structure. The genetic variation is achieved through specific glycosyltransferases responsible for synthesis of the heterosaccharide determinants. For example, the *H* gene codes for a fucosyltransferase, which adds fucose to a peripheral galactose in the heterosaccharide precursor. The *A, B*, and *O* genes are located on chromosome 9. The *A* gene encodes an *N*-acetylgalactosamine glycosyltransferase, the *B* gene encodes a galactosyltransferase, and the *O* gene encodes an inactive enzyme. The sugars are added to the *H*-specific oligosaccharide. The Lewis (*Le*) gene codes for another fucosyltransferase, which adds fucose to a peripheral *N*-acetylglucosamine residue in the precursor. Absence of the *H* gene gives rise to the Le^a-specific determinant, whereas in the presence of both the *H* and *Le* genes, the interaction product responsible for the Le^b specificity is found. The elucidation of the structures of these oligosaccharide determinants represents a milestone in carbohydrate chemistry. This knowledge is essential to blood transfusion practices and for legal and historical purposes. For example, tissue dust containing complex carbohydrates has been used in serological analysis to establish the blood group of Tutankhamen and his probable ancestral background.

Watkins, W. M. Blood group substances. *Science* 152:172, 1966.

structural elucidation of oligosaccharides, interest in this class of enzymes exists because many genetic diseases of complex carbohydrate metabolism result from defects in glycosidases (see Clin. Corr. 8.7 and 8.8).

8.5— Glycoproteins

Glycoproteins have been restrictively defined as conjugated proteins that contain, as a prosthetic group, one or more saccharides lacking a serial repeat unit and bound covalently to a peptide chain. This definition excludes proteoglycans, which are discussed in Section 8.6.

The functions of glycoproteins in the human are of great interest. Glycoproteins in cell membranes may have an important role in the group behavior of cells and other important biological functions of the membrane. Glycoproteins form a major part of the mucus that is secreted by epithelial cells, where they perform an important role in lubrication and in the protection of tissues lining the body's ducts. Many other proteins secreted from cells into extracellular fluids are glycoproteins. These proteins include hormones found in blood, such as follicle-stimulating hormone, luteinizing hormone, and chorionic gonadotropin; and plasma proteins such as the orosomucoids, ceruloplasmin, plasminogen, prothrombin, and immunoglobulins (see Clin. Corr. 2.7).

Glycoproteins Contain Variable Amounts of Carbohydrate

The percentage of carbohydrate in glycoproteins is highly variable. Some glycoproteins such as IgG contain low amounts (4%) of carbohydrate by weight, while glycophorin, the human red cell membrane glycoprotein, contains 60% carbohydrate. Human ovarian cyst glycoprotein contains 70% carbohydrate, and human gastric glycoprotein is 82% carbohydrate. The carbohydrate can be distributed fairly evenly along the polypeptide chain or concentrated in defined regions. For example, in human glycophorin A the carbohydrate is found in the NH_2-terminal half of the polypeptide chain that lies on the outside of the cellular membrane.

The carbohydrate attached at one or at multiple points along a polypeptide chain usually contains less than 12–15 sugar residues. In some cases the carbohydrate component consists of a single sugar moiety, as in the submaxillary gland glycoprotein (single *N*-acetyl-α-D-galactosaminyl residue) and in some types of

CLINICAL CORRELATION 8.7

Aspartylglycosylaminuria: Absence of 4-L-Aspartylglycosamine Amidohydrolase

A group of human inborn errors of metabolism involving storage of glycolipids, glycopeptides, mucopolysaccharides, and oligosaccharides exists. These diseases are caused by defects in lysosomal glycosidase activity, which prevents the catabolism of oligosaccharides. The disorders involve gradual accumulation in tissues and urine of compounds derived from incomplete degradation of the oligosaccharides and may be accompanied by skeletal abnormalities, hepatosplenomegaly, cataracts, or mental retardation. One disorder resulting from a defect in catabolism of asparagine-*N*-acetylglucosamine-linked oligosaccharides is aspartylglycosylaminuria. A deficiency in the enzyme 4-L-aspartylglycosylamine amidohydrolase allows accumulation of aspartylglucosamine-linked structures. (See accompanying table.)

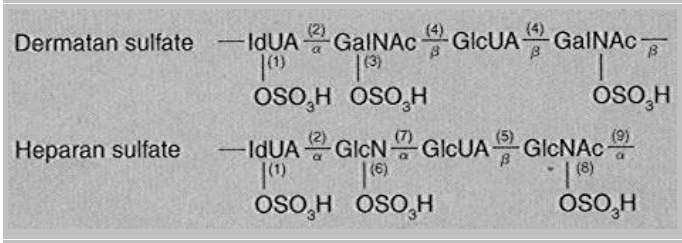
Other disorders have been described involving accumulation of oligosaccharides derived from both glycoproteins and glycolipids, which may share common oligosaccharide structures (see table). Examples of genetic diseases include mannosidosis (α-mannosidase), the GM_2 gangliosidosis variant O (Sandhoff–Jatzkewitz disease; β-*N*-acetylhexosaminidases A and B), and GM_1 gangliosidosis (β-galactosidase). Mucolipidosis II ("I-Cell Disease") is a generalized degradative disorder resulting from a deficiency of UDP-GlcNAc: lysosomal enzyme precursor GlcNAc phosphotransferase, which attacks Man-6-PO_4 (see also Chapter 10).

Sewell, A. C. Urinary oligosaccharide excretion in disorders of glycolipid, glycoprotein, and glycogen metabolism: a review of screening for differential diagnosis. *Eur. J. Pediatr* 134:183, 1980.

Enzymic Defects in Degradation of Asn-GlcNAc Type Glycoproteins[a]

Disease	*Deficient Enzyme*[b]
Aspartylglycosylaminuria	4-L-Aspartylglycosylamine amidohydrolase (a)
β-Mannosidosis	β-Mannosidase (7)
α-Mannosidosis	α-Mannosidase (3)
GM_2 Gangliosidosis variant O (Sandhoff–Jatzkewitz disease)	β-*N*-Acetylhexosaminidases (A and B) (4)
GM_1 Gangliosidosis	β-Galactosidase (5)
Mucolipidosis I (Sialidosis)	Sialidase (6)
Fucosidosis	α-Fucosidase (8)

[a] A typical Asn-GlcNAc oligosaccharide structure.



[b] The numbers in parentheses refer to the enzymes that hydrolyze those bonds.

mammalian collagens (single α-D-galactosyl residue). In general, glycoproteins contain sugar residues in the D form, except for L-fucose, L-arabinose, and L-iduronic acid. A glycoprotein from different animal species often has an identical primary structure in the protein component, but a variable carbohydrate component. This heterogeneity of a given protein may even be true within a single organism. For example, pancreatic ribonuclease A and B forms have identical amino acid sequences and a similar kinetic specificity toward substrates, but they differ significantly in their carbohydrate composition.

Carbohydrates Are Covalently Linked to Glycoproteins by N- or O-Glycosyl Bonds

At present, the structures of a limited number of oligosaccharide components have been elucidated completely. **Microheterogeneity** of glycoproteins, arising from incomplete synthesis or partial degradation, makes structural analyses

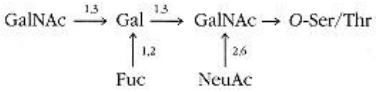
CLINICAL CORRELATION 8.8

Heparin Is an Anticoagulant

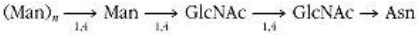
Heparin is a naturally occurring sulfated polysaccharide that is used to reduce the clotting tendency of patients. Both *in vivo* and *in vitro* heparin prevent the activation of clotting factors but do not act directly on the clotting factors. Rather, the anticoagulant activity of heparin is brought about by the binding interaction of heparin with an inhibitor of the coagulation process. Presumably, heparin binding induces a conformational change in the inhibitor that generates a complementary interaction between the inhibitor and the activated coagulation factor, thereby preventing the factor from participating in the coagulation process. The inhibitor that interacts with heparin is antithrombin III, a plasma protein inhibitor of serine proteases. In the absence of heparin, antithrombin III slowly (10–30 min) combines with several clotting factors, yielding complexes devoid of proteolytic activity. In the presence of heparin, inactive complexes are formed within a few seconds. Antithrombin III contains an arginine residue that combines with the active site serine of factors Xa and IXa; thus the inhibition is stoichiometric. Heterozygous antithrombin III deficiency results in an increased risk of thrombosis in the veins and resistance to the action of heparin.

Rosenberg, R. D., and Rosenberg, J. S. Natural anticoagulant mechanisms. *J. Clin. Invest.* 74:1, 1984.

extremely difficult. However, certain generalities about the structure of glycoproteins have emerged. Covalent linkage of sugars to the peptide chain is a central part of glycoprotein structure, and only a limited number of bonds are found (see Chapter 2). The three major types of **glycopeptide bonds**, as shown in Figure 8.9 and Figure 2.45, are *N*-glycosyl to **asparagine** (Asn), *O*-glycosyl to **serine** (Ser) or **threonine** (Thr), and *O*-glycosyl to **5-hydroxylysine**. The latter linkage, representing the carbohydrate side chains of either a single galactose or the disaccharide glucosylgalactose covalently bonded to hydroxylysine, is generally confined to the collagens. The other two linkages occur in a wide variety of glycoproteins. Of the three major types, only the *O*-glycosidic linkage to serine or threonine is labile to alkali cleavage. By this procedure two types of oligosaccharides (simple and complex) are released. Examination of the simple class from porcine submaxillary mucins reveals some general structural features. A core structure exists, consisting of galactose (Gal) linked β(1 3) to *N*-acetylgalactosamine (GalNAc) *O*-glycosidically linked to serine or threonine residues. Residues of L-fucose (Fuc), sialic acid (NeuAc), and another *N*-acetylgalactosamine are found at the nonreducing periphery of this class of glycopeptides. The general structure of this type of glycopeptide is as follows:

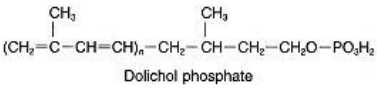


More complex heterosaccharides are also linked to peptides via serine or threonine residues and are exemplified by the blood group substances. Study of these determinants has shown how complex and variable these structures are, as well as how the oligosaccharides of cell surfaces are assembled and how that assembly pattern is genetically determined. An example of how oligosaccharide structures on the surface of red blood cells determine blood group specificity is presented in Clin. Corr. 8.6. Certain common structural features of the oligosaccharide *N*-glycosidically linked to asparagine have also emerged. These glycoproteins commonly contain a core structure consisting of mannose (Man) residues linked to *N*-acetylglucosamine (GlcNAc) in the following structure:



Synthesis of N-Linked Glycoproteins Involves Dolichol Phosphate

While the synthesis of *O*-glycosidically linked glycoproteins involves the sequential action of a series of glycosyltransferases, the synthesis of *N*-glycosidically linked peptides involves a somewhat different and more complex mechanism (Figure 8.10). A common core is preassembled as a **lipid-linked oligosaccharide** prior to incorporation into the polypeptide. During synthesis, the oligosaccharide intermediates are bound to derivatives of **dolichol phosphate**.



Dolichols are polyprenols (C_{80}–C_{100}) containing 16–20 isoprene units, in which the final isoprene unit is saturated. These lipids participate in two types of reactions in core oligosaccharide synthesis. The first reaction involves formation of *N*-acetylglucosaminylpyrophosphoryldolichol with release of UMP from the respective nucleotide sugar. The second *N*-acetylglucosamine and the mannose transferase reactions proceed by sugar transfer from the nucleotide without formation of intermediates. Subsequent addition of mannose units occurs via

a dolichol-linked mechanism. In the final step, the oligosaccharide is transferred from the dolichol pyrophosphate to an asparagine residue in the polypeptide chain.

After synthesis of the specific core region, the oligosaccharide chains are completed by action of glycosyltransferases without further participation of lipid intermediates. Extensive processing in the Golgi body, involving addition and subsequent removal of certain glycosyl residues, occurs during the course of synthesis of asparagine-*N*-acetylglucosamine-linked glycoproteins. Just as the synthesis of oligosaccharides requires specific glycosyltransferases, degradation requires specific glycosidases. Exoglycosidases remove sugars sequentially from the nonreducing end, exposing the substrate for the subsequent glycosidase. The absence of a particular glycosidase prevents the action of the next enzyme, resulting in cessation of catabolism and accumulation of the product (see Clin. Corr. 8.7). Endoglycosidases with broader specificity also exist and the action of endo- and exoglycosidases results in catabolism of glycoproteins. Although the primary degradation process occurs in lysosomes, there are specific endo-plasmic reticulum glycosidases involved in processing of glycoproteins during synthesis as well.

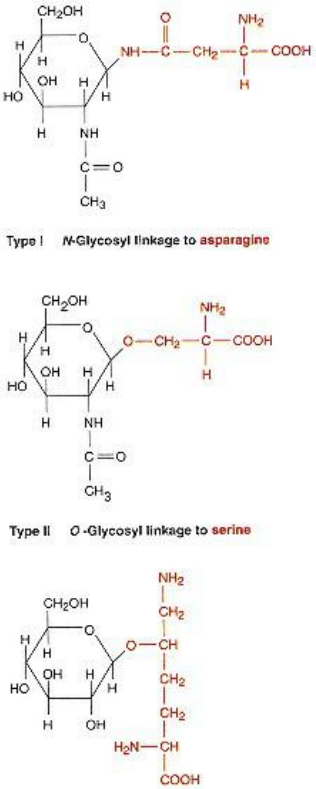


Figure 8.9
Structure of three major glycopeptide bonds.

8.6— Proteoglycans

In addition to glycoproteins, which usually contain proportionally less carbohydrate than protein by weight, there is another class of complex macromolecules, which can contain as much as 95% or more carbohydrate. Their properties resemble polysaccharides more than proteins. To distinguish these compounds

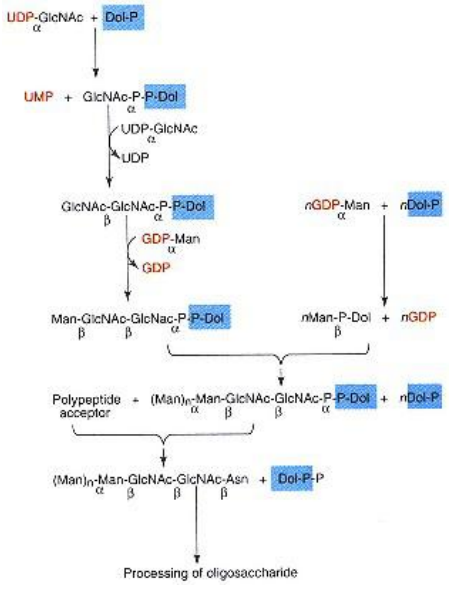


Figure 8.10
Biosynthesis of the oligosaccharide core in asparagine-*N*-acetylgalactosamine-linked glycoproteins.
Dol, dolichol.

from other glycoproteins, they are referred to as **proteoglycans** and their carbohydrate chains as **glycosaminoglycans**. An older name, mucopolysaccharides, is still in use, especially in reference to the group of storage diseases, mucopolysaccharidoses, which result from an inability to degrade these molecules (see Clin. Corr. 8.9).

Proteoglycans are high molecular weight polyanionic substances consisting of many different glycosaminoglycan chains linked covalently to a protein core. Although six distinct classes of glycosaminoglycans are now recognized,

CLINICAL CORRELATION 8.9

Mucopolysaccharidoses

A group of human genetic disorders characterized by excessive accumulation and excretion of the oligosaccharides of proteoglycans exists, collectively called mucopolysaccharidoses. These disorders result from a deficiency of one or more lysosomal hydrolases responsible for the degradation of dermatan and/or heparan sulfate. The enzymes lacking in specific mucopolysaccharidoses that have been identified are presented in the accompanying table.

Although the chemical basis for this group of disorders is similar, their mode of inheritance as well as clinical manifestations may vary. Hurler's syndrome and Sanfilippo's syndrome are transmitted as autosomal recessives, whereas Hunter's syndrome is X-linked. Both Hurler's syndrome and Hunter's syndrome are characterized by skeletal abnormalities and mental retardation, which in severe cases may result in early death. In contrast, in the Sanfilippo syndrome, the physical defects are relatively mild, while the mental retardation is severe. Collectively, the incidence for all mucopolysaccharidoses is 1 per 30,000 births.

In addition to those listed in the table, some others exist. Morquio's syndrome involves impaired degradation of keratan sulfate, and two types have been identified: type A due to deficiency of galactose 6-sulfatase and type B due to deficiency of β-galactosidase. Multiple sulfatase deficiency (MSD) is characterized by decreased activity of all known sulfatases. Recent evidence suggests that a co- or posttranslational modification of a cysteine to a 2-amino 3-oxopropionic acid is required for active sulfatases and that a lack of this modification results in MSD.

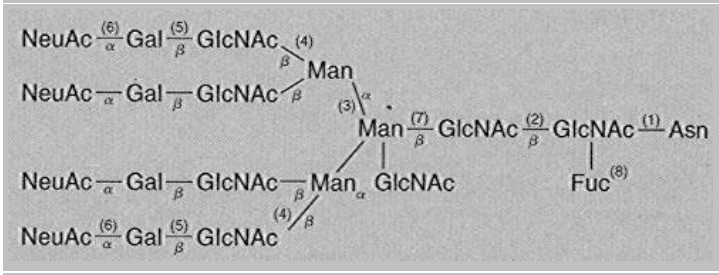
These disorders are amenable to prenatal diagnosis, since the pattern of metabolism by affected cells obtained from amniotic fluid is strikingly different from normal.

McKusick5th ed. New York: McGraw-Hill, 1983, , V., and Neufeld, E. F. The mucopolysaccharide storage diseases. In: J. B. Stansbury, J. B. Wyngaarden, D. S. Frederickson, J. L. Goldstein, and M. S. Brown (Eds.). *The Metabolic Basis of Inherited Disease,* p. 751.

Enzymic Defects in the Mucopolysaccharidoses

Disease	*Accumulated Products*[a]	*Deficient Enzyme*[b]
Hunter	Heparan sulfate	Iduronate sulfatase (1)
	Dermatan sulfate	
Hurler + Scheie	Heparan sulfate	-L-Iduronidase (2)
	Dermatan sulfate	
Maroteaux–Lamy	Dermatan sulfate	*N*-Acetylgalactosamine (3) sulfatase
Mucolipidosis VII	Heparan sulfate	-Glucuronidase (5)
	Dermatan sulfate	
Sanfilippo A	Heparan sulfate	Heparan sulfamidase (6)
Sanfilippo B	Heparan sulfate	*N*-Acetylglucosaminidase (9)
Sanfilippo D	Heparan sulfate	*N*-Acetylglucosamine 6-sulfatase (8)

[a] Structures of dermatan sulfate and heparan sulfate.



[b] The numbers in parentheses refer to the enzymes that hydrolyze those bonds.

certain features are common to all classes. The long unbranched heteropolysaccharide chains are made up largely of disaccharide repeating units, in which one sugar is a hexosamine and the other a uronic acid. Other common constituents of glycosaminoglycans are sulfate groups, linked by ester bonds to certain monosaccharides or by amide bonds to the amino group of glucosamine. An exception, hyaluronate, is not sulfated and has not been shown to exist covalently attached to protein. The carboxyl from uronic acids and sulfate groups contribute to the highly charged polyanionic nature of glycosaminoglycans. Both their electrical charge and macromolecular structure aid in their biological role as lubricants and support elements in connective tissue. Proteoglycans form solutions with high viscosity and elasticity by absorbing large volumes of water. This allows them to act in stabilizing and supporting fibrous and cellular elements of tissues, as well as contributing to the maintenance of water and salt balance in the body. Increasingly more dynamic roles as receptors for growth factors, transport proteins, and viruses are being elucidated for the proteoglycans.

Hyaluronate Is a Copolymer of N-Acetylglucosamine and Glucuronic Acid

Hyaluronate is very different from the other five types of glycosaminoglycans. It is unsulfated, not covalently complexed with protein, and the only glycosaminoglycan not limited to animal tissue; it is also produced by bacteria. It is classified as a glycosaminoglycan because of its structural similarity to these other polymers, since it consists solely of repeating disaccharide units of *N*-acetylglucosamine and glucuronic acid (Figure 8.11). Although hyaluronate has the least complex chemical structure of all the glycosaminoglycans, the chains can reach molecular weights of 10^5–10^7 The large molecular weight, polyelec-

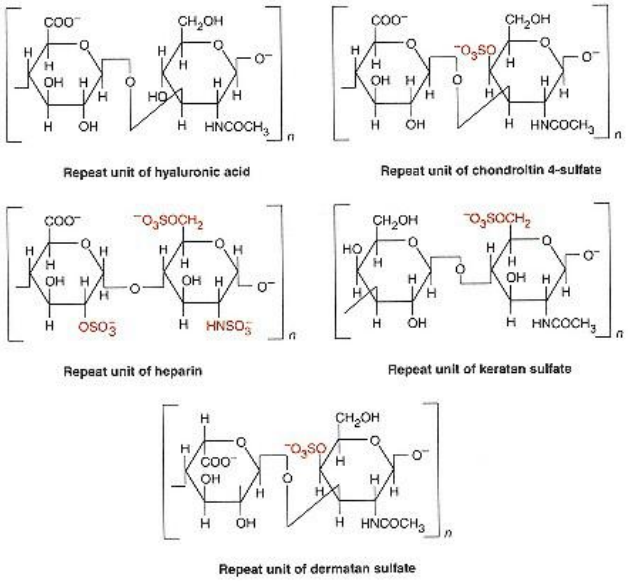


Figure 8.11
Major repeat units of glycosaminoglycan chains.

trolyte character, and large volume it occupies in solution all contribute to the properties of hyaluronate as a lubricant and shock absorbent. Hence it is found predominantly in synovial fluid, vitreous humor, and umbilical cord.

Chondroitin Sulfates Are the Most Abundant Glycosaminoglycans

The most abundant glycosaminoglycans in the body are the chondroitin sulfates. Individual polysaccharide chains are attached to specific serine residues in a protein core of variable molecular weight through a tetrasaccharide linkage region.

$$\text{GlcUA} \xrightarrow[1,3]{} \text{Gal} \xrightarrow[1,3]{} \text{Gal} \xrightarrow[1,4]{} \text{Xyl} \rightarrow \textit{O}\text{-Ser}$$

The characteristic repeating disaccharide units of *N*-acetylgalactosamine and glucuronic acid are covalently attached to this linkage region (Figure 8.11). The disaccharides can be sulfated in either the 4 or 6 position of *N*-acetylgalactosamine. Each polysaccharide chain contains between 30 and 50 such disaccharide units, corresponding to molecular weights of 15,000–25,000. An average chondroitin sulfate proteoglycan molecule has approximately 100 chondroitin sulfate chains attached to the protein core, giving a molecular weight of $1.5–2 \times 10^6$. Proteoglycan preparations are, however, extremely heterogeneous, differing in length of protein core, degree of substitution, distribution of polysaccharide chains, length of chondroitin sulfate chains, and degree of sulfation. Chondroitin sulfate proteoglycans have also been shown to aggregate noncovalently with hyaluronate, forming much larger structures. They are prominent components of cartilage tendons, ligaments, and aorta and have also been isolated from brain, kidney, and lung.

Dermatan Sulfate Contains L-Iduronic Acid

Dermatan sulfate differs from chondroitin 4- and 6-sulfates in that its predominant uronic acid is L-iduronic acid, although D-glucuronic acid is also present in variable amounts. The glycosidic linkages have the same position and configuration as in chondroitin sulfates, with average polysaccharide chains of molecular weights of 2–5 × 104. Unlike the chondroitin sulfates, dermatan sulfate is antithrombic like heparin, but in contrast to heparin, it shows only minimal whole-blood anticoagulant and blood lipid-clearing activities. As a connective tissue macromolecule, dermatan sulfate is found in skin, blood vessels, and heart valves.

Heparin and Heparan Sulfate Differ from Other Glycosaminoglycans

Heparin differs from other glycosaminoglycans in a number of important respects. Glucosamine and D-glucuronic acid or L-iduronic acid form the characteristic disaccharide repeat unit, as in dermatan sulfate (Figure 8.11). In contrast to most other glycosaminoglycans, heparin contains α-glycosidic linkages. Almost all glucosamine residues contain sulfamide linkages, but a small number of glucosamine residues are *N*-acetylated. The sulfate content of heparin, although variable, approaches 2.5 sulfate residues per disaccharide unit in preparations with the highest biological activity. In addition to *N*-sulfate and *O*-sulfate on C-6 of glucosamine, heparin can also contain sulfate on C-3 of the hexosamine and C-2 of the uronic acid. Unlike other glycosaminoglycans, heparin is an intracellular component of mast cells and functions predominantly as an anticoagulant and lipid-clearing agent (see Clin. Corr. 8.8 on p. 350).

Heparan sulfate contains a similar disaccharide repeat unit but has more *N*-acetyl groups, fewer *N*-sulfate groups, and a lower degree of *O*-sulfate groups. Heparan sulfate may be extracellular or an integral and ubiquitous component of the cell surface in many tissues including blood vessel walls, amyloid, and brain.

Keratan Sulfate Exists in Two Forms

Keratan sulfate is composed principally of the repeating disaccharide unit of *N*-acetylglucosamine and galactose, with no uronic acid in the molecule (Figure 8.11). Sulfate content is variable, with ester sulfate present on C-6 of both galactose and hexosamine. Two types of keratan sulfate differ in overall carbohydrate content and tissue distribution. Both contain as additional monosaccharides, mannose, fucose, sialic acid, and *N*-acetylgalactosamine. Keratan sulfate I, isolated from cornea, is linked to protein by an *N*-acetylglucosamine–asparaginyl bond, typical of glycoproteins. Keratan sulfate II, isolated from cartilage, is attached to protein through *N*-acetylgalactosamine in *O*-glycosidic linkage to either serine or threonine. Skeletal keratan sulfates are often found covalently attached to the same core protein as are the chondroitin sulfate chains.

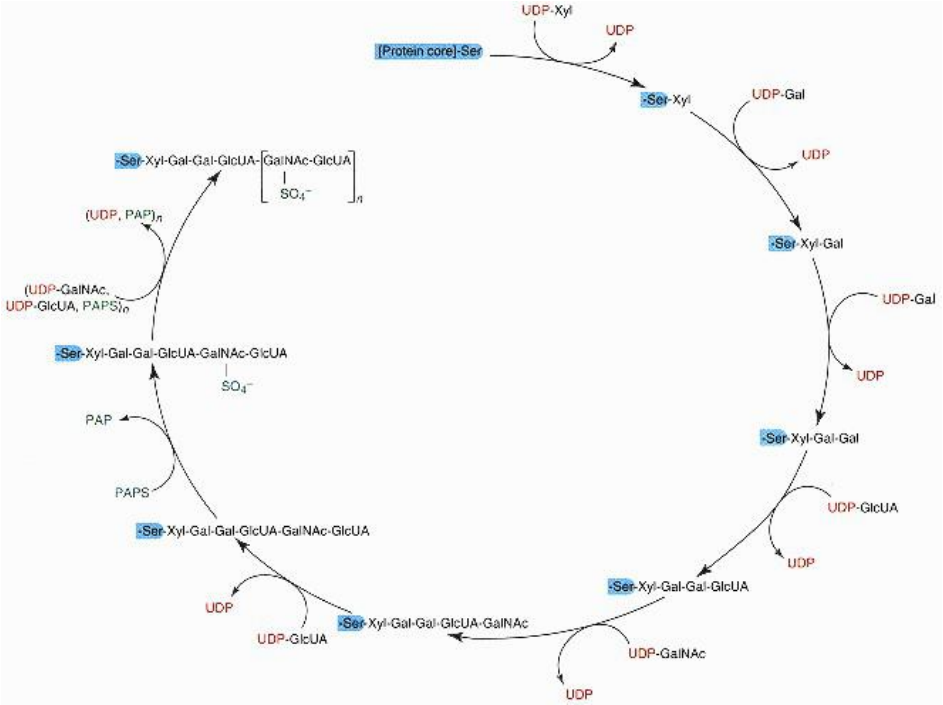


Figure 8.12
Synthesis of chondroitin sulfate proteoglycan.
Xyl, xylose; Gal, galactose; GlcUA, glucuronic acid; GalNAc, *N*-acetylgalactosamine; PAPS, 3 -phosphoadenosine 5 -phosphosulfate.

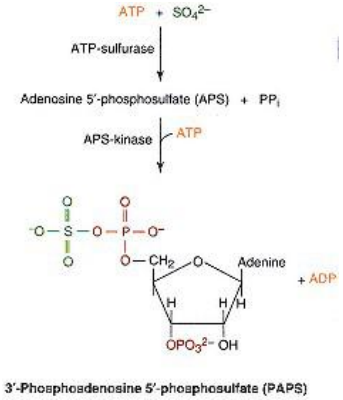


Figure 8.13
Biosynthesis of 3 -phosphoadenosine 5 -phosphosulfate (PAPS).

Biosynthesis of Chondroitin Sulfate Is Typical of Glycosaminoglycan Formation

The polysaccharide chains of proteoglycans are assembled by sequential action of a series of glycosyltransferases in the endoplasmic reticulum, which catalyze the transfer of a monosaccharide from a nucleotide sugar to an appropriate acceptor, either the nonreducing end of another sugar or a polypeptide. Since the biosynthesis of the chondroitin sulfates is most thoroughly understood, this pathway will be discussed as the prototype for glycosaminoglycan formation (Figure 8.12 on p. 355).

Formation of the core protein of the chondroitin sulfate proteoglycan is the first step in this process, followed by assembly of the polysaccharide chains catalyzed by six different glycosyltransferases in the lumen of the endoplasmic reticulum. Strict substrate specificity is required for completion of the unique tetrasaccharide linkage region. Polymerization then results from the concerted action of two glycosyltransferases, an *N*-acetylgalactosaminyltransferase and a glucuronosyltransferase, which alternately add the two monosaccharides, forming the characteristic repeating disaccharide units. Sulfation of *N*-acetylgalactosamine residues in either the 4 or 6 position apparently occurs along with chain elongation. The sulfate donor, as in other biological systems, is 3 -phosphoadenosine 5 -phosphosulfate (PAPS), which is formed from ATP and sulfate in two steps (Figure 8.13).

Synthesis of other glycosaminoglycans requires additional transferases specific for the sugars and linkages found in these molecules. Completion often involves modifications in addition to *O*-sulfation, including epimerization, acetylation, and *N*-sulfation. Interestingly, the epimerization of D-glucuronic acid to L-iduronic acid occurs after incorporation into the polymer chain and is coupled with the process of sulfation.

Synthesis of both proteoglycans and glycoproteins is regulated by the same mechanism at the level of hexosamine synthesis. The fructose 6-phosphate-glutamine transamidase reaction (Figure 8.4) is subject to feedback inhibition by UDP-*N*-acetylglucosamine, which is in equilibrium with UDP-*N*-acetylgalactosamine. More specific to proteoglycan synthesis, the levels of UDP-xylose and UDP-glucuronic acid are stringently controlled by the inhibition by UDP-xylose of the UDP-glucose dehydrogenase conversion of UDP-glucose to UDP-glucuronic acid (Figure 8.4). Since xylose is the first sugar added during synthesis of chondroitin sulfate, dermatan sulfate, heparin, and heparan sulfate, the earliest effect of decreased core protein synthesis would be accumulation of UDP-xylose, which aids in maintaining a balance between synthesis of protein and polysaccharide moieties of these complex macromolecules.

Proteoglycans, like glycoproteins, are degraded by the sequential action of proteases and **glycosidases,** as well as **deacetylases** and **sulfatases.** Much of the information about metabolism and degradation of proteoglycans has been derived from the study of **mucopolysaccharidoses** (see Clin. Corr. 8.9). This group of human genetic disorders is characterized by accumulation in tissues and excretion in urine of oligosaccharide products derived from incomplete breakdown of the proteoglycans, due to a deficiency of one or more lysosomal hydrolases. In the diseases for which the biochemical defect has been identified, a product accumulates that has a nonreducing terminus that would have been the substrate for the deficient enzyme.

Although proteoglycans continue to be defined on the basis of the glycosaminoglycan chain they contain, new ones are increasingly being described based largely on functional properties or location. Aggrecan and versican are the predominant extracellular species; syndecan, CD44, and thrombomodulin are integral membrane proteins; neurocan, brevican, cerebrocan, and phosphacan are largely restricted to the nervous system; while many proteoglycans (i.e., aggrecan, syndecan, and betaglycan) carry two types of glycosaminoglycan

chains, whose size and ratio may change with development, age, or disease. Thus it appears that the versatile structure of these abundant carbohydrate-containing molecules is well exploited by cells in many as yet undiscovered ways.

Bibliography

Dutton, G. J. (Ed.). *Glucuronic Acid, Free and Combined*. New York: Academic Press, 1966.

Ginsburg, V., and Robbins, P. (Eds.). *Biology of Carbohydrates*. New York: Wiley, 1984.

Horecker, B. L. *Pentose Metabolism in Bacteria*. New York: Wiley, 1962.

Kornfeld, R., and Kornfeld, S. Assembly of Asn-linked oligosaccharides. *Annu. Rev. Biochem.* 54:631, 1985.

Lennarz, W. J. (Ed.). *The Biochemistry of Glycoproteins*. New York: Plenum Press, 1980.

Menkes, J. A. Metabolic diseases of the nervous system. In: *Textbook of Child Neurology*, 4th ed. Philadelphia: Lea & Febiger, 1990, p. 28.

Nyhan, W. L., and Sakati, N. A. Mucopolysaccharidoses and related disorders. In: *Diagnostic Recognition of Genetic Disease*. Philadelphia: Lea & Febiger, 1987, p. 371.

Rademacker, T. W., Parck, R. B., and Dwek, R. A. Glycobiology. *Annu. Rev. Biochem.* 57:285, 1988.

Schwartz, N. B., and Smalheiser, N. Biosynthesis of glycosaminoglycans and proteoglycans. In: *Neurobiology of Glycoconjugates*. New York: Plenum Press, 1989, p. 151.

Scriver, C. R., Beaudet, A. L., Sly, W. S. and Valle, D. (Eds.) *The Metabolic and Molecular Bases of Inherited Disease*, 7th ed. New York: McGraw-Hill, 1995.

Questions

C. N. Angstadt and J. Baggott

1. [NADPH/$NADP^+$] is maintained at a high level in cells primarily by:

   A. lactate dehydrogenase.

   B. the combined actions of glucose-6-phosphate dehydrogenase and gluconolactonase.

   C. the action of the electron transport chain.

   D. shuttle mechanisms such as the α-glycerophosphate dehydrogenase shuttle.

   E. the combined actions of transketolase and transaldolase.

2. Transketolase:

   A. transfers a C_2 fragment to an aldehyde acceptor.

   B. transfers a C_3 ketone-containing fragment to an acceptor.

   C. converts the ketose sugar ribulose 5-phosphate to ribose 5-phosphate.

   D. is part of the irreversible oxidative phase of the pentose phosphate pathway.

   E. converts two C_5 sugar phosphates to fructose 6-phosphate and erythrose 4-phosphate.

3. If a cell requires more NADPH than ribose 5-phosphate:

   A. only the first phase of the pentose phosphate pathway would occur.

   B. glycolytic intermediates would flow into the reversible phase of the pentose phosphate pathway.

   C. there would be sugar interconversions but no net release of carbons from glucose 6-phosphate.

   D. the equivalent of the carbon atoms of glucose 6-phosphate would be released as 6 CO_2.

   E. only part of this need could be met by the pentose pathway, and the rest would have to be supplied by another pathway.

4. All of the following interconversions of monosaccharides (or derivatives) require a nucleotide-linked sugar intermediate EXCEPT:

   A. galactose 1-phosphate to glucose 1-phosphate.

   B. glucose 6-phosphate to mannose 6-phosphate.

   C. glucose to glucuronic acid.

   D. glucuronic acid to xylose.

   E. glucosamine 6-phosphate to *N*-acetylneuraminic acid (a sialic acid).

5. Fructose:

   A. unlike glucose, cannot be catabolized by the glycolytic pathway.

   B. in the liver, enters directly into glycolysis as fructose 6-phosphate.

   C. must be isomerized to glucose before it can be metabolized.

   D. is converted to a UDP-linked form and then epimerized to UDP-glucose.

   E. catabolism in liver uses fructokinase and a specific aldolase that recognizes fructose 1-phosphate.

6. Galactosemia:

   A. is a genetic deficiency of a uridylyltransferase that exchanges galactose 1-phosphate for glucose on UDP-glucose.

   B. results from a deficiency of an epimerase.

   C. is not apparent at birth but symptoms develop in later life.

   D. is an inability to form galactose 1-phosphate.

   E. would be expected to interfere with the use of fructose as well as galactose because the deficient enzyme is common to the metabolism of both sugars.

7. All of the following are true about glucuronic acid EXCEPT:

   A. it enhances the water solubility of compounds to which it is conjugated.

   B. as a UDP derivative, it can be decarboxylated to a component used in proteoglycan synthesis.

   C. it is a precursor of ascorbic acid in humans.

   D. its formation from glucose is under feedback control by a UDP-linked intermediate.

   E. it can ultimately be converted to xylulose 5-phosphate and thus enter the pentose phosphate pathway.

8. The conversion of fructose 6-phosphate to glucosamine 6-phosphate:

   A. is a transamination reaction with glutamate as the nitrogen donor.

   B. is stimulated by UDP-*N*-acetylglucosamine.

   C. requires that fructose 6-phosphate first be linked to a nucleotide.

   D. is a first step in the formation of *N*-acetylated amine sugars.

   E. occurs only in the liver.

9. Roles for the complex carbohydrate moiety of glycoproteins include all of the following EXCEPT:

A. determinant of blood type.

B. template for the synthesis of glycosaminoglycans.

C. cell surface receptor specificity.

D. determinant of the rate of clearance from the circulation of certain molecules.

E. targeting of cells to certain tissues.

10. Glycoproteins:

A. may contain 95% or more carbohydrate.

B. always contain a serially repeating carbohydrate unit.

C. are found only intracellularly.

D. from different animal species may have a different primary sequence in the protein but have identical carbohydrate chains.

E. have the carbohydrate linked to the protein by either *N*- or *O*-glycosidic bonds.

11. The carbohydrate core structure is assembled on dolichol phosphate before transfer to the protein for:

A. *N*-linked glycoproteins.

B. *O*-linked glycoproteins.

C. proteoglycans.

D. glycosaminoglycans.

E. all of the above.

12. Fucose and sialic acid:

A. are found most commonly in *N*-linked glycoproteins.

B. are the parts of the carbohydrate chain that are covalently linked to the protein.

C. can be found in the core structure of certain *O*-linked glycoproteins.

D. are transferred to a carbohydrate chain when it is attached to dolichol phosphate.

E. are the repeating unit of proteoglycans.

13. Glycosaminoglycans:

A. are the carbohydrate portion of glycoproteins.

B. contain large segments of a repeating unit typically consisting of a hexosamine and a uronic acid.

C. are low molecular weight cations.

D. exist in only two forms.

E. are bound to protein by ionic interaction.

Refer to the following for Questions 14–16.

A. chondroitin sulfate

B. dermatan sulfate

C. heparin

D. hyaluronate

E. keratan sulfate

14. Differs from other glycosaminoglycans in being predominantly intracellular rather than extracellular.

15. Only glycosaminoglycan not covalently linked to protein.

16. The most abundant glycosaminoglycan in the body.

17. All of the following are true of proteoglycans EXCEPT:

A. specificity is determined, in part, by the action of glycosyltransferases.

B. synthesis is regulated, in part, by UDP-xylose inhibition of the conversion of UDP-glucose to UDP-glucuronic acid.

C. synthesis involves sulfation of carbohydrate residues by PAPS.

D. synthesis of core protein is balanced with synthesis of the polysaccharide moieties.

E. degradation is catalyzed in the cytosol by nonspecific glycosidases.

Answers

1. B Although the glucose-6-phosphate dehydrogenase reaction, specific for NADP, is reversible, hydrolysis of the lactone assures that the overall equilibrium lies far in the direction of NADPH. A, C, and D: These all use NAD, not NADP. E: These enzymes are part of the pentose phosphate pathway but catalyze freely reversible reactions that do not involve NADP (pp. 337–338).

2. A Both reactions catalyzed by transketolase are of this type. B and E describe transaldolase. C describes an isomerase. D: Transketolase is part of the reversible phase of the pentose phosphate pathway that also allows glycolytic intermediates to be converted to pentose sugars, if necessary (p. 338).

3. D A, C, D, E: Glucose 6-phosphate yields ribose 5-phosphate + CO_2 in the oxidative phase. If this is multiplied by six, the six ribose 5-phosphates can be rearranged to five glucose 6-phosphates by the second, reversible phase. B: If more ribose 5-phosphate than NADPH were required, the flow would be in this direction to supply the needed pentoses (pp. 338–340).

4. B The glucose and mannose phosphates are both in equilibrium with fructose 6-phosphate by phosphohexose isomerases. A: This occurs via an epimerase at the UDP-galactose level. C and D: This oxidation of glucose is catalyzed by UDP-glucose dehydrogenase and the product can be decarboxylated to UDP-xylose. E: Again, an epimerization occurs on the nucleotide intermediate (p. 342, Fig. 8.4).

5. E A, C, E: Fructokinase produces fructose 1-phosphate. Since this cannot be converted to fructose 1,6-bisphosphate, a specific aldolase cleaves it to dihydroxyacetone phosphate and glyceraldehyde. The first product is a glycolytic intermediate; the second requires modification to enter glycolysis. D: Glucose and fructose are not epimers (p. 342, Clin. Corr. 8.2).

6. A B: The epimerase is normal. C: Galactose is an important sugar for infants. E: Fructose metabolism does not use the uridylyltransferase that is deficient in galactosemia (p. 353, Clin. Corr. 8.3).

7. C Humans do not make ascorbic acid. A: Enhancing water solubility is a major physiological role for glucuronic acid,

for example, bilirubin metabolism. B and D: Decarboxylation of UDP-glucuronic acid gives UDP-xylose, which is a potent inhibitor of the oxidation of UDP-glucose to the acid. E: The reduction of D-glucuronic acid to L-gulonic acid leads to ascorbate as well as xylulose 5-phosphate for the pentose phosphate pathway (p. 345, Figure 8.7).

8. D Glucosamine 6-phosphate is acetylated. UDP-*N*-acetylglucosamine is formed, and the UDP derivative can be epimerized to the galactose derivative. A and C: This conversion is a transamidation of the amide nitrogen of glutamine and does not involve nucleotide intermediates. B: This is a feedback inhibitor of the transamidase reaction, thus controlling formation of the nucleotide sugars. E: May account for 20% of the glucose flux in skin (p. 344).

9. B Synthesis of complex carbohydrates is not template directed but determined by the specificity of individual enzymes. A, C, D, and E: Because of the diversity possible with oligosaccharides, they play a significant role in determining the specificity of many biological molecules (pp. 345–347).

10. E A and B: This is true of proteoglycans. C: Some hormones in blood are glycoproteins. D: Heterogeneity of carbohydrates is common (pp. 348–350).

11. A This is characteristic of *N*-linked glycoproteins. B, C, and D: Synthesis of *O*-linked glycoproteins involves the sequential addition to the *N*-acetylgalactosamine linked to serine or threonine (p. 350).

12. C Core also contains galactose and *N*-acetylgalactosamine. A and D: Core structure of *N*-linked carbohydrates contains mannose and *N*-acetylglucosamine. B: Usually found at the periphery of the carbohydrate. E: Repeating unit is hexosamine and uronic acids (pp. 350 and 353).

13. B This is a major distinction from glycoproteins, which, by definition, do not have a serial repeating unit. A: These are carbohydrate of proteoglycans. C: They have anionic character contributed by carboxyl and sulfate (another common feature) groups. D: There are at least six different classes. E: Carbohydrates are bound by covalent links (p. 353).

14. C (p. 354).

15. D Classified as a glycosaminoglycan because of its structural similarity to the others (p. 353).

16. A (p. 354).

17. E Degradation is lysosomal; deficiencies of one or more lysosomal hydrolases lead to accumulation of proteoglycans in the mucopolysaccharidoses. A: Strict substrate specificity of the enzymes is important in determining the type and quantity of proteoglycans synthesized. Formation of specific protein acceptors for the carbohydrate is also important. B and D: Both xylose and glucuronic acid levels are controlled by this; xylose is the first sugar added in the synthesis of four of the six types and would accumulate if core protein synthesis is decreased. C: This is necessary for the formation of all proteoglycans (hyaluronic acid is not part of a proteoglycan) (p. 356).