

# GLYCOGENOLYSIS AND GLYCOLYSIS IN MUSCLE

## The Cellular Degradation of Sugar and Carbohydrate to Pyruvate and Lactate

**A**s described in Chapter 3, speed-power activities such as 400-m sprinting, and court and field games such as basketball and football, are energetically driven by the combination of immediate and nonoxidative energy sources. However, the importance of nonoxidative, glycolytic energy sources described in this chapter extend far beyond a role in sustaining activities lasting a few minutes or less. Glycolytic metabolism provides the monocarboxylate substrates (lactate and pyruvate) that are the main fuels for oxidative metabolism. Glycolytic metabolism so dominates fuel selection in muscle and other tissues that fuel-energy substrate partitioning—that is, the use of other fuels such as fatty acids—is largely determined by the availability of monocarboxylates from glycolysis. Further, lactate and pyruvate are the main gluconeogenic precursors used in gluconeogenesis, a process that helps maintain blood glucose homeostasis in prolonged exercise.

Of the three main foodstuffs (carbohydrates, fats, and proteins), only carbohydrates can be degraded without the direct participation of oxygen. The main product of dietary sugar and starch digestion is glucose, which is released into the blood of the systemic circulation. The simple sugar glucose enters cells, including muscle and liver cells,



**Figure 5-1** Michael Johnson, once one of the world's premier long sprinters, in action. Success in activities such as sprinting requires great muscular power and the ability of muscles to operate from chemical energy stores which do not immediately require  $O_2$  for metabolism. PHOTO: © Steven E. Sutton/Duomo/Corbis.

and is either used directly or stored for later use. The first stage of cellular glucose catabolism is called glycolysis. Glucose molecules not undergoing glycolysis can be linked together to form the carbohydrate storage form called glycogen. Glycogen stored in muscle is broken down in a process called glycogenolysis. These processes occur in most cells but are specialized in muscle cells. Glycolysis is the only energy source in red blood cells. In Chapter 5, we emphasize the muscular use of carbohydrate (CHO), but this is only part of a contemporary view of CHO metabolism. In Chapter 9, roles of the endocrines and the gluconeogenic organs (liver and kidneys) in maintaining blood glucose homeostasis will be emphasized.

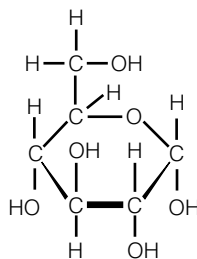
Readers of *Exercise Physiology* will find treatment of the relationship between glycolysis and oxidative metabolism to be very different from presentations of these topics found in most other texts on physiology and biochemistry. Differences in presentation are due to discoveries by the coauthors (KMB and GAB) concerning muscle fiber type heterogeneity, the presence of lactate transporters, their distribution in cells and tissues of humans and other species, and the ability of mitochondria to take up and oxidize lactate directly. As will be described in the following section, since the 1920s, it has been believed that lactate is produced in muscle and other cells as the result of insufficient oxygen. However, it is now clear that lactate is continuously produced and removed. Hence, in resting muscle there is 10 times more lactate than pyruvate and the lactate/pyruvate ratio increases many times during contractions. Even though glycolysis inevitably results in lactate formation, this formation is of little consequence so long as the lactate is removed. Lactate is removed mainly by oxidation ( $\approx 75\%$ ), with the remainder removed by gluconeogenesis ( $\approx 25\%$ ). As such, lactate is a major energy source. In addition to new primary references (Bergman, 1992; Dubouchaud, 1999; Miller et al., 2002a, 2002b), readers are referred to recent reviews by Brooks and Gladden (2003) and Gladden (2004).

Recognition that lactate moves between lactate-producing and lactate-consuming cells led to for-

mulation of the *lactate shuttle concept*. Highly glycolytic (white, type IIb) muscle fibers were thought to be the primary sites of lactate formation. The classic, cell-cell lactate shuttle concept posited that highly oxidative cells (red, slow oxidative and cardiac muscle cells) were the sites of lactate removal. Recent discoveries indicating that mitochondria take up and oxidize lactate directly have led to formulation of the *"intracellular lactate shuttle concept."* According to this concept, glycolysis results in the formulation of lactate because of the abundance of the terminal glycolytic enzyme (lactate dehydrogenase, LDH), in which the equilibrium constant ( $K_{eq}$ ) of LDH is  $3.6 \times 10^4 \text{ mol}^{-1}$ . Thus, glycolysis in the cytosol results in lactate production, most of which is consumed by mitochondria that have the enzymatic apparatus to take up and oxidize lactate. The cell-cell and intracellular lactate shuttles function because some cells, such as those found in red skeletal muscle and cardiac muscles and liver, contain high mitochondrial densities.

## ■ The Dietary Sources of Glucose

*Glucose*, a six-carbon sugar, is the primary product of photosynthesis (Figure 5-2). Glucose is produced by plants that use it as a fuel, much as we do, and store it by linking the molecules together to form starch. Plants such as potatoes that store large



**Figure 5-2** Structure of glucose, a simple sugar. Five carbons and an oxygen atom serve to create a hexagonal ring conformation. Shaded lines represent the three-dimensional platelike structure.

amounts of starch are very useful to us as foodstuffs. Plants also link glucose molecules together in a complex pattern to form cellulose for structural purposes, but humans lack the enzymes necessary to digest this glucose polymer.

There are numerous dietary sources of glucose, including starches, such as rice, pasta, and potatoes, and dietary sugars, such as granulated sugar and brown sugar. Most of these enter the bloodstream in the form of glucose and lactate. Large starch molecules are split fairly rapidly into disaccharides and glucose by the action of the pancreatic enzymes known as amylases. Sugars other than glucose are largely isomerized—that is, converted to glucose by the wall of the small intestine or by the liver. In Chapter 28, we shall see that the form of delivery influences the rate of glucose delivery to the systemic circulation, and so physiological responses to sugar and complex carbohydrate feeding can differ, even if the carbon content of meals is the same.

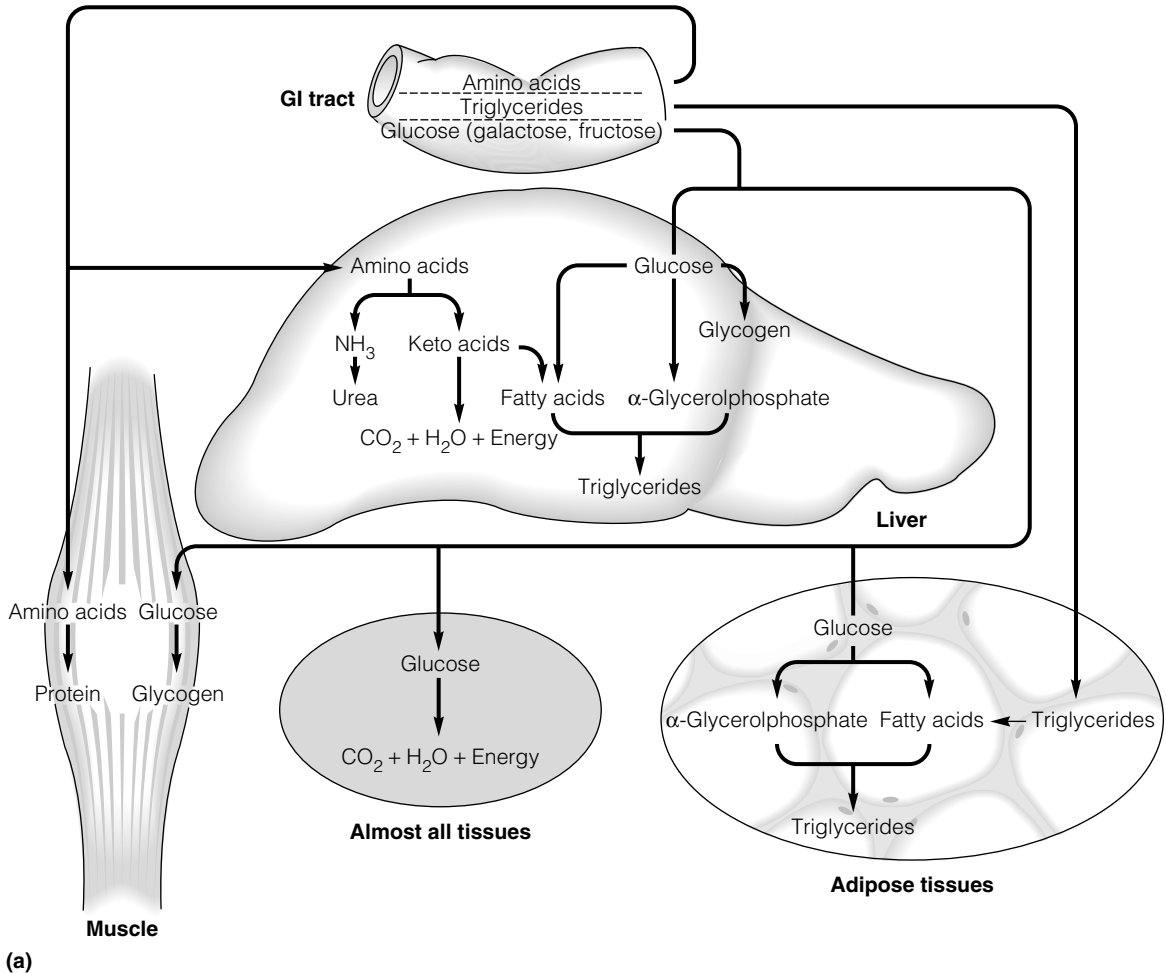
The uptake of glucose by cells depends on several factors, including the type of tissue, the levels of glucose in the blood and tissue, the presence of the hormone insulin, and the physiological status of the tissue. Most tissues—with the notable exception of muscles during contraction—require insulin in order to take in glucose. The unique mechanism by which muscles take up glucose during exercise is currently being studied (Chapter 9). Nerve and brain tissues usually consume large amounts of glucose; the liver also usually takes up large amounts even if insulin is low. In the past, it was thought that the liver only stores and releases glucose but does not utilize it as a fuel. We now know, however, that under appropriate circumstances—that is, when the blood levels of both glucose and insulin are high—liver cells can use glucose. In adipose cells, the presence of glucose stimulates fat synthesis (Figure 5-3a).

The storage of dietary glucose under postprandial (after eating) conditions is illustrated in Figure 5-3a. Besides use as an energy substrate (fuel), glucose is directed mainly to storage as glycogen in liver and muscle tissues and as triglyceride

in adipose tissue. Figure 5-3b illustrates that the mobilization of substrates for energy and gluconeogenesis (the making of glucose) under postabsorptive (fasting) conditions is maintained by degradation of glycogen in the liver (i.e., glycogenolysis) and production of glucose in the liver and kidneys from precursors (mainly lactate) delivered in the circulation.

### ■ Direct vs. Indirect Pathways of Liver Glycogen Synthesis: The “Glucose Paradox”

Figure 5-4 diagrams the “new glucose to liver glycogen pathway.” Of course, this pathway itself is not “new,” as it likely has been inherited from our earliest ancestors; it is recognition of the pathway’s importance that is “new.” In contrast to Figure 5-3, Figure 5-4 shows that liver glycogen can be synthesized indirectly from three-carbon precursors, mainly lactate. When the pathway of liver glycogen synthesis from dietary carbohydrate involves hepatic uptake of glucose and addition of glucose to already existing glycogen molecules, the pathway of liver glycogen synthesis is said to be *direct*. However, when the pathway involves the degradation of glucose to lactate, and then conversion of lactate to glycogen, the pathway is said to be *indirect*. Because skeletal muscle is the largest tissue containing enzymes of glycolysis, much of the glucose-to-lactate conversion is thought to occur in muscle. Other cells and tissues containing enzymes of glycolysis, including red blood cells (erythrocytes), adipose cells (adipocytes), and portions of the liver itself, are also likely to contribute to lactate formation in the indirect pathway. Because of the circuitous route of dietary carbohydrate carbon flow—that is, the use of lactate (an indirect precursor) rather than glucose (a direct precursor)—in liver glycogen synthesis, the phenomenon is sometimes also called the “glucose paradox.” Current best estimates are that 60% of liver glycogen synthesis is by the direct pathway, whereas 40% is by the indirect, paradoxical pathway. However,

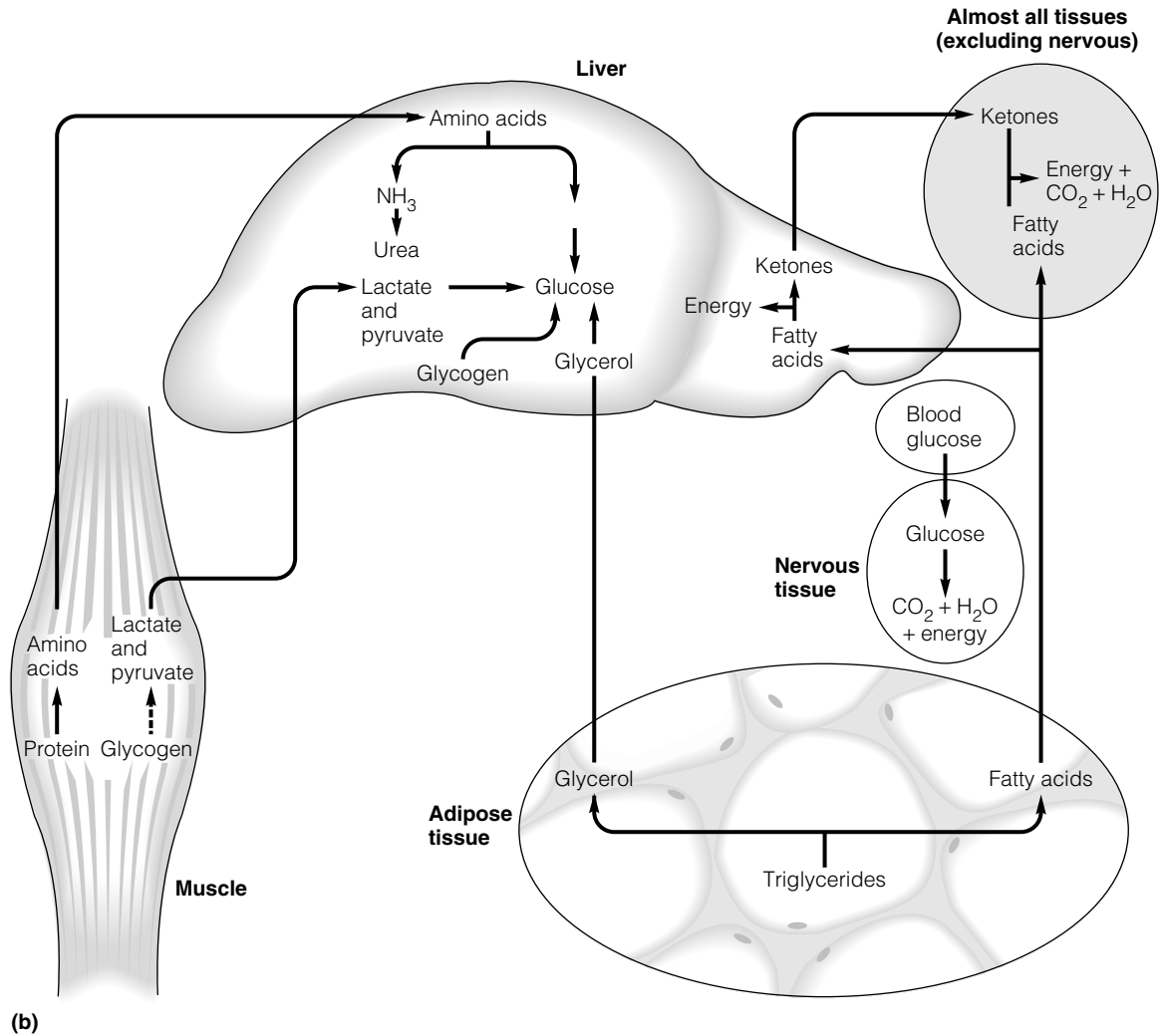


**Figure 5-3** Flowchart of glucose and other metabolites in an individual (a) immediately after a meal and (b) during fasting. Modified from Vander, Sherman, and Luciano, 1980. Used with permission.

conditions prior to and immediately after eating are likely to greatly affect the pathway of liver glycogen synthesis.

Recognition by biochemists that lactate is important in the synthesis of liver glycogen after eating comes as little surprise to exercise physiologists, who have long known that during and after exercise blood glucose supply is maintained by gluconeogenesis via the Cori cycle (discussed in more detail

later in this chapter). However, it is important to note here that, like the Cori cycle, the concept of the indirect pathway is part of a revision in current thinking which indicates that the formation, distribution, and utilization of lactate is a central means by which carbohydrate metabolism in diverse tissues is coordinated. This overall conceptual scheme, termed the *lactate shuttle*, is discussed more in a later section.



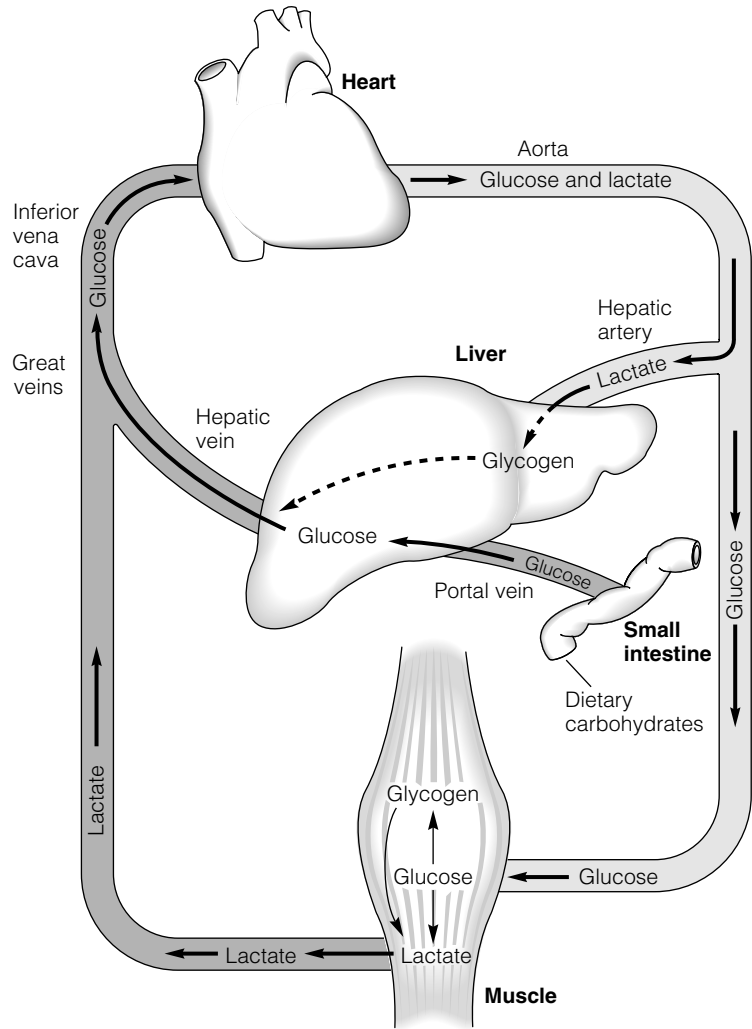
**(b)** Figure 5-3 (continued)

### ■ Blood Glucose Concentration During Rest and Exercise

The concentration of glucose in plasma is one of the most precisely regulated physiological variables (Chapter 9). Normal blood glucose (“euglycemia”) in postabsorptive humans is approximately  $100 \text{ mg} \cdot \text{dl}^{-1}$  (100 mg %, or 5.5 mM). This level of glucose is required for function of the central nervous system

(CNS) and other glucose-requiring systems, organs, and cells. For this reason, the Recommended Daily Allowance for dietary carbohydrate is 130 g/day (equivalent to 520 kcal/day). During exercise, maintenance of blood glucose homeostasis becomes a major physiological challenge as skeletal muscle suddenly switches from a situation of little glucose uptake to a situation of greatly increased glucose uptake. Therefore, release of glucose by the liver

**Figure 5-4** Diagram of the new glucose to hepatic glycogen pathway (“glucose paradox”) by which the liver prefers to make glycogen from lactate as opposed to glucose. Glucose released into the blood from the digestion of dietary carbohydrate bypasses the liver and is taken up by skeletal muscle. The muscle can either synthesize glycogen or produce lactate. The lactate then recirculates to the liver and stimulates glucose and glycogen formation. See Foster (1984) for additional information.

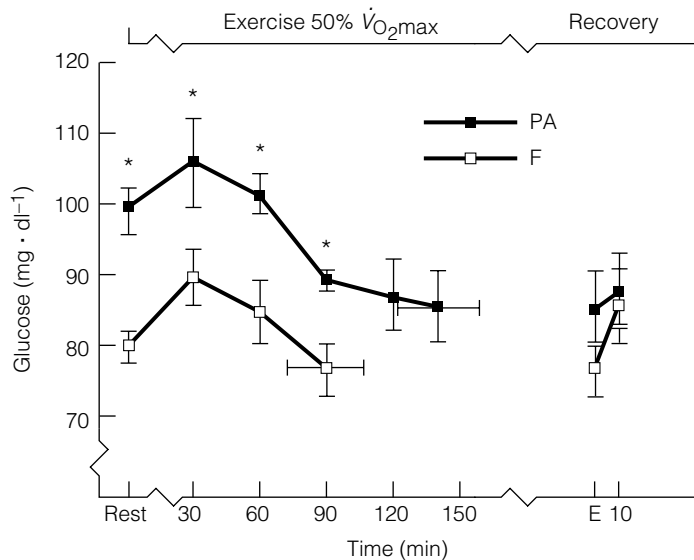


(the liver being the main organ of glucose production) must rise from a value of approximately  $1.8 \text{ mg} \cdot \text{kg body weight}^{-1} \cdot \text{min}^{-1}$  to a much higher value. For instance, during exercise at  $50\% \dot{V}_{O_{2\max}}$ , hepatic glucose production (HGP) rises to a value of approximately  $3.5 \text{ mg} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ . Even greater values of HGP can be observed in maximal exercise.

A typical response of blood glucose in postabsorptive men is shown in Figure 5-5. From the normal value of about  $100 \text{ mg/dl}$ , glucose rises when

exercise starts. This rise is due to a hormonally, and perhaps neurally, mediated feed-forward mechanism (Chapter 9). Then, over time, depending on liver glycogen reserves and other factors, glucose may fall, but remain within approximately 10% of the normative value until exercise stops.

Figure 5-5 also shows the blood glucose response in the same subjects after a 36-hour fast. The concentration of glucose at rest is decreased approximately 20%, but this decrease is compensated for by doubling the levels of alternative fuels such



**Figure 5-5** Blood glucose concentration at rest, during exercise to exhaustion at 50%  $\dot{V}O_{2max}$  and 10-min postrecovery under postabsorptive (PA), over night, 12h fast and 36h fasting (F) conditions. SE vertical bars are included for each sampling point, and horizontal bars are shown for time to exhaustion. \* Significant difference between means (PA vs. F,  $P < 0.05$ ). SOURCE: Zinker et al., 1990. Used with permission of the American Physiological Society.

as free fatty acids and ketones (Chapter 7). Even in the fasting state, glucose rises for a time during exercise but then falls, probably because liver glycogen reserves are depleted by the 36-hour fast, and gluconeogenesis cannot fully compensate. Low and falling blood glucose concentrations (hypoglycemia, glucose of  $\leq 3.5$  mM,  $65 \text{ mg} \cdot \text{dl}^{-1}$ ) are frequently associated with fatigue.

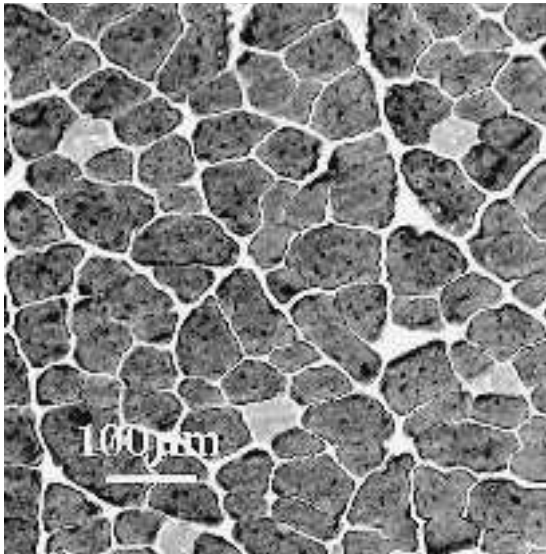
Although but one of many illustrations in this chapter, Figure 5-5 is one of the most important as it demonstrates blood glucose homeostasis under multiple stresses. Thus, the figure links Chapter 5 (on glucose use) to Chapter 9 (on glucose production to maintain euglycemia). Now, as we delve into details of cell carbohydrate use to maintain cell ATP homeostasis, remember also that maintenance of blood glucose homeostasis is an equally important physiological priority.

## ■ Glycolysis

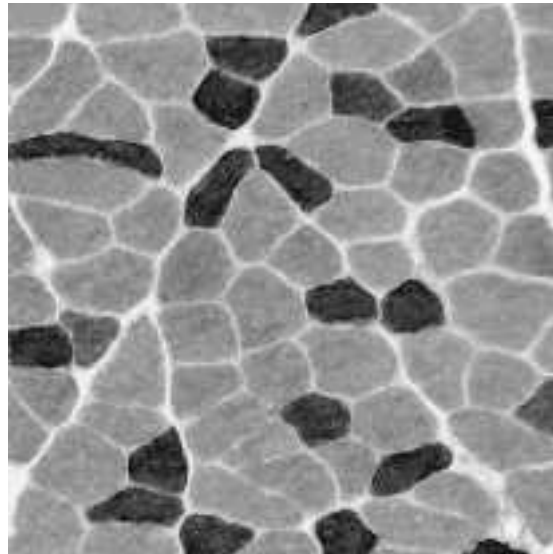
The metabolic pathway of glucose breakdown in mammalian cells is called *glycolysis* (glyco/lysis), the dissolution of sugar. The process of glycolysis is

frequently referred to as a metabolic pathway because the process occurs over a specific route, in either 11 or 12 specific steps, each of which is catalyzed and regulated by a specific enzyme. The pathway is sometimes called *anaerobic*, because oxygen is not directly involved.

The study and appreciation of glycolysis predates recorded history, thanks to the process of fermentation. Fermentation occurs when yeast carry glycolysis a few extra steps and produces ethyl alcohol,  $\text{CO}_2$ , and vinegar. There is some evidence that the earliest communities in ancient Egypt were organized around structures that served as bakeries and breweries. It was probably discovered quite by accident that grain left to soak fermented and became beer, and that leavening (yeast) added to dough fermented and yielded bread after baking. The practice of fermentation technology, however, has been extremely important in the development of many cultures. Louis Pasteur was famous even before he developed any vaccines to prevent diseases. Pasteur discovered a method for preventing ordinary table wine from fermenting to vinegar. Today, his process, called *pasteurization*, is more frequently used to preserve milk.



(a)



(b)

**Figure 5-6** Transverse sections of skeletal muscle from a rodent, incubated for myofibrillar-ATPase at pH 10.3. Panel (a) is a section from the extensor digitorum longus (EDL) muscle, which is composed primarily of fibers that have high glycolytic capacity. Note the high proportion of dark-appearing fibers in the EDL muscle. In contrast, panel (b) is a section from the soleus (SOL) muscle, which is composed primarily of fibers lower in glycolytic capacity (but high in oxidative type I fibers). Note the large number of light-appearing fibers. The details regarding the diversity of fiber types are developed in Chapter 18. SOURCE: Micrographs, courtesy of T. P. White.

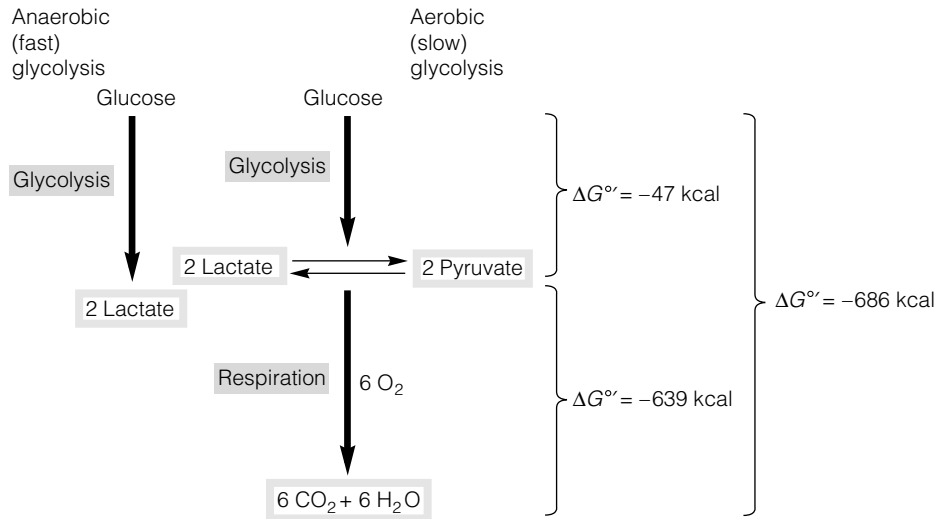
### Glycolysis in Muscle

The process of glycolysis is very active in skeletal muscle, which is often termed a *glycolytic tissue*. In particular, pale or white skeletal muscles contain large quantities of glycolytic enzymes. As we will see later, highly oxidative red muscle fibers and heart cells are capable of rapid glycolysis, but glycolysis is the main energy source for white muscle fibers during exercise (Figure 5-6). Because it is the predominant metabolic pathway of energy conservation in amphibians and reptiles, glycolysis is frequently considered a “primitive pathway.” The hearts and lungs of these animals are poorly developed and cannot utilize oxidative metabolism for bursts of activity; they must rely, therefore, on glycogenolysis and glycolysis.

### Aerobic, Anaerobic, and “Nonrobic” Glycolysis in the Cytosol

There are two forms of glycolysis: aerobic (“with oxygen”) and anaerobic (“without oxygen”). Historically, these terms were developed by scientists, such as Pasteur, who studied glycolysis in yeast and other unicellular organisms in test tubes where air was either present or was flushed out by gassing with substances like nitrogen. Pasteur noted that when oxygen was present (aerobic), glycolysis was slow and acid products did not accumulate. When oxygen was removed (anaerobic), Pasteur found that glycolysis was rapid. Today, we also know that in most cells glycolysis proceeds the additional step beyond pyruvic acid to form lactic acid even when oxygen is present in abundance (Figure 5-7).



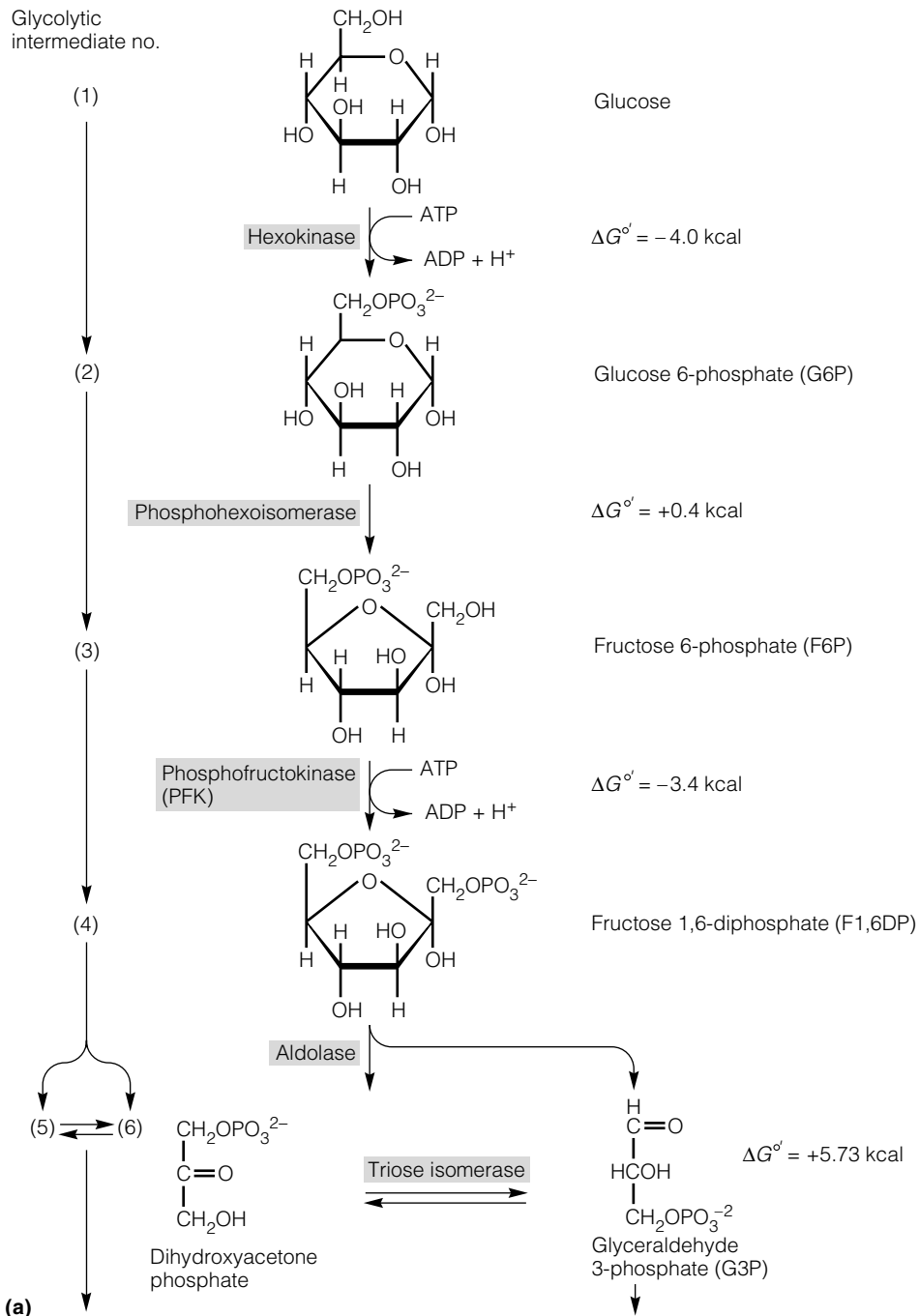


**Figure 5-7** In anaerobic (fast) glycolysis, lactic acid is the product. In aerobic (slow) glycolysis, pyruvic acid is the main product. The terms *aerobic* (with O<sub>2</sub>) and *anaerobic* (without O<sub>2</sub>) refer to the test tube conditions used by early researchers to speed up or slow down glycolysis. In real life, pyruvate and lactate pools are in equilibrium, and the rapidity of glycolysis largely determines the product formed. Note the far greater energy released under aerobic conditions. Modified from Lehninger, 1973. Used with permission.

The early experimentation and terminology has led to some confusion in contemporary physiology, because when pioneer scientists observed increased levels of lactic acid in muscle and blood as the result of exercise, they assumed that the tissue was anaerobic (without oxygen) during exercise. As we will see, however, there are several reasons for lactic acid formation; no oxygen or insufficient oxygen supply is seldom one of them. For instance, maximal exercise under the low oxygen condition of high altitude exposure results in little lactate accumulation compared to maximal exercise at sea level. Further, a constant level of lactic acid in the blood does not mean that no lactic acid is formed, only that production and removal are equivalent. Also, there is good evidence that lactic acid is always produced, even at rest, as in the indirect pathway of liver glycogen synthesis after eating. Recently, in experiments on men studied during rest and exercise, using infusion of a lactic acid/sodium lactate cocktail containing glucose and lactate tracers, to clamp

blood [lactate] at 4 mM Miller et al. showed that lactate was the preferred fuel during exercise. Therefore, the terms *aerobic* and *anaerobic* as applied to mammalian systems are archaic. We can deal with these terms and use them as long as we keep in mind their actual meanings. Perhaps more descriptive terms such as “rapid” (for anaerobic) and “slow” (for aerobic) will eventually come into use. Because much of glycolysis has little to do with the presence of O<sub>2</sub>, the process itself is essentially “nonrobic”; that is, it does not involve O<sub>2</sub>. *Note:* Before closing this section, it is important to mention that “nonrobic” is used for emphasis and is *not* a generally appropriate or widely accepted and used term.

Glycolysis is a process that occurs mainly in the cytosol, where the enzymes are concentrated. However, some glycolytic enzymes, such as lactate dehydrogenase (LDH), exist in other cellular organelles, such as mitochondria and peroxisomes. The glycolysis pathway is depicted in two ways in Figures 5-8 and 5-9. Figure 5-8 is a detailed presentation that



**Figure 5-8** Detailed presentation of the glycolytic pathway. Glycolytic intermediates are identified by name to the right of each structure and by number to the left; catalyzing enzymes are noted on the left of each reaction step. Free energy changes are  $\text{mol}^{-1}$ .

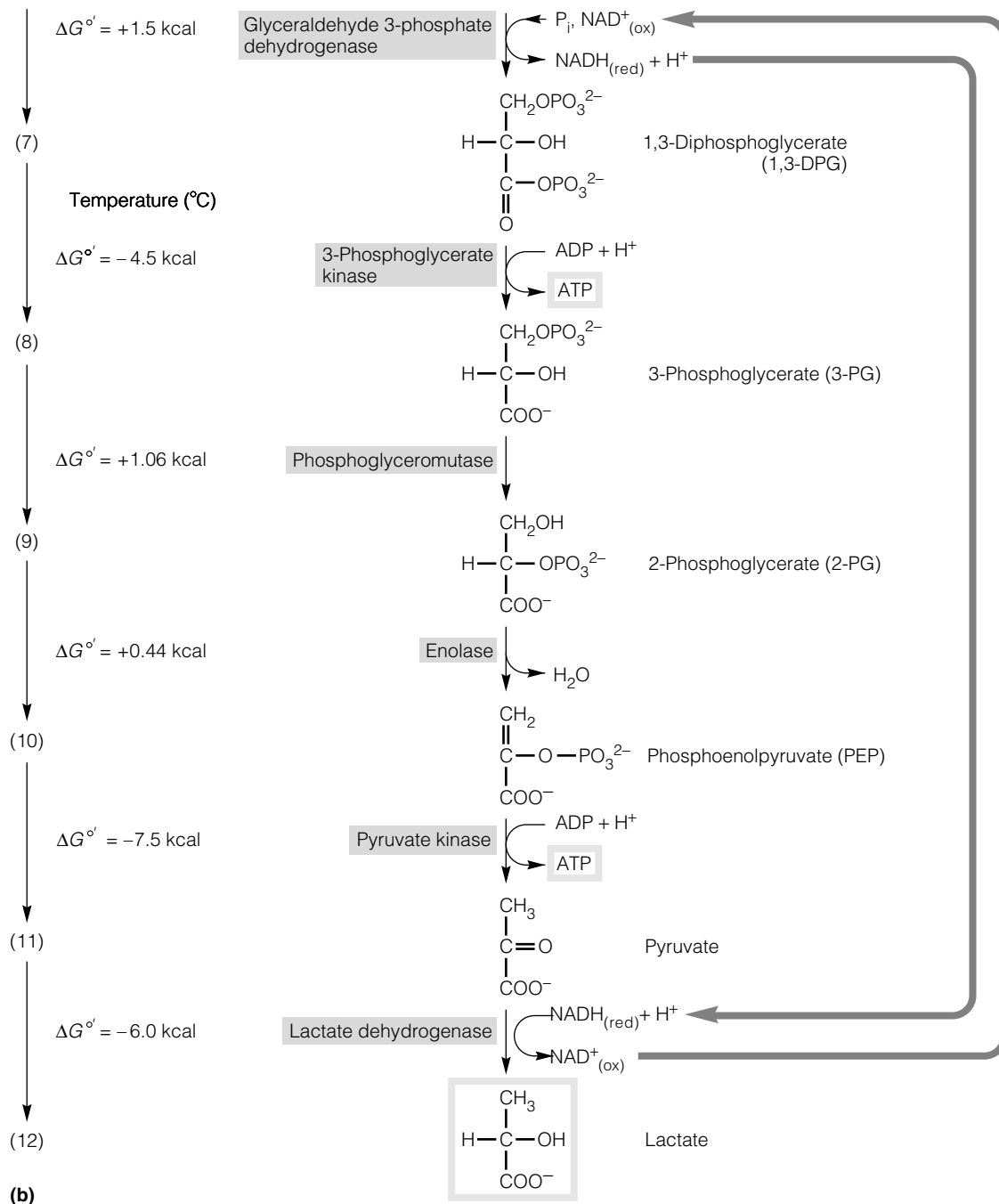
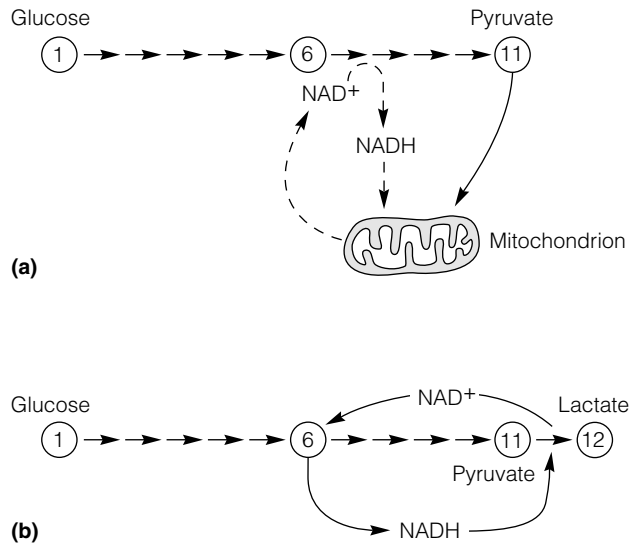


Figure 5-8 (continued)

**Figure 5-9** Simplified and conventional portrayal of glycolysis showing the beginning and ending substances for (a) slow and (b) rapid glycolysis. In real life, however, the lactate/pyruvate ratio is  $\geq 10.0$ . Hence, glycolysis always proceeds to lactate.

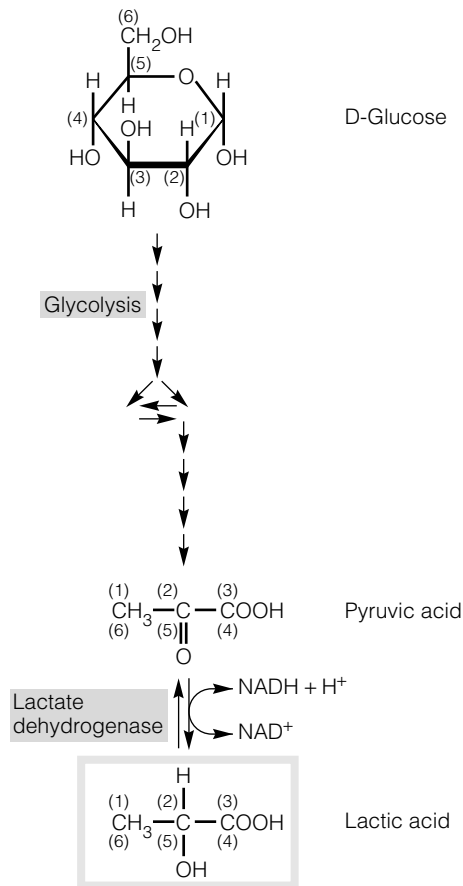


will serve as a reference. Figure 5-9 is designed to contribute to an understanding of how the pathway is controlled when the rate (flux) of glycolysis is slow (Figure 5-9a), and when flux is rapid (5-9b). In glycolysis, one six-carbon sugar is split into two three-carbon carboxylic acids. Pyruvic and lactic acid each possess a carboxyl group (Figure 5-10). At physiological pH, these molecules dissociate a hydrogen ion ( $H^+$ ) and therefore are acids. The terms *pyruvate* and *lactate*, properly meaning salts of the respective acids, are generally used interchangeably with pyruvic acid and lactic acid. Figure 5-10 illustrates why glycolysis inevitably results in lactate production. The standard free-energy change for the reaction catalyzed by lactate dehydrogenase ( $\Delta G^\circ$ ) is very high ( $-6.0$  kcal/mol), as is the equilibrium constant ( $K_{eq}$   $3.6 \times 10^4$  mol<sup>-1</sup>). Consequently, even considering  $K_M$  differences among the various LDH isoforms, in the cytosol, pyruvate not immediately entering mitochondria is reduced to lactate. For this reason, lactate accumulation is typical in red blood cells (erythrocytes), which do not have mitochondria, and type IIb muscle fibers with high glycolytic flux rates and low mitochondrial density.

The substance diagrammed in Figure 5-11a is called nicotinamide adenine dinucleotide ( $NAD^+$ ). Because of its unique structure,  $NAD^+$  can exist in

two forms:  $NAD^+$  (oxidized) and  $NADH$  (reduced, Figure 5-11b). We shall encounter  $NAD^+$  frequently, because it transfers hydrogen ions and electrons within cells and also because the cellular ( $NADH/NAD^+$ ) ratio (or redox) is important in the control of metabolism. Examination of its structure (Figure 5-11a) reveals that  $NAD^+$  contains the nucleotides adenine and nicotinamide. We have already encountered adenine in the structure of ATP (Figure 2-7). Nicotinamide is the product of a B vitamin. The dietary deficiency disease pellagra is typified by poor energy metabolism. Flavine adenine dinucleotide (FAD) is a compound similar to  $NAD^+$ . FAD can be reduced to  $FADH_2$  and, like  $NADH$ , functions to conserve and transport reducing equivalents within the cell.

The simplified flowchart for glycolysis (Figure 5-9) reveals that step 6 involves the reduction of  $NAD^+$  (adding of hydrogen and electrons) to yield  $NADH$ . When glycolysis proceeds slowly (Figure 5-9a),  $NADH$  transports or “shuttles” the hydrogen and electron to mitochondria (those cellular organelles where most oxygen is consumed). Under these circumstances, the end product of glycolysis, pyruvate, is also consumed by the mitochondria. However, if there is insufficient mitochondrial activity to accept the glycolytic flux,

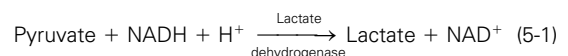


**Figure 5-10** Chemical structures of glucose, pyruvic acid, and lactic acid. Small numbers in parentheses identify the carbon atoms in the original glucose structure. At physiological pH, lactic and pyruvic acids dissociate a hydrogen ion. In glycolytic cells, such as erythrocytes and muscle cells, lactate is inevitably formed because the  $K_{eq}$  of lactate dehydrogenase (LDH) is 36,000 and  $\dot{V}_{max}$  is high.

which can occur in type IIb fibers or type I fibers during maximal exercise or if the mitochondria are defective or poisoned (Figure 5-9b), then NADH is oxidized and pyruvate reduced to form lactate in the cytoplasm as the result of rapid glycolysis (Eq. 5-1).

The net formation of lactate or pyruvate, then, depends on relative glycolytic and mitochondrial activities, and not on the presence of oxygen. As in the indirect pathway (Figure 5-4), which occurs during rest under fully aerobic conditions, glycolysis leading to lactate production occurs readily in fully aerobic contracting red skeletal muscle. Thus, in relation to mitochondrial respiration, which is tightly controlled and directly related to energy demand, glycolysis and glycogenolysis are less tightly controlled. Glycolytic flux in excess of mitochondrial

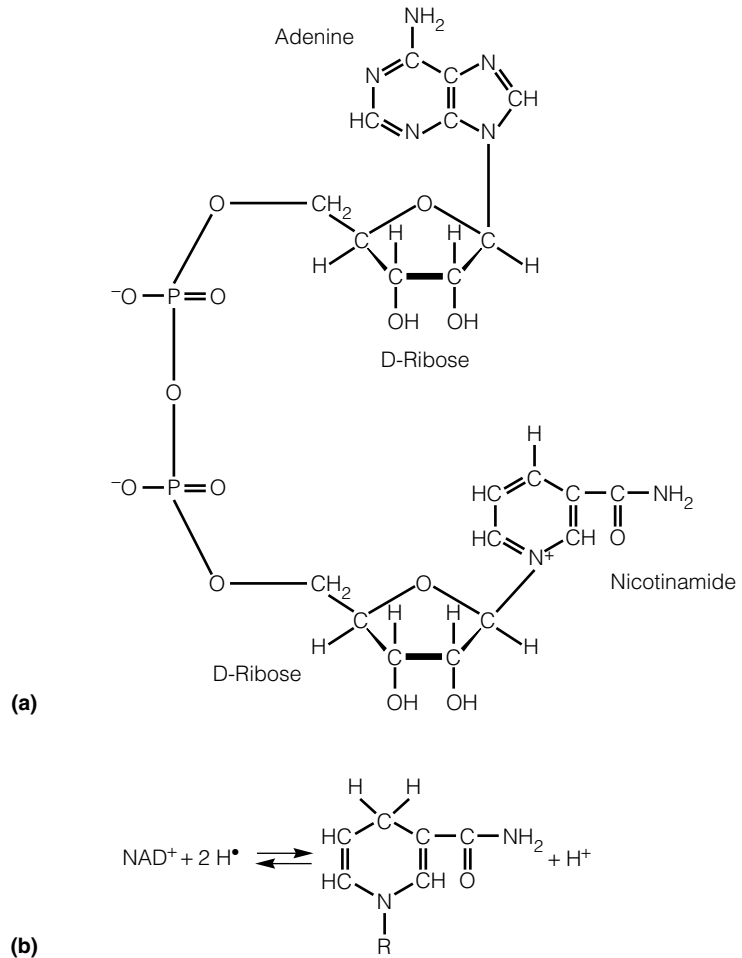
demand results in lactate production simply because LDH has the highest  $V_{max}$  of any glycolytic enzyme and because the  $K_{eq}$  and  $\Delta G$  (Chapter 2) of pyruvate-to-lactate conversion favors product formation.



### Malate-Aspartate and Glycerol-Phosphate Cytoplasmic-Mitochondrial Shuttle Systems

Scientists have elucidated the pathways and controls of the three glycolytic-mitochondrial shuttle systems. Because NADH and  $\text{NAD}^+$  poorly diffuse across the inner mitochondrial membrane, nature utilizes three reduced compounds (malate, glycerol

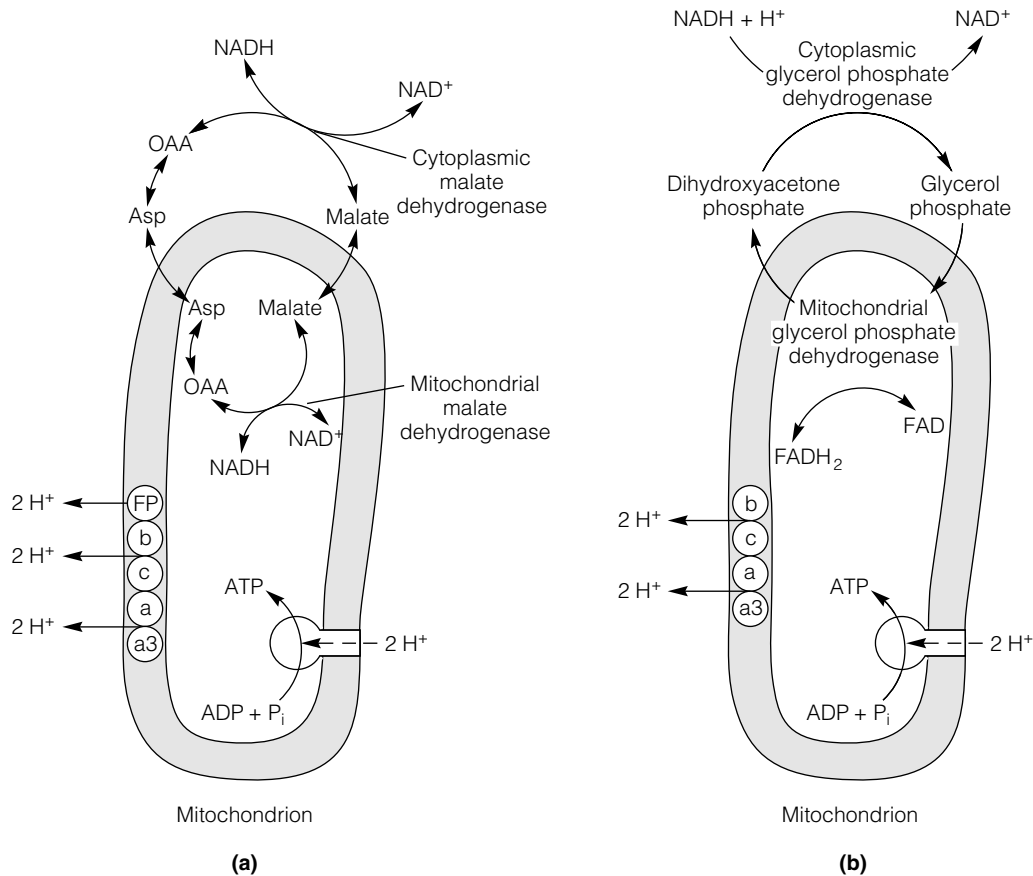
**Figure 5-11** Structures of  $\text{NAD}^+$  and  $\text{NADH}$ .  $\text{NAD}^+$  can exist in two forms: (a) without added hydrogen and electrons—that is, oxidized—or (b) with added hydrogen and electrons—that is, reduced.  $\text{NAD}^+$  serves to transfer these high-energy species within cells:  $\text{NAD}^+ + 2\text{H} \rightleftharpoons \text{NADH} + \text{H}^+$ .



phosphate, and lactate) to transfer reducing equivalents from cytosol to the mitochondrial electron transport chain. The first two shuttles discovered are diagrammed in simple form in Figure 5-12. The malate-aspartate shuttle predominates in the heart, and the glycerol-phosphate shuttle predominates in skeletal muscle. In addition to differences in mechanism and tissue specificity, the shuttles differ in the energy level of the product that becomes available within mitochondria. As we will see,  $\text{NADH}$  generates three ATP molecules within mitochondria for each atom of oxygen consumed ( $\text{ATP} : \text{O} = \text{ADP} : \text{O} = \text{P}_i : \text{O} = 3$ ), and  $\text{FADH}$  generates two ATPs within mitochondria ( $\text{P}_i : \text{O} = 2$ ).

### The Intracellular Lactate Shuttle

Since the 1970s, electron microscope and biochemical evidence have indicated that mitochondria in skeletal muscles, liver, sperm, and other cells contain lactate dehydrogenase (LDH). Unfortunately, these results were either ignored or misinterpreted. However, recent observations that tissue homogenates and that mitochondria isolated from liver, heart, and skeletal muscle oxidize lactate at rates greater than pyruvate (Brooks et al., 1999) have rekindled interest in studying mitochondrial LDH and lactate-pyruvate (monocarboxylate) transporters. More importantly, the realization that mito-

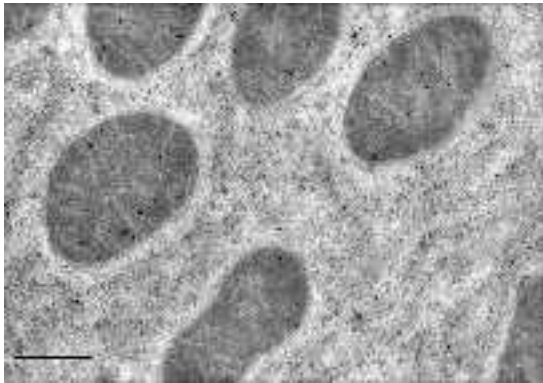


**Figure 5-12** Shuttle systems for moving reducing equivalents generated from glycolysis in the cytoplasm to mitochondria. (a) The malate-aspartate shuttle is the main mechanism in the heart. (b) The glycerol-phosphate shuttle is the main mechanism in skeletal muscle. Each proton pair pumped out of mitochondria results in a sufficient chemical and osmotic energy gradient to form an ATP molecule.  $\text{NADH} \approx 3\text{ATP}$ ;  $\text{FADH} \approx 2\text{ATP}$ . Modified from Lehninger, 1971. Used with permission.

chondria consume and oxidize lactate completely changes our understanding of the relationship between glycolytic and oxidative metabolism. Rather than the conclusion that lactate is a dead-end metabolite formed as the result of  $\text{O}_2$  insufficiency, we now realize that lactate, more than pyruvate, is the link between glycolytic (anaerobic) and oxidative (aerobic) metabolism.

Figure 5-13 is a transmission electron micrograph (TEM) of rat liver showing mitochondria, rough ER, and cytosol. The presence of the LDH-5

(M4) isoform of lactate dehydrogenase in mitochondria and cytosol is indicated by gold particles that appear as black dots. Similar results have been obtained on heart and skeletal muscle, and together these results have given rise to realization of an *intracellular lactate shuttle* (Figure 5-14). According to this model, when glycolysis is rapid and cytosolic lactate concentration rises, lactate enters mitochondria; lactate is oxidized to pyruvate by mitochondrial LDH with participation of the mitochondrial electron transport chain, and the resulting pyruvate

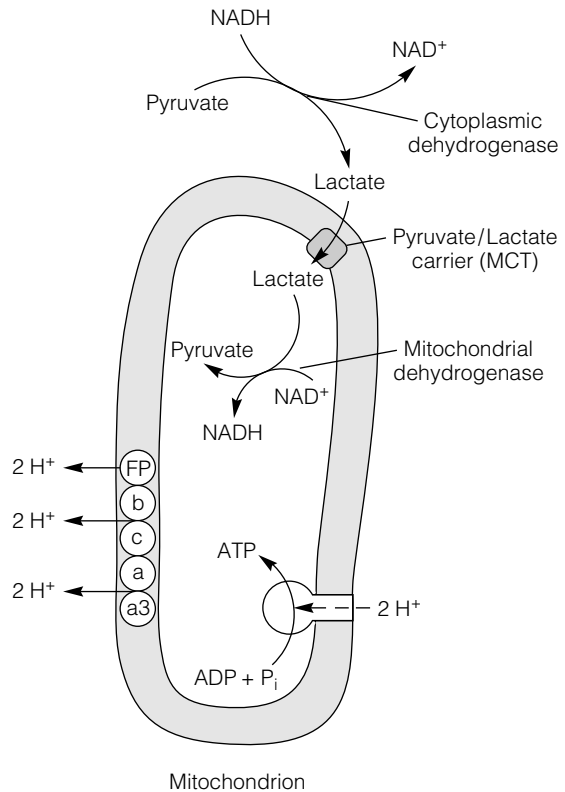


**Figure 5-13** Transmission electron micrograph (TEM) of high pressure frozen rat liver showing mitochondria, rough ER, and cytosol. Immunolocalization of anti-LDH-5 (M4) antibodies is indicated by the 15-nm gold particles which appear as black dots. Magnification = 58,300  $\times$  and scale bar = 400 nm. Note presence of LDH-5 in mitochondria and surrounding matrix and organelles. From Brooks et al., 1999. Used with permission of the National Academy of Sciences.

is oxidized by the mitochondrial tricarboxylic acid cycle (Chapter 6).

In important ways, the intracellular lactate shuttle (Figure 5-15) is similar to the malate-aspartate and glycerol-phosphate shuttles (see Figure 5-12). Specifically, the shuttles are similar in that NADH-equivalent potential energy is transported into mitochondria by means of a compound for which there is a specific mitochondrial carrier protein. As already stated, this transport-carrier mechanism is necessary because NADH and  $\text{NAD}^+$  poorly diffuse across mitochondrial membranes. The shuttle systems differ in a major way in that while the malate-aspartate and glycerol-phosphate shuttles allow transfer of reducing equivalents from cytosol to mitochondria, there is no net transfer of substrate for oxidation. In contrast, the intracellular lactate shuttle transports from cytosol to mitochondria both NADH and a substrate for oxidation by the tricarboxylic acid cycle (TCA cycle). In this case, however, lactate, not malate or glycerol phosphate, is the carrier.

The intracellular lactate shuttle has a major advantage for working muscle in that it permits rapid



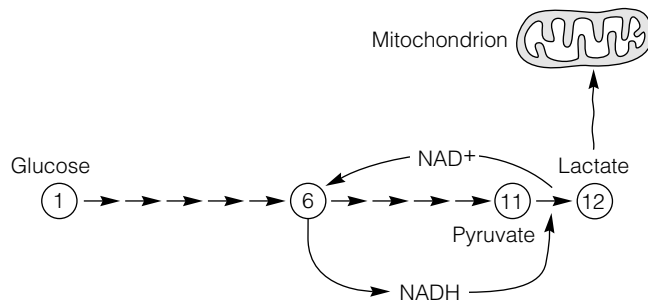
**Figure 5-14** The *intracellular lactate shuttle* permits both reducing equivalents as well as oxidizable substrate generated from glycolysis in the cytosol to gain entry into mitochondria. The malate-aspartate and glycerol-phosphate shuttles (Figure 5-12) are believed to function when glycolysis is slow, whereas the lactate shuttle operates when glycolysis is rapid and lactate accumulates. MCT stands for monocarboxylate transporter.

*aerobic glycolysis* involving lactate formation to occur simultaneously with high rates of oxygen consumption. Thus, Figures 5-9a and 5-9b are combined in Figure 5-14 to illustrate how the intracellular lactate shuttle supports aerobic glycolysis.

### ATP Yield by Glycolysis

At the beginning of glycolysis (Figure 5-8), there are two activating steps where ATP is consumed. However, following the cleavage of the six-carbon molecule into two three-carbon molecules, there are two





**Figure 5-15** Diagram of the intracellular lactate shuttle by which lactate formed as the result of rapid glycolysis is shuttled into mitochondria and oxidized. By this means, glycolytic (“anaerobic”) and oxidative (“aerobic”) metabolism are linked. Comprehension of the intracellular lactate shuttle makes possible understanding of many observations of so-called *aerobic glycolysis* in which fully oxygenated cells consume glucose and produce lactate, or consume lactate and reduce glucose consumption.

steps where an ATP molecule is formed. Because these steps occur twice each for each six-carbon glucose that enters the pathway, the gross ATP yield is  $2 \times 2 = 4$ . However, if we then subtract the two ATP molecules used for activation, the net yield is 2.

Under “aerobic” conditions, the formation of two ATPs per glucose is complemented by the formation for mitochondrial consumption of two NADHs. Depending on the shuttle system used, these are equivalent to another four to six ATPs.



“Anaerobic” glycolysis is summarized in Equation 5-4 and “aerobic” glycolysis in Equation 5-5.

### The Efficiency of Glycolysis

The formation of only two ATPs from each glucose in glycolysis seems small (Eqs. 5-1 and 5-2). For this reason, glycolysis is sometimes called an “inefficient” pathway. Fortunately, however, skeletal muscle (white and red glycolytic fibers) can break down glucose rapidly and can produce significant quantities of ATP for short periods during glycolysis.

Nevertheless, an appropriate energetic consideration reveals that, in fact, the efficiency of glycol-

ysis is excellent. The energy change ( $\Delta H$ ) from glucose to lactate is  $-47 \text{ kcal} \cdot \text{mol}^{-1}$ .

If two ATPs are formed, and  $\Delta G^\circ$  for ATP =  $-7.3 \text{ kcal} \cdot \text{mol}^{-1}$ , then

$$\text{Efficiency} = \frac{2(-7.3)}{-47} = 31\% \quad (5-6)$$

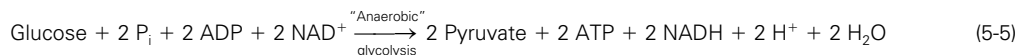
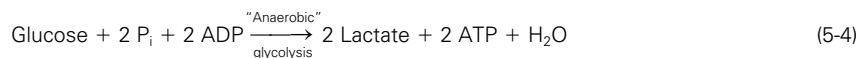
If  $\Delta G$  for ATP is  $-11 \text{ kcal} \cdot \text{mol}^{-1}$ ,

$$\text{Efficiency} = \frac{2(-11)}{-47} = 46.8\% \quad (5-7)$$

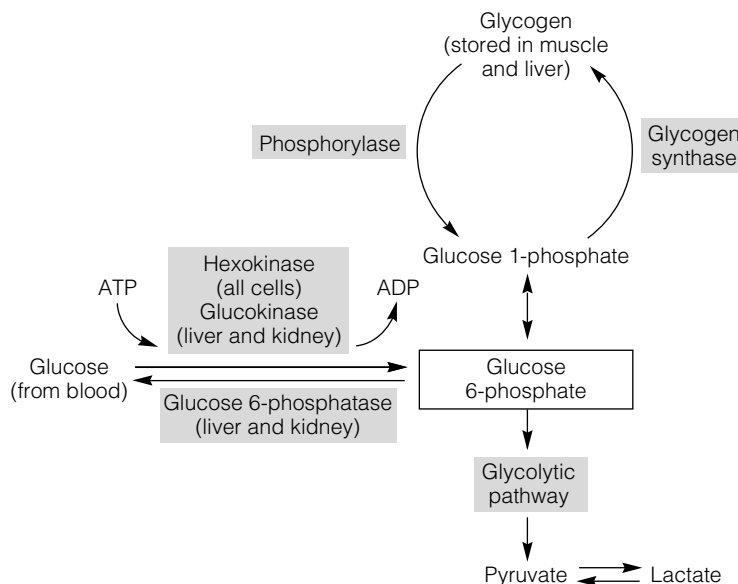
This latter result of almost 50% efficiency compares favorably with that of oxidative enzymatic processes. It is not correct, therefore, to assume that glycolysis, or “anaerobic metabolism,” is inefficient—the enzymes actually conserve a good deal of the energy released.

### The Control of Glycolysis

Glycolysis is a pathway controlled by many factors but, in general, two kinds of controls predominate: “feed-forward” and “feedback” controls. In general, feed-forward mechanisms set the coarse gain in metabolic regulation, whereas feedback mechanisms accomplish the fine-tuning of energy supply to demand. Feed-forward and feedback controls are



**Figure 5-16** Illustration of the central role of glucose 6-phosphate in determining the direction of carbon flow in glycolysis (catabolism of glucose), glycogenolysis, glycogen synthesis, lactate and pyruvate production, and glucose release.



illustrated by their various effects on the level and flux (movement of molecules) through the glucose 6-phosphate (G6P) pool (Figure 5-16).

In feed-forward control of glycolysis, factors that increase G6P levels tend to stimulate glycolysis. Feed-forward factors include stimulation of glycogenolysis (by epinephrine and contractions) and glucose uptake (by contractions and insulin). Thus, as seen in Figure 5-5 with exercise of moderate to high intensity, blood glucose rises because of the stimulation of hepatic glucose production, which increases faster than the increase in muscle glucose uptake. Feedback controls involve changes in levels of metabolites that result from glycolysis (e.g., citrate) or from muscle contraction (e.g., ADP). Also, a decline in blood glucose concentration, such as occurs at the end of the exercise (Figure 5-5), is probably the most important feedback control in normal, healthy subjects. Feedback control usually resides at the phosphofruktokinase (PFK) step and can either speed (stimulate) or slow (inhibit) regulatory enzymes.

**The Cellular “Energy Charge”** Earlier, in Chapters 2 and 3 (Eq. 3-11), it was shown that the contractile actin-myosin system splits ATP to ADP

and  $P_i$  and that adenyl kinase maintains ATP levels by forming ATP and AMP from two ADPs. The contents of the ATP-ADP-AMP system therefore exist in one of three forms. The adenylate energy charge is high when ATP concentration is high relative to AMP and ADP, and the energy charge falls when levels of the ATP degradation products rise. As we shall see in Chapter 6, ADP and AMP affect mitochondrial respiration as well as glycolysis.

**GLUT-4 and Glucose Transporter Translocation** Recent advances indicate that glucose uptake into muscle and other cells occurs via glucose transport proteins. Transporter-mediated cell glucose uptake is discussed more fully in Chapter 9. (See Figure 9-6 and Table 9-1). Here, it needs to be emphasized that muscle and adipose cells possess both noninsulin (GLUT-1) and insulin-mediated (GLUT-4) glucose uptake transporters. In resting muscle, most glucose enters by the GLUT-1 carrier. However, when glucose and insulin levels are high, or during exercise, most glucose enters muscle cells by the insulin (and contraction) regulatable (GLUT-4) glucose transporter protein. Under resting conditions, insulin binding to the muscle cell surface receptor causes release of some messen-

TABLE 5-1

## Control Enzymes of Glycolysis

Enzyme	Stimulators	Inhibitors
Phosphofructokinase	ADP, $P_i$ , $\uparrow$ pH, $(NH_4^+)$	ATP, CP, citrate
Pyruvate kinase		ATP, CP
Hexokinase		Glucose 6-phosphate
Lactic dehydrogenase		ATP

ger or messengers, which cause the GLUT-4 transporters to move (translocate) from intracellular locations to the cell surface. Translocation of GLUT-4 transporters to the muscle cell surface also occurs as the result of contractions. Results of Friedman, Dohm, and associates (1991) suggest that in the resting state GLUT-4 transporters reside near T-tubules and lateral cisternae of the sarcoplasmic reticulum. This finding offers the possibility that events associated with the ion fluxes of contraction ( $Na^+$  or  $Ca^{2+}$ ) may influence transporter translocation to T-tubules, which are extensions of the sarcolemma. Such a mechanism would explain the results of Richter and associates (1982, 1985, 1988) which show that contracting muscle can take up glucose with “no need for insulin.” Thus, with both insulin-dependent and insulin-independent mechanisms of glucose transport, working muscle can take up glucose even when insulin falls during exercise.

**Phosphofructokinase** Usually a dominant factor in the regulation of glycolysis is the activity of the enzyme phosphofructokinase (PFK). As we shall see in Chapter 9, two forms of PFK exist: one for glycolysis (PFK-1) and one for gluconeogenesis (PFK-2). In muscle, PFK-1 predominates. PFK-1, which catalyzes the third step of glycolysis, is a multivalent, allosteric enzyme. This means that several metabolites bind to the enzyme and affect its catalytic capacity. Known modulators of PFK-1 are given in Table 5-1. PFK is probably the rate-limiting enzyme in muscle when glycolysis is rapid during exercise, but factors such as ATP, CP, and citrate affect the conformation of PFK to slow its activity dur-

ing rest. When exercise starts, immediate changes in the relative concentrations of PFK modulators increase its activity. Pyruvate kinase, hexokinase, and lactic dehydrogenase are other important controlling enzymes whose activities are modulated.

**Thermodynamic Control** As indicated in Figures 5-7 and 5-8, significant free energy changes occur in several exergonic steps. These reactions are catalyzed by the enzymes hexokinase, phosphofructokinase, pyruvate kinase, and lactic dehydrogenase. The reactions keep glycolysis going in the direction of product; the other steps have small free-energy changes and are freely reversible. The reverse of glycolysis is not possible without energy input and the intervention of specific enzymes. This reversal process is called *gluconeogenesis*, meaning “making new glucose.” Gluconeogenesis is a function of tissues such as liver and kidney. It is a capability of muscle, but at present is not believed to be a primary function of muscle.

**Control by Lactate Dehydrogenase (LDH)** The terminal enzyme of glycolysis, which results in the formation of lactic acid from pyruvic acid, is lactate dehydrogenase (LDH). As shown earlier (Figure 5-9a), when glycolysis is slow, LDH is in competition with mitochondria for pyruvate. However, LDH is an enzyme of significant content in muscle, especially white skeletal muscle. As already mentioned, the  $K'_{eq}$  and the  $\Delta G'^{\circ}$  of LDH are large and the reaction proceeds actively to completion. Therefore, some lactate is always formed. As a consequence, resting muscle always produces and releases lactate on a net basis (Figure 10-5).

TABLE 5-2

Distribution of lactate dehydrogenase (LDH) isoenzymes in various mammalian tissues<sup>a</sup>

Tissue	M <sub>4</sub> (LDH <sub>5</sub> )	M <sub>3</sub> H <sub>1</sub> (LDH <sub>4</sub> )	M <sub>2</sub> H <sub>2</sub> (LDH <sub>3</sub> )	M <sub>1</sub> H <sub>3</sub> (LDH <sub>2</sub> )	H <sub>4</sub> (LDH <sub>1</sub> )
Liver	94.0	4.0	1.0	0.8	0.2
Heart	2	3	5	30	60
Fast white muscle (tibialis anterior)	80	14	3	2	1
Fast red muscle (red gastroc)	58	10	11	13	8
Slow red muscle (soleus)	11	13	18	30	28
Kidney	6	11	21	34	28
Red blood cells	5	10	15	30	40
Lungs	21	23	38	18	10
Spleen	18	31	31	15	5

<sup>a</sup>Each enzyme molecule is comprised of four subunits, thus yielding five isoenzymes according to relative composition of muscle (M) and heart (H) subunits. Data are for whole tissues, not cytosolic and other cell compartments.

SOURCE: Dubouchaud and Brooks (1999), Lott and Wolfe (1986), McCullagh et al. (1996), York et al. (1975).

There are two basic types of LDH: muscle (M) and heart (H). These LDH types are found predominantly in white skeletal muscle and heart, respectively. The equilibria of the two types are identical, but they differ in their affinities for reactants (substrates) and products. The M type has a high affinity for the substrate pyruvate and therefore has higher biological activity than the H type, which has a lower affinity for pyruvate.

Each molecule of LDH has four subunits. Considering the two basic types of LDH, there are then five possible arrangements: M<sub>4</sub>, M<sub>3</sub>H<sub>1</sub>, M<sub>2</sub>H<sub>2</sub>, M<sub>1</sub>H<sub>3</sub>, and H<sub>4</sub>. The population of these *isozymes* of the LDH varies among tissues, with M<sub>4</sub> being highest in white skeletal muscle and lowest in heart (Table 5-2).

Because of the distribution of LDH isoenzymes in various cells and tissues, the biological activity of LDH depends to some extent on its concentration and isoenzyme type. An older idea was that lactate-consuming tissues contained predominantly LDH1 (H<sub>4</sub>) isoenzymes, whereas lactate-producing cells and tissues contained predominantly LDH5 (M<sub>4</sub>) isoenzymes. However, while the heart and red skeletal muscles can be net lactate consumers, liver and red skeletal muscle, which contain predominantly LDH5 (Table 5-2), can be net lactate consumers as well. Therefore, in determining whether a

tissue is a net lactate consumer or producer, the presence of LDH in mitochondria, where lactate oxidation takes place by the intracellular lactate shuttle mechanism (Figures 5-14 and 5-15), is more important than the presence of LDH isoform in the cytosol of the tissue.

**Control by Pyruvate Dehydrogenase** Although a mitochondrial and not a glycolytic enzyme, pyruvate dehydrogenase (PDH) is a key enzyme whose activity, which is regulated by phosphorylation (Chapter 9), can affect the rate of lactate production. As we will see in Chapter 6, when PDH is active, pyruvate can be diverted to the mitochondria for oxidation. By competing with LDH for pyruvate, PDH indirectly affects the NADH/NAD<sup>+</sup> ratio and, therefore, the rate of glycolysis.

**Control by Cytoplasmic Redox (NADH/NAD<sup>+</sup>)** Glycolytic flux and the competition between cytoplasmic LDH and mitochondrial PDH for pyruvate affect the ratio of pyruvate to lactate as well as the ratio of NADH to NAD<sup>+</sup>. The cytoplasmic NADH/NAD<sup>+</sup> ratio (or redox) affects the activity of glyceraldehyde 3-phosphate dehydrogenase, which requires NADH as a cofactor. In

general, cytoplasmic reduction ( $\uparrow\text{NADH}/\text{NAD}^+$ ) slows glycolysis, whereas oxidation ( $\downarrow\text{NADH}/\text{NAD}^+$ ) speeds glycolysis.

**Control by Glycogenolysis** To a certain extent, the rate at which glycolysis proceeds depends on the rate of glycogen breakdown. In resting muscle, little glycogen is broken down, so the rate of glycolysis is limited by muscle glucose uptake. However, during exercise, glycogen breakdown is greatly accelerated, and glycogen, not glucose, is the major precursor for glycolysis. For instance, during steady-rate exercise at 65% of  $\dot{V}_{\text{O}_2\text{max}}$ , glycogen breakdown can exceed glucose uptake by four to five times. Thus, glycolysis is said to be under “feed-forward” control.

## ■ Glycogenolysis

Skeletal muscle glycolysis is heavily dependent on the intramuscular storage form, glycogen. During heavy muscular exercise, glycogen may supply most of the immediate glucosyl residues for glycolysis. Because 80% or more of the carbon for glycolysis in muscle comes from the glycogen in muscle, and not from blood glucose, glycogen depletion results in fatigue (Chapter 33).

The structure of glycogen (Figure 5-17) consists mostly of end-to-end (C1–C4) linkages, with a few cross (C1–C6) linkages. The storage of glucose units as glycogen is dependent on the activity of the enzyme glycogen synthase (Figure 5-18). Breakdown of glycogen is dependent on the enzyme phosphorylase, which hydrolyzes the C1–C4 linkages. Another enzyme, called “debranching enzyme,” hydrolyzes the C1–C6 branching, or side linkages.

The activity of phosphorylase appears to be controlled by two mechanisms. One system is hormonally mediated and depends on the extracellular action of epinephrine and the intracellular action of cyclic AMP (cAMP), the “intracellular hormone” (Figure 5-19). This mechanism is too slow to explain the rapid glycolysis during onset of heavy exercise.

Therefore, faster mechanisms mediated by phosphate ( $\text{P}_i$ , or  $\text{PO}_4^{2-}$ ) and calcium ion ( $\text{Ca}^{2+}$ ) provide important control mechanisms for mobilizing glycogen in working skeletal muscle. These signals arise from the causes and consequences of muscle contraction and not extracellular, endocrine signaling.

### Control by Phosphate

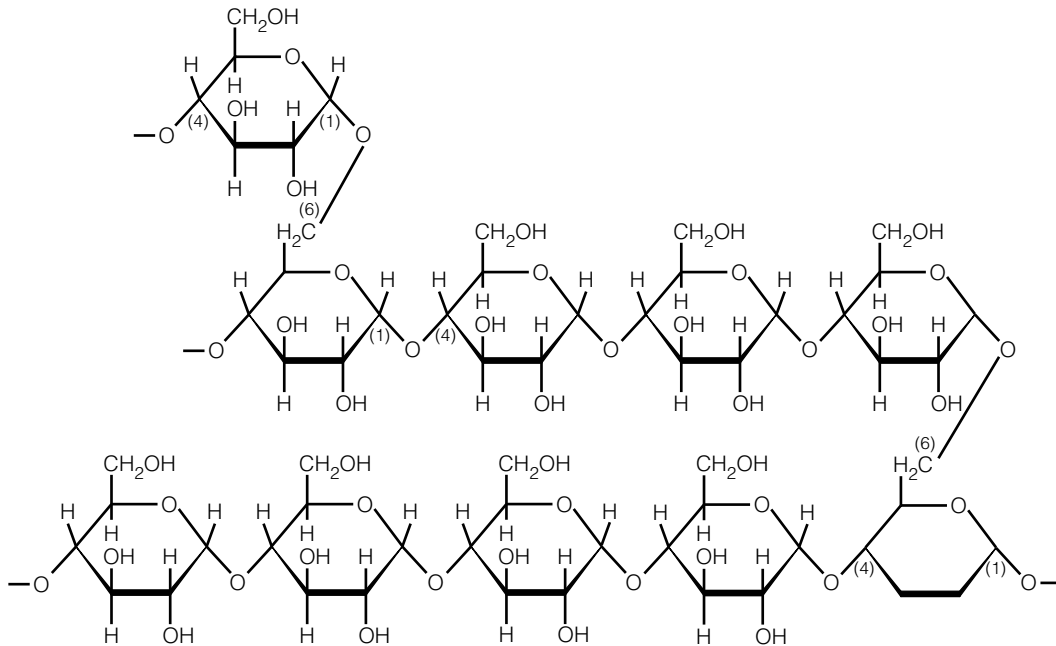
According to the work of J. O. Holloszy and associates, probably the control mechanism most important for mobilizing glycogen is the free inorganic phosphate ( $\text{P}_i$ ) that comes from the breakdown of ATP (Eq. 3-2). Phosphate provides a potent stimulus for glycogen degradation in muscle because it is a substrate for phosphorylase *a* (Figure 5-19). The important role of phosphate in the regulation of muscle glycogenolysis is usually overlooked by biochemists, but is given attention by exercise physiologists, who know that epinephrine stimulation, that increases cyclic AMP (cAMP), in the absence of contraction does not result in appreciable glycogen breakdown because  $\text{P}_i$  level does not change without contraction.

### Control by Calcium Ion

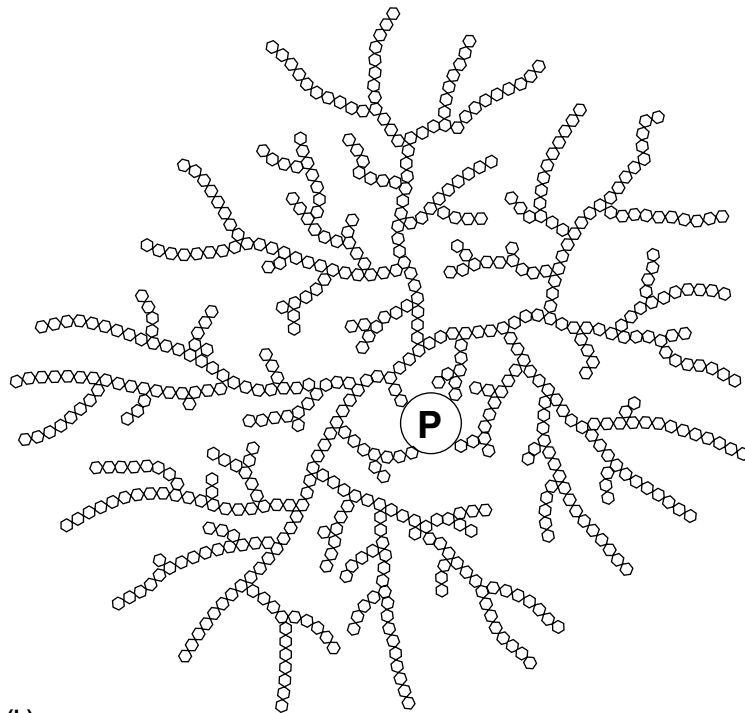
In addition,  $\text{Ca}^{2+}$ , which is released from the sarcoplasmic reticulum, constitutes another control mechanism (Figure 5-20) regulating glycogen catabolism.

The hormonally mediated, cAMP-dependent mechanism serves two purposes during exercise and recovery: (1) It amplifies the local,  $\text{P}_i^{-2}$ , and  $\text{Ca}^{2+}$ -mediated process in *active* muscle, and (2) it mobilizes glycogen in *inactive* muscle to provide lactate as a fuel and as a glyconeogenic (Cori cycle) precursor (Chapter 9).

During rest, cellular uptake of glucose is usually sufficient to support glycogen synthesis and glycolysis. During maximal exercise, glycogenolysis and glucose uptake are sufficient to support rapid glycolysis. During prolonged exercise, the depletion of intramuscular glycogen and liver glycogen can result in the decreased capability of substrate for

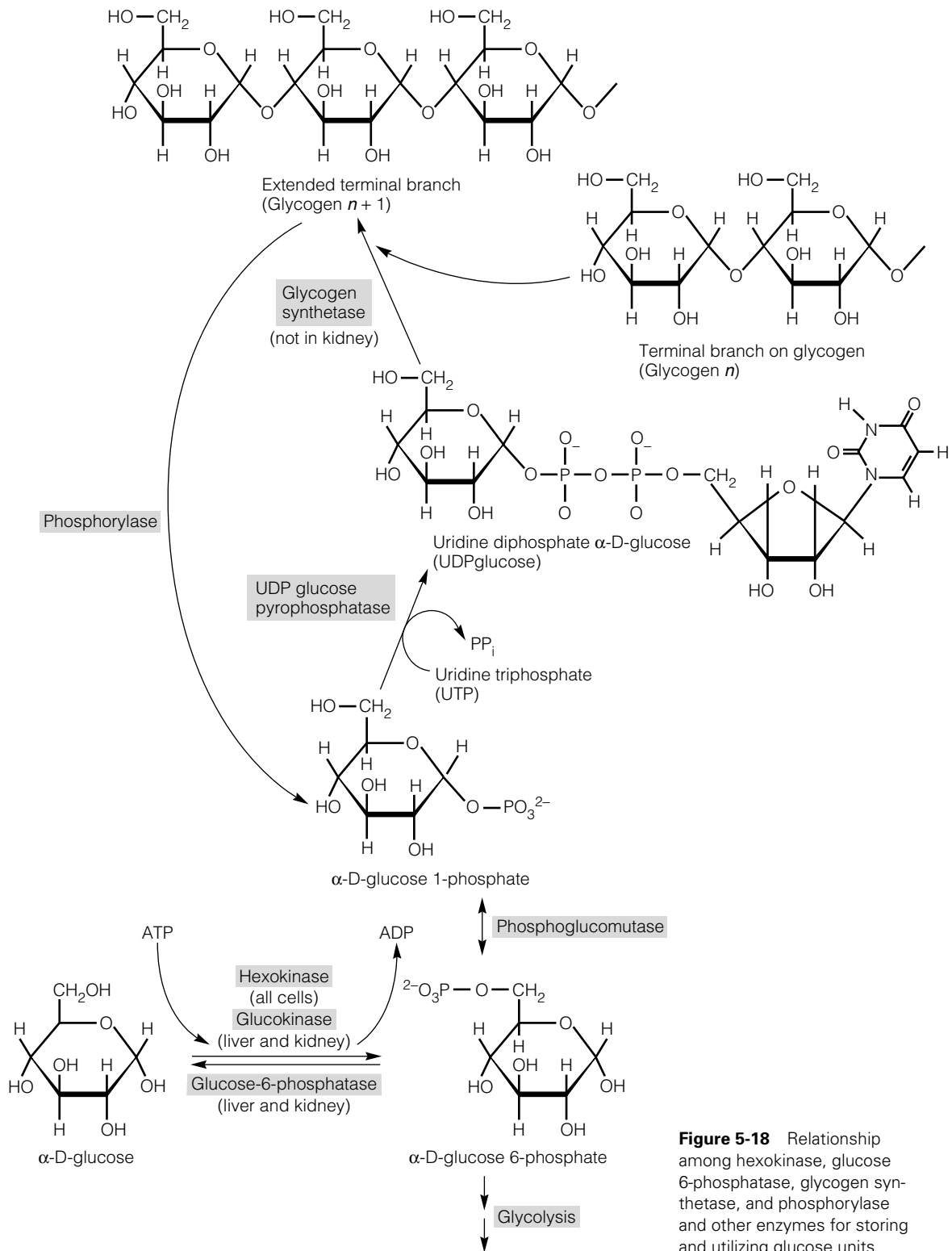


(a)



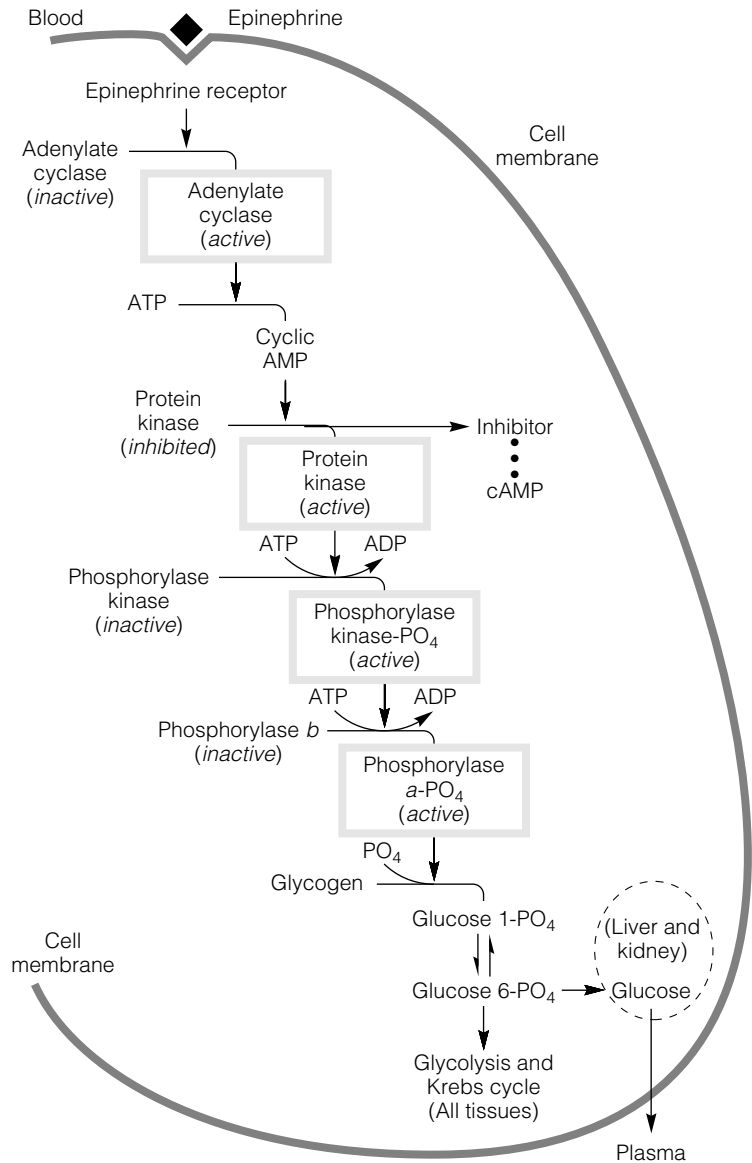
(b)

**Figure 5-17** (a) The structure of glycogen is seen to be a polymer of glucose units. The linkages exist mainly end to end (C1–C4 linkages), but there is also some cross-bonding (C1–C6 linkages). (b) A pinwheel-like structure results from C1–C4 and C1–C6 linkages. The hexagons represent glucosyl units. Under high magnification in the electron microscope (e.g., Figure 5-6), the pinwheel-like structures of glycogen appear as dark granules. Glycogen forms around a “foundation” protein P-glycogenin.



**Figure 5-18** Relationship among hexokinase, glucose 6-phosphatase, glycogen synthetase, and phosphorylase and other enzymes for storing and utilizing glucose units.

**Figure 5-19** The breakdown of glycogen in muscle is heavily influenced by the enzyme phosphorylase *a*. Phosphorylase *b* (the inactive form) can be converted to phosphorylase *a* (the active form) through a series of events initiated by the hormone epinephrine. This mechanism involves cyclic AMP (cAMP), the intracellular hormone. During sudden bursts of activity, the cAMP mechanism is too slow to account for the observed glycogenolysis. Intracellular factors such as  $\text{Ca}^{2+}$  and AMP increase the catalytic activity of phosphorylase. As well, phosphate ion ( $\text{PO}_4$ , or  $\text{P}_i$ ) is thought to be regulatory; see Figure 3-7). Modified from Vander, Sherman, and Luciano, 1980. Used with permission.

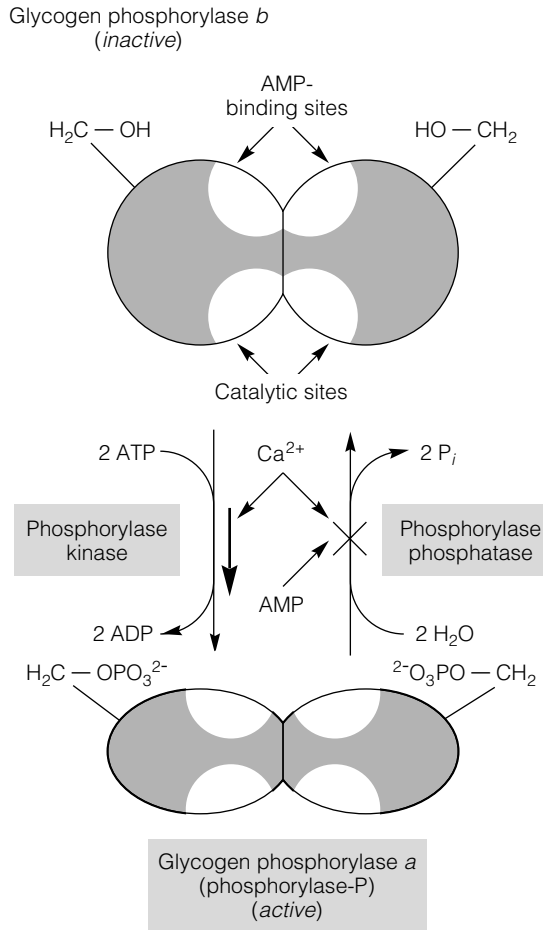


muscle glycolysis. Moreover, it is also likely that the ability to glycolyze limits the ability to utilize fat. Therefore, glycogen depletion during exercise may be doubly important. The interaction between carbohydrate and fat metabolism is discussed later (Chapter 7) in more detail.

### Energy Flux and Metabolic Regulation

In summary on the controls of glycogenolysis and glycolysis, it is necessary to reflect on all the details in the text and reassert the necessity to match energy supply to demand in the body. Here, as well as





**Figure 5-20** The conversion of phosphorylase *b* (the inactive form of the enzyme) to phosphorylase *a* (the active form) depends on the stimulation of phosphorylase kinase by  $\text{Ca}^{2+}$ . Calcium ions are released immediately when muscles contract, and this mechanism helps to link pathways of ATP supply with those of ATP utilization. During exercise, the levels of AMP increase; this helps to minimize the reconversion of phosphorylase *a* to *b* by inhibiting phosphorylase phosphatase. Modified from McGilvery, 1975.

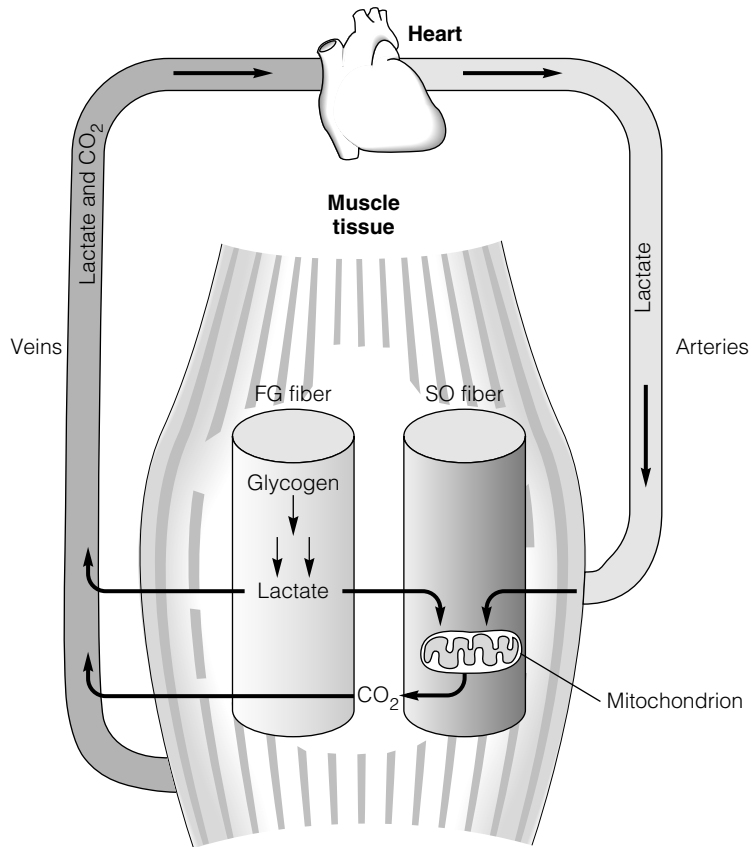
in Chapter 7 where the crossover concept is elaborated upon, it needs to be emphasized that *energy flux is the most important regulator of metabolism*. For instance, consider the following experiments. First, infuse a volunteer with a dose of epinephrine suf-

ficient to stimulate glycogen breakdown, but below a dose damaging to the heart. Muscle glycogen will break down and glycolysis will be stimulated, but only slightly as there is only a minuscule effect on energy use and the AEC is little affected. Interestingly, a rise in muscle glucose 6-phosphate (G 6-P) (Figure 5-16) will block muscle glucose uptake and the excess glycolytic flux will appear as lactate in the venous blood. Next, with a different person or the same person on another occasion, stimulate muscles to contract by electrical stimulation or by having the person do mild to moderate intensity exercise. Muscle glycogen will break down and glucose uptake will be observed, but without changes in epinephrine or insulin. So, in terms of metabolic regulation in muscle, neural mechanisms determine the intensity of contractions, or in other words, the rate of ATP hydrolysis, or alternatively stated, the muscle energy output. Thus, feed-forward neural control sets the coarse gain in the metabolic response. In contrast, the other compensatory feedback and endocrine feed-forward mechanisms described earlier in this chapter serve to fine-tune the metabolic responses in the overall effort to maintain the AEC and preserve ATP homeostasis in cells stressed by exercise and hormonal stimulation.

## ■ The Cell-Cell Lactate Shuttle

Isotope tracer studies and arteriovenous difference measurements across muscles and other tissues have allowed precise estimation of the rates of lactate and glucose production and oxidation during sustained, submaximal exercise. The results indicate that lactate is actively oxidized in working muscle beds and may be a preferred fuel in heart and red skeletal muscle fibers. In the original (cell-cell) lactate shuttle concept, the idea was that within a muscle tissue during sustained exercise, lactate produced at some sites, such as type IIb (FG) fibers, diffuses or is transported into type I (SO) fibers (Figure 5-21). Some of the lactate produced in type IIb fibers shuttles directly to adjacent type I fibers. Alternatively, other lactate produced in type IIb fibers

**Figure 5-21** Diagram of the cell-cell lactate shuttle. Lactate produced in some cells (e.g., fast glycolytic [FG, type IIb] muscle cells) can shuttle to other cells (e.g., slow oxidative [SO, type I] fibers) and be oxidized. Also, lactate released into the venous blood can recirculate to the active muscle tissue bed and be oxidized. During exercise, the lactate shuttle can provide significant amounts of fuel. Muscle cell membrane lactate transport proteins (MCT1 and MCT4) facilitate lactate release and uptake. (See Brooks [1985] and Brooks et al. [1999] for additional information.)



can reach type I fibers by recirculation through the blood. Thus, by this mechanism of shuttling lactate between cells, glycogenolysis in one cell can supply a fuel for oxidation to another cell. Skeletal muscle tissue then becomes not only the major site of lactate production, but also the major site of its removal. In addition, much of the lactate produced in a working muscle is consumed within the same tissue and never reaches the venous blood.

### ■ Monocarboxylate (Lactate/Pyruvate) Transport Proteins in Muscle Cell Membranes and Mitochondria

The original cell-cell lactate shuttle concept (Figure 5-21) predicted that lactate exchange occurred between lactate-producing and -consuming muscle

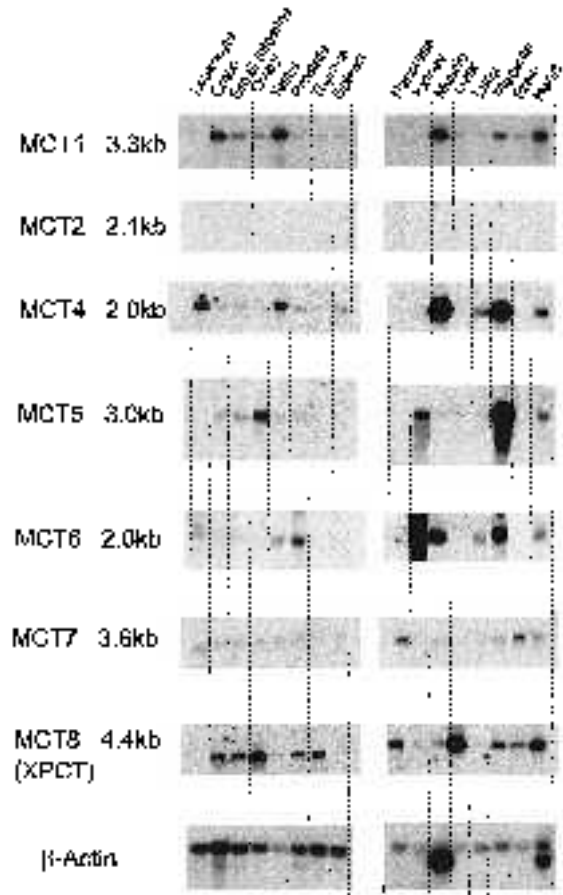
fibers during exercise and predicted presence of sarcolemmal lactate transport proteins to facilitate lactate exchange. The concept was well supported by results obtained from isotope tracer and limb metabolite balance studies. The concept has also been well supported by results of subsequent physiological, biochemical, and molecular studies. Some of these deserve mention here.

Working independently, Juel (1988) and Watt and associates (1988) provided convincing evidence of facilitated and proton-linked lactate transport into mammalian muscle tissue. Those observations were followed by work of Roth and Brooks (1990a, 1990b), who provided the first evidence of a lactate transporter in sarcolemmal membranes.

The field of study of cell membrane lactate transport proteins received a major boost when, in extending their earlier work on the mevalonate

transporter (Mev) in Chinese hamster ovary cells, Garcia and associates cloned and sequenced a monocarboxylate transporter, which they termed MCT1, or monocarboxylate transporter 1. MCT1 differed from Mev by only one amino acid substitution (Cys for Phe), and the amino acid structures of MCT1 and Mev predicted a membrane-bound protein with 12 membrane-spanning regions. Transfection of a breast cancer cell line lacking MCT1 with a plasmid containing cDNA encoding for MCT1 conferred properties reported for the erythrocyte transporter, including increased pyruvate uptake, proton symport, transstimulation, partial inhibition by other monocarboxylates (including lactate), and sensitivity to the known inhibitor of lactate cinnamate (CIN). Garcia and associates also raised rabbit polyclonal antibodies against the C-terminus of MCT1, and conducted immunofluorescence studies to locate MCT1 and succinic dehydrogenase (SDH, a mitochondrial marker) on several hamster tissues. MCT1 was found to be abundant in erythrocytes and heart and basolateral intestinal epithelium. In skeletal muscle, MCT1 was detectable only in oxidative muscle fiber types and not at all in liver. With an interest in describing a role for MCT isoforms in the Cori cycle (see below), Garcia and associates subsequently described isolation of a second isoform (MCT2) by screening a Syrian hamster liver library; and MCT2 was found in liver and testes.

Independent of Garcia and associates, Vicki Jackson, Andrew Halestrap, and associates cloned and sequenced MCT1 and MCT2 isoforms from rabbit and rat tissues, respectively. Subsequently, a unique isoform (MCT3) was found in the chicken eye. Candidates for several putative cell membrane monocarboxylate transporters (MCT4–MCT8) were later cloned and sequenced by Price, Jackson, and Halestrap (1998). The distribution of RNAs for seven of eight known MCTs is shown in Figure 5-22. This number may change in the near future, and it is likely to be discovered that *not* all the MCTs are lactate- or pyruvate-specific transporters. For the present, it is certain that muscle cell membrane lactate and other monocarboxylate transporters are highly abundant in diverse tissues. Again, MCT1 appears more abundant in oxidative striated muscle fibers, whereas



**Figure 5-22** Two human multitissue Northern blots showing distribution of RNAs encoding for MCT isoforms in human tissues. Sizes of transcripts were estimated by comparison with the position of RNA standards marked on the blot. The break in identification of RNAs encoding for MCT isoforms is due to the discovery of MCT3, a unique isoform so far found only in chicken eye. To date, MCT1, -2, -3, and -4 are lactate/pyruvate transporters, and it is unknown which monocarboxylic acids are transported by other isoforms. MCT1 is abundant in numerous cell types, including erythrocytes, red skeletal muscle cells, and heart cells. In muscle and heart cells, MCT1 is present in mitochondria as well as cell membranes. MCT4 is a sarcolemmal lactate transporter and is more abundant in white, glycolytic striated muscle. From Price and associates (1998), courtesy of A. Halestrap and Portland Press.

Wilson and associates (1998) have shown MCT4 to be more abundant in white, glycolytic fibers.

### The Muscle Mitochondrial Lactate/Pyruvate Transporter Is MCT1

The original cell-cell lactate shuttle concept successfully predicted results obtained from isotope tracer and limb metabolite balance studies as well as results of subsequent biochemical and molecular studies. However, while the original concept worked for exercise, it was less satisfactory for rest, during which type IIb fibers were (obviously) not recruited; glycolysis was slow and oxygen was present in abundance. Therefore, Brooks and associates turned their attention to a model that would permit glycolysis leading to lactate formation and mitochondrial lactate oxidation to occur simultaneously in noncontracting, fully oxygenated muscle. Subsequently, they showed that isolated mitochondria readily oxidize lactate and that mitochondrial lactate oxidation is blocked by known LDH and MCT inhibitors. In addition to showing that mitochondria from heart and skeletal muscle contain LDH (Figure 5-13), Brooks and associates (1999) reported that MCT1 is abundant in mitochondria of skeletal and cardiac muscle. Further, Hervé Dubouchaud and Brooks (2000) have found that training increases both the amounts of mitochondrial and sarcolemmal MCT1 in human muscle. Thus, not only are there variations in the distribution of MCTs, but tissues can express multiple isoforms that have different domains (locations) in the cells.

### ■ Gluconeogenesis

Although red mammalian skeletal muscle itself has only a vestigial enzymatic capacity to make glucose or glycogen, mammalian muscle does indirectly participate in gluconeogenesis, the making of new glucose.

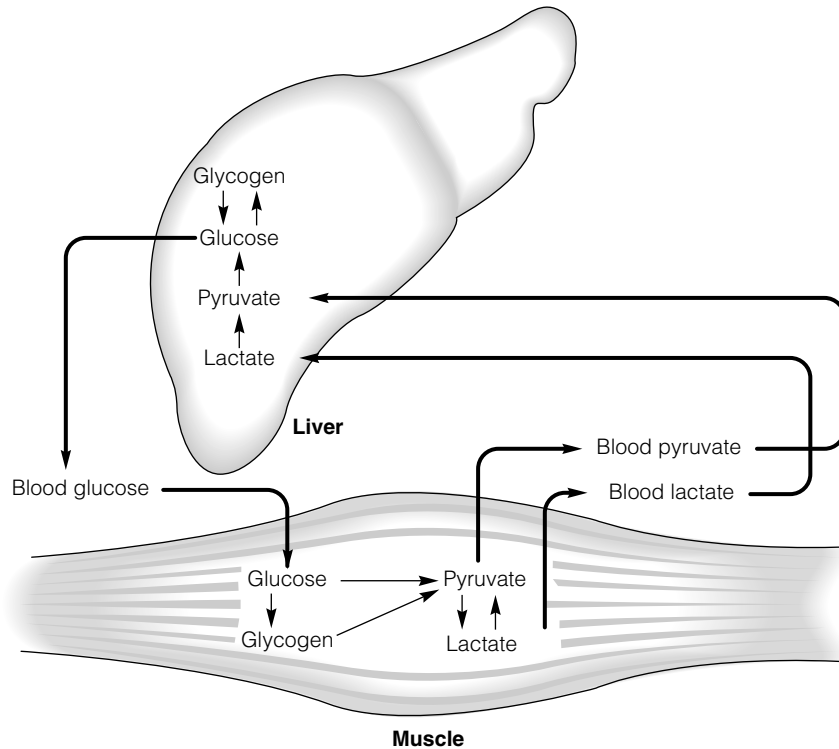
Carl and Gerty Cori (1947 Nobel Prize in physiology and medicine) were among the first to recognize that the lactate and pyruvate produced by skeletal muscle could circulate to the liver and be made into glucose. The glucose so produced could

then recirculate to muscle. This cycle of carbon flow is called the *Cori cycle* (Figure 5-23). Rapid glycolysis in skeletal muscle inevitably results in lactate production because of the activity and  $K'_{eq}$  of LDH, gluconeogenesis is an efficient way to reutilize the products of glycolysis, thereby providing for the maintenance of blood glucose and prolonged muscle glycolysis. The Cori cycle is complemented by the glucose-alanine cycle, which is discussed in Chapter 8.

Because glycolysis is an exergonic process, gluconeogenesis must be endergonic. Gluconeogenesis is under endocrine control, and gluconeogenic tissues such as liver and kidney possess enzymes that bypass the controlling exergonic reactions of glycolysis. Gluconeogenesis is discussed more in Chapter 9.

Mammalian skeletal muscle has poor capacity to convert pyruvate or lactate to glycogen, but Todd Gleeson and associates (1993) showed that reptilian and probably amphibian muscles are capable of glycogen synthesis (glyconeogenesis) from lactate *in situ*. In white reptilian muscle fibers, contractions produce extremely high lactate levels, but circulation is poor, so lactate needs to be removed within the tissue bed of formation. During recovery, lactate moves from white to red fibers by facilitated diffusion. In reptilian red fibers, lactate undergoes gluconeogenesis, as opposed to oxidation in mammalian fibers (see Figure 5-21 and Chapter 10). Thus, the reptilian lactate shuttle supports gluconeogenesis mainly, and lactate oxidation to a lesser extent.

In mammalian white, not red, fibers, some of the lactate resulting from maximal exercise can be converted to glycogen *in situ*. However, in contrast to reptilian muscle, which is poorly perfused with blood, lactate present in mammalian muscle is either removed by oxidation within the tissue bed, or the lactate circulates to the heart or active red muscle, where it is oxidized, or to the liver, where it is converted to glucose. Some of the newly formed glucose can recirculate to recovering muscle and be synthesized to glycogen. However, restoration of muscle glycogen to preexercise levels in mammalian muscle depends on carbohydrate feeding (Chapters 10 and 28). Thus, in mammalian muscle, the lactate shuttle favors direct muscle lactate oxi-



**Figure 5-23** The Cori cycle, showing that pyruvate and lactate formed in muscle can circulate to liver and kidney. There, carboxylic acids can be synthesized to glucose. The glucose thus formed can then reenter the circulation. At rest, the lactate/pyruvate ratio (L/P) approximates 10, but rises an order of magnitude during exercise making lactate the more important gluconeogenic precursor.

dation and, to a minor extent, gluconeogenesis and glycogen synthesis by a version of the indirect pathway (i.e., muscle glycogen  $\rightarrow$  muscle lactate  $\rightarrow$  blood lactate  $\rightarrow$  liver glucose  $\rightarrow$  blood glucose  $\rightarrow$  muscle glycogen).

### Glycogen Synthesis

Work by Wehlan and associates (Lomako et al., 1990) indicates that glycogen is synthesized around a core protein called *glycogenin*. Glycogenin is autocatalyzed, or self-activated, by addition of glucose molecules.

Studies of the role of glycogenin in regulating glycogen synthesis in resting muscle and during recovery from exercise are in their infancy, with the initial work conducted by Terry Graham of the University of Guelph (Adamo et al., 1998a, 1998b). The analyses of human muscle biopsies taken after exercise by Graham, Adamo, and others indicates that large molecular glycogen (i.e., macroglycogen) is degraded to smaller glycogen particles

(i.e., proglycogen), but that exercise does not unmask glycogenin itself. Hence, total muscle glycogen restoration mainly involves building pro- to macroglycogen.

### ■ Effects of Training on Glycolysis

Studies on the effects of training on the glycolytic capability of skeletal muscle have mainly utilized the technique of catalysis. In this experimental approach, a muscle sample of the experimental individual (animal or human) is taken. Then, either an attempt is made to isolate the enzyme or, more usually, the muscle sample is homogenized and the activity of the enzyme is studied. This can be done by observing the disappearance of a substrate or the appearance of a product.

Compared to its considerable effect on oxidative enzymes of muscle (Chapter 6), endurance training appears to have relatively insignificant effects on catalytic activities of glycolytic enzymes. This may

be because skeletal muscle, especially fast-twitch muscle (see Figure 5-6 and Chapter 17), has an intrinsically high glycolytic capability.

Endurance training apparently has little effect on most glycolytic enzymes. Reports of the effects of endurance training on PFK vary, so we may conclude for the present that endurance training has no significant effect on PFK activity.

Hexokinase activity increases significantly in endurance-trained animals. This adaptation, which is thought to facilitate the entry of sugars from the blood into the glycolytic pathway of muscle, would be of benefit in prolonged exercise, where the liver serves to supply muscle with glucose.

Endurance training has been observed to *decrease* total LDH activity in fast glycolytic muscle and to influence the LDH isozymes in muscle to include more of the heart type. However, by increasing muscle mitochondrial mass (Chapter 6), training actually increases mitochondrial LDH content. These adaptations serve to decrease lactate accumulation per unit glycolytic carbon flow in endurance-trained muscle during exercise, and to allow some muscle fibers to take up and oxidize lactate produced in other fibers.

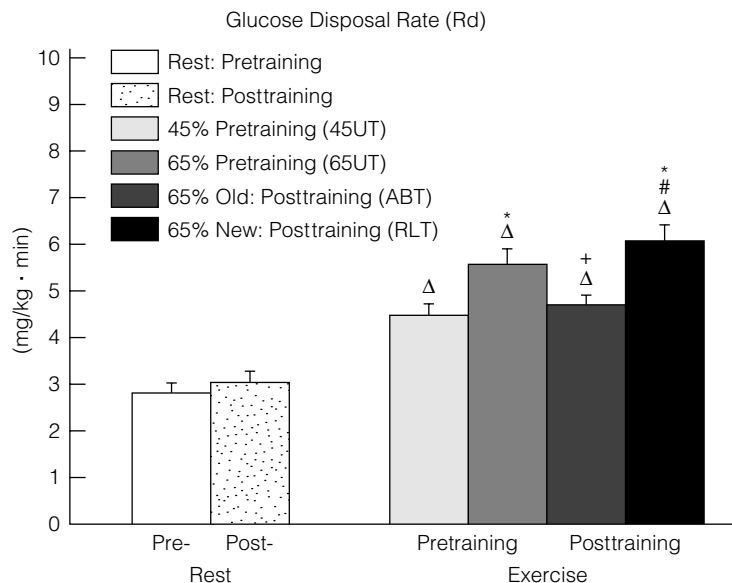
Studies of the effects of speed and power training on the activities of glycolytic enzymes are not as

numerous as studies on the effects of endurance training. However, as with endurance training, the effects of speed and power training on the specific activities of glycolytic enzymes are not great. If the muscle hypertrophies as the result of speed and power training, then the total catalytic activity of the muscle improves.

### ■ Quantitative and Relative Uses of Glucose, Glycogen, and Other Substrates During Exercise

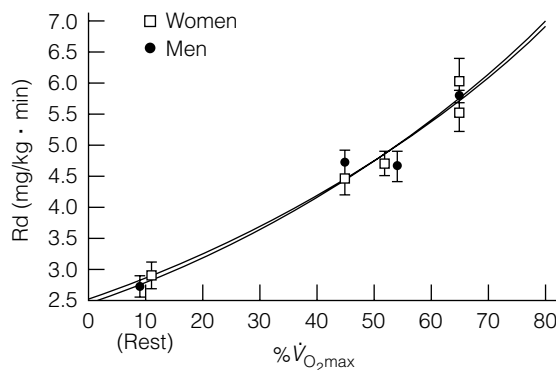
With the advent of stable (nonradioactive) isotope tracer technology, and with simultaneous application of tracer and classical arterial-venous difference (a-v) measurements of metabolites across resting and exercising limbs, much recent progress has been made in measuring use of glucose and other substrates during exercise. Data in Figure 5-24 were obtained in the Berkeley Exercise Physiology Laboratory (Friedlander et al., 1998a, 1998b). These results show several important things about the effects of exercise and training on blood glucose flux during exercise among young women in the mid-follicular menstrual phase using [6,6-<sup>2</sup>H]glucose. The results show that glucose-use rate (Rd, or rate of

**Figure 5-24** The effect of exercise intensity and training on the plasma glucose rate of disappearance (Rd) measured with stable (non-radioactive) isotope [6,6-<sup>2</sup>H]glucose tracer. Values are mean  $\pm$  SEM of the last 15 and 30 minutes for rest and exercise, respectively; n = 17;  $\Delta$  significantly different from rest; \* significantly different from 45UT; + significantly different from 65UT; # significantly different from ABT ( $p < 0.05$ ). Data on young women from Friedlander et al., 1998. Used with permission of the American Physiological Society.

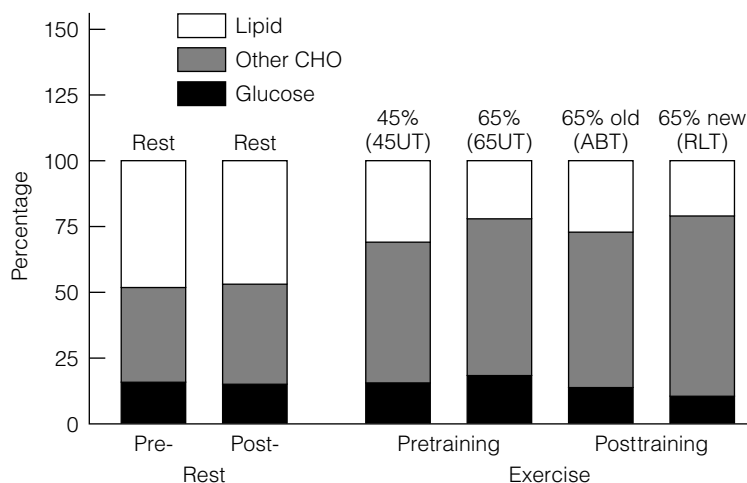


disposal) increases as an exponential function of relative power output. This is also illustrated in Figure 5-25, which contains data on both men and women. Training decreased glucose Rd for a given specific (absolute) power output, but when normalized to  $\% \dot{V}_{O_{2max}}$ , an exponential relationship for both men and women is clear. Thus, at high relative intensity power outputs (in this case, 65% of  $\dot{V}_{O_{2max}}$ ), glucose use is high, even after training.

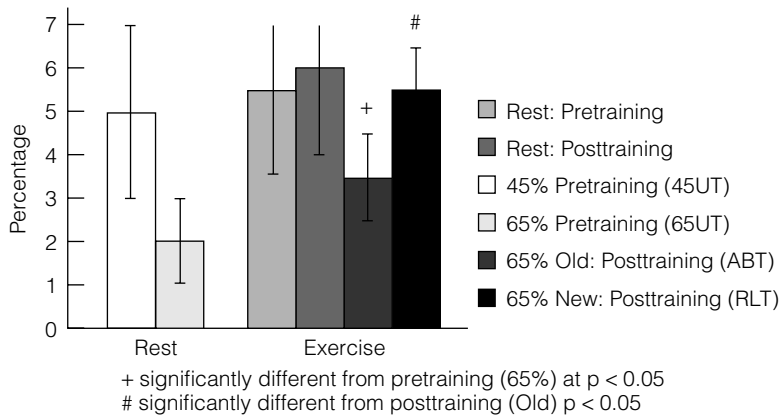
Despite the exponential rise in glucose use as a function of exercise power output, in reality, the rise compared to  $\dot{V}_{O_2}$  rise is relatively small. For instance, in the studies referenced,  $\dot{V}_{O_2}$  rose more than sixfold in the transition from rest to exercise at 65% of  $\dot{V}_{O_{2max}}$  after training; however, glucose disposal and oxidation rates little more than doubled. As shown in Figure 5-26, use of other carbohydrate



**Figure 5-25** Exponential relationship between glucose use (disposal rate, Rd) and relative exercise intensity ( $\dot{V}_{O_{2max}}$ ) before and after training in young men and women. Data from Friedlander et al., 1998. Used with permission of the American Physiological Society.



**Figure 5-26** Relative percentages of substrate supply by percentage of energy supplied in resting and exercising men, before and after ten weeks of endurance training. Subjects studied at 45% and 65% of  $\dot{V}_{O_{2max}}$  before training, and after training at the same power output that elicited 65% of  $\dot{V}_{O_{2max}}$  before training (then 54%  $\dot{V}_{O_{2max}}$ ), and 65% of the posttraining  $\dot{V}_{O_{2max}}$ . Even though use of glucose increases in relation to relative exercise intensity (Figure 5-25), glycogen and lactate are more important as substrates. Lipid use is discussed in Chapter 7. RLT is the relative exercise intensity; ABT is the absolute power output. Values are mean  $\pm$  SEM of the last 15 and 30 minutes for rest and exercise, respectively;  $n = 17$ ;  $\Delta$  significantly different from rest; \* significantly different from 45UT; + significantly different from 65UT; # significantly different from ABT ( $p < 0.05$ ). Data on young women from Friedlander et al., 1998. Used with permission of the American Physiological Society.



**Figure 5-27** Human leg glucose fractional extraction as determined from arterial-venous difference measurements of blood glucose contents during rest and exercise, before and after endurance training. Values are mean  $\pm$  SEM of the last 30 minutes of 90 minutes of rest and 60 minutes of exercise, respectively;  $n = 6$ . Modified from Bergman et al., 1999b. Data show that only a small percentage of glucose is taken up by resting and working human skeletal muscle. Because leg blood flow is increased during exercise as compared to during rest, and after training at given relative exercise intensities, delivery of glucose to working muscle increases. Still, the results show that availability of glucose is unlikely to limit muscle glucose uptake. Muscle glucose uptake is thought to be limited by factors within the cell such as sarcolemmal transport and downstream metabolic factors.

energy sources, mainly glycogen and lactate, increases far more during exercise than does use of glucose.

That glucose supplies only 10–25% of the carbohydrate energy used for exercise, and that working muscle relies on endogenous glycogen and the lactate shuttle probably indicates a protective mechanism preventing the fall in blood glucose during exercise. Recall from earlier in this chapter that the physiological setpoint for blood [glucose] is 100 mg/dl (5.5 mM). Our capacity for hepatic glucose production from liver glycogen breakdown and gluconeogenesis is limited to a maximum fourfold increase. Therefore, should glucose demand by muscle increase too much during exercise, the supply of glucose in the blood could be drained in a matter of minutes during exercise, thus leaving the brain starved for substrate and the person hypoglycemic and unable to think or function physically.

Figure 5-27 illustrates relative (fractional) glucose consumption rates in resting and working limbs. These results were determined from the product of the arterial-venous concentration difference for glucose and limb blood flow. The results are also from the Berkeley Exercise Physiology Laboratory (Bergman et al., 1999c) and are comparable with those obtained in Figures 5-24 and 5-25 because experimental protocols were identical. Note in Figure 5-27 that fractional glucose extraction is restricted to the range of 2–8%. In other words, most (92–98%) glucose courses through working tissue beds without being taken up. Again, this “pseudo-glucose resistance” by working muscle is possible only because of the use of intramuscular glycogen and other energy sources. The results also mean that muscle glucose uptake is not limited by supply, but is determined by intracellular factors such as sarcolemmal glucose transport and downstream metabolic regulation.

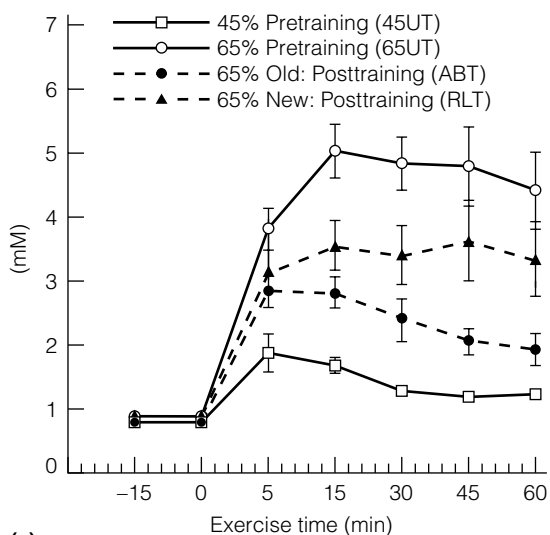


## ■ Effects of Exercise and Endurance Training on Blood Lactate Concentration, Appearance, and Clearance Rates

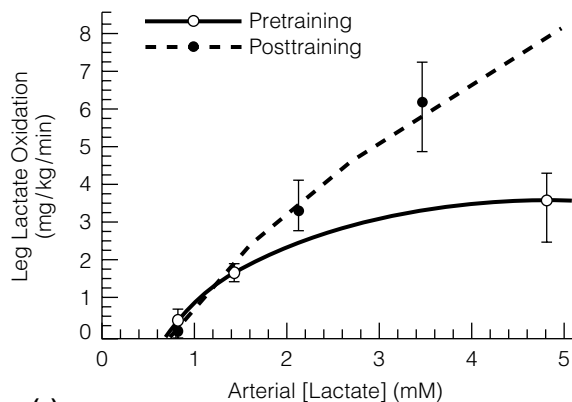
Using a combination of stable (nonradioactive) [ $3\text{-}^{13}\text{C}$ ]lactate tracer technology, and simultaneous arterial and arterial-venous difference measurements of lactate across resting and exercising limbs, Brooks and colleagues have provided long needed data on the effects of training on the blood lactate re-

sponse to exercise. The results in Figure 5-28a reproduce what has long been known: that exercise increases the blood lactate concentration, but that endurance training decreases blood lactate concentration whether measured during given absolute or relative exercise intensities.

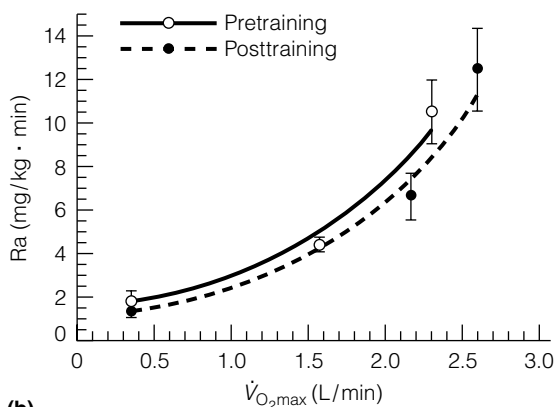
Consistent with results of previous studies on laboratory rats by Casey Donovan and Brooks using radioactive tracers (Chapter 10), results in Figure 5-28b show that blood lactate appearance (entry) rate is exponentially related to the metabolic



(a)



(c)



(b)

**Figure 5-28** Arterial blood lactate concentration and blood lactate kinetics in resting and exercising men, before and after ten weeks of endurance training. Subjects studied at 45% and 65% of  $\dot{V}_{O_2\max}$  before training and after training at the same absolute power output (ABT) that elicited 65% of  $\dot{V}_{O_2\max}$  before training (then 54%  $\dot{V}_{O_2\max}$ ), and 65% of the posttraining  $\dot{V}_{O_2\max}$  (i.e., the same relative exercise intensity (RLT)). In (a), the blood lactate response during exercise is given; training reduces blood lactate concentration at given relative and absolute power outputs after training. In (b), the appearance rate of lactate as determined by infusion of [ $3\text{-}^{13}\text{C}$ ]lactate is given as a function of metabolic rate ( $\dot{V}_{O_2}$ ) during rest and exercise; note the absence of a training effect. (c) After endurance training, the clearance of lactate by oxidation in working human muscle is increased. Values are mean  $\pm$  SEM of the last 15 and 90 minutes of rest exercise, respectively;  $n = 6$ . Modified from Bergman et al., 1999c, and used with permission of the American Physiological Society.

rate during exercise. Importantly, there is no physiologically significant training effect on blood lactate appearance during rest or exercise.

How then are the seemingly disparate results in Figures 5-28a and 5-28b to be understood? The answers are in Figure 5-28c. Blood lactate appearance ( $R_a$ ) rises during even mild exercise (Figure 5-28b) with little effect on blood lactate concentration (Figure 5-28a) because blood lactate disposal rate ( $R_d$ ) rises to compensate for the increased  $R_a$ . After training, the slope of the  $R_d$ /[lactate] curve in Figure 5-28c is greater than before training, indicating

improved lactate clearance. Further, before training the lactate-clearance curve shows a limitation (saturation), whereas after training the clearance curve rose continuously over the range of power outputs studied. In other words, after training blood lactate has to rise less to achieve a given removal rate. The results in Figure 5-28c are consistent with training increasing the number of sarcolemmal and mitochondrial lactate transport proteins (MCTs), thereby facilitating function of the cell-cell and intracellular lactate shuttles (Figures 5-14 and 5-21).

## SUMMARY

Glycolysis and glycogenolysis are specialized functions in skeletal muscle. Large quantities of glycolytic and glycogenolytic enzymes in muscle can support powerful contractions for brief periods. In resting muscle, little glycogenolysis occurs, so the muscle depends primarily on exogenously supplied glucose and free fatty acids (Chapter 7). However, when exercise starts, muscle glycogen provides most (80–90%) of the carbohydrate requirement for exercise. Working muscle glucose uptake rises exponentially as a function of the relative exercise intensity, but the scope of the rise (two- to fourfold) is small in comparison to the increment in overall energy supply. Working muscle takes up an average of only 4% of the glucose circulated to it. The “pseudo-glucose resistance” of working muscle is considered a protective mechanism, allowing glucose to be available for the brain, which depends on it.

Recent discoveries that mitochondria in muscle, liver, and other tissues consume and oxidize lactate, along with documentation of the existence of mitochondrial LDH and MCT, allow a new view of how glycolytic (nonoxidative or anaerobic) metabolism is linked to oxidative (or aerobic) metabolism. Lactate is always produced in muscle and other tissues because of the abundance, activity, and characteristics of cytoplasmic lactic dehydrogenase. The lac-

tate to pyruvate ratio ( $L/P$ ) is approximately 10 in resting muscle, and the ratio can rise many times during exercise even when there is no limitation in oxygen supply. Because of the *intracellular lactate shuttle mechanism*, lactate produced as the result of glycolysis in the cytosol is balanced by oxidation in mitochondria of the same cell. Hence, while resting muscle produces and releases lactate on a net basis, the release is small compared to the rate of production. In the steady state, working muscle releases little lactate so long as mitochondrial respiration is adequate to keep pace with cytosolic lactate production.

At the start of exercise, large amounts of lactate are released from muscle until the rate of oxygen consumption can rise and balance lactate production and removal. Thereafter, working muscle stops releasing lactate and, like the heart, becomes a net lactate consumer (Chapter 10). During exercise, the intracellular lactate shuttle is assisted in removing lactate by the cell-cell lactate shuttle. By this mechanism, lactate moves through the interstitium and vasculature from sites of production and net release (e.g., type IIb fibers) to highly oxidative (type I and cardiac fibers) as well as liver where removal is via gluconeogenesis as opposed to oxidation.

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