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### 11.3 Digestion and Absorption of Carbohydrates

In humans and other mammals, carbohydrate absorption from food occurs in the small intestine, primarily the jejunum. The substrate-specific transport proteins in the membranes of the epithelial cells transport only monosaccharides. Disaccharides and polysaccharides must be hydrolyzed into monosaccharides by specific enzymes in order to be absorbed.

**EXAMPLE 11.4** The milk disaccharide lactose is hydrolyzed in the small intestine prior to the absorption of the constituent monosaccharides. Describe this process.

Lactose is hydrolyzed by the membrane-bound enzyme *lactase*. The enzyme is also known as a  $\beta$ -*galactosidase* because its action involves the cleavage of the  $\beta$ -anomer of galactose from its 1 $\rightarrow$ 4 bond with an  $\alpha$ -anomer of glucose. Note this arrow nomenclature for the bond or linkage between the two monosaccharides.

**EXAMPLE 11.5** What is the consequence of an acquired or *inherited* deficiency of lactase?

If the enzyme lactase is deficient, then lactose is not hydrolyzed in the small intestine and it passes unchanged into the large intestine. There it is fermented by bacteria that release volatile organic acids that irritate the mucosa of the bowel; and large volumes of gas such as hydrogen and methane are produced in the process. This, in turns, leads to the clinical signs of flatulence and loose stools, plus abdominal discomfort and a feeling of being “bloated.” This negative response to the ingestion of lactose is called *lactose intolerance*, and if it occurs in babies prior to weaning, the condition is manifested as *explosive diarrhea*. Interestingly, the extent of lactose intolerance is seen to be strongly linked to ethnicity, with some populations having very low incidence (3% in Scandinavians) while other groups show very high prevalence (>90% in Thais).

In the small intestine, sucrose is cleaved to glucose and fructose by *sucrase*. In contrast to lactase, this enzyme is rarely deficient.

Starch digestion also occurs primarily in the small intestine, but some cleavage begins in the mouth and gastric contents since saliva contains the enzyme *ptyalin*, which is a form of  $\alpha$ -amylase. It attacks the 1 $\rightarrow$ 4 glycosidic bond of an adjacent  $\alpha$ -anomer of glucose. Ptyalin hydrolyzes the starch chains down into *di-* and *trisaccharides* (maltose and maltodextrins) that are further hydrolyzed by *maltase* to glucose in the small intestine.

Note that there are two forms of starch: amylase and amylopectin. In amylase, the glucose residues are joined end to end by 1 $\rightarrow$ 4 glycosidic bonds between  $\alpha$ -anomers of glucose. This produces a linear, unbranched polysaccharide. Hydrogen bonds between amylose molecules twist the chains into large helical rodlike structures that stack to form sheets. These sheets impede digestion by amylase.

In contrast, amylopectin contains 1 $\rightarrow$ 6 glycosidic branch points at regular intervals. These result in an open, treelike structure. Amylopectin is more readily hydrolyzed by amylase, and this results in a mixture of small, branched maltodextrin molecules that are often referred to as *limit dextrins*. Limit dextrins are further hydrolyzed by maltase.

**EXAMPLE 11.6** What are the respective dietary sources of the starches amylose and amylopectin?

Amylose is mainly found in legumes and pulses (such as peas, lentils, chick peas, and kidney beans). Older strains of grain contain more amylose than modern, agriculturally developed ones. Through successive generations, grain crops have been bred to contain a higher proportion of *amylopectin* because this form of starch makes flour that yields a more pleasing outcome when cooked (e.g., it makes lighter bread). Most of the pasta and bread used in the Western diet contains a high proportion of amylopectin. Increasing interest in the potential health benefits of amylose starch has led grain producers to explore the use of grains that contain a higher proportion of amylose.

As the di- and trisaccharides are hydrolyzed to glucose by *maltase* and *dextrinases*, respectively, in the small intestine, the glucose molecules enter the epithelial cells through specialized sodium-dependent GLUT transporters (Prob. 10.32). From there, the glucose crosses the enterocytes and enters the bloodstream via different GLUT transport proteins, thus elevating the blood glucose concentration.

### 11.4 Blood Glucose Homeostasis

The concentration of glucose in the blood of normal individuals is usually ~5 mM, a state that is referred to as *euglycemia* (Greek *eu-* = good or well, *glycemia* = glucose in the blood). If the concentration falls below 2–3 mM, this state is referred to as *hypoglycemia*; and because the brain has an obligatory requirement for glucose, hypoglycemia can lead rapidly to *unconsciousness*. Glucose is a relatively reactive molecule,

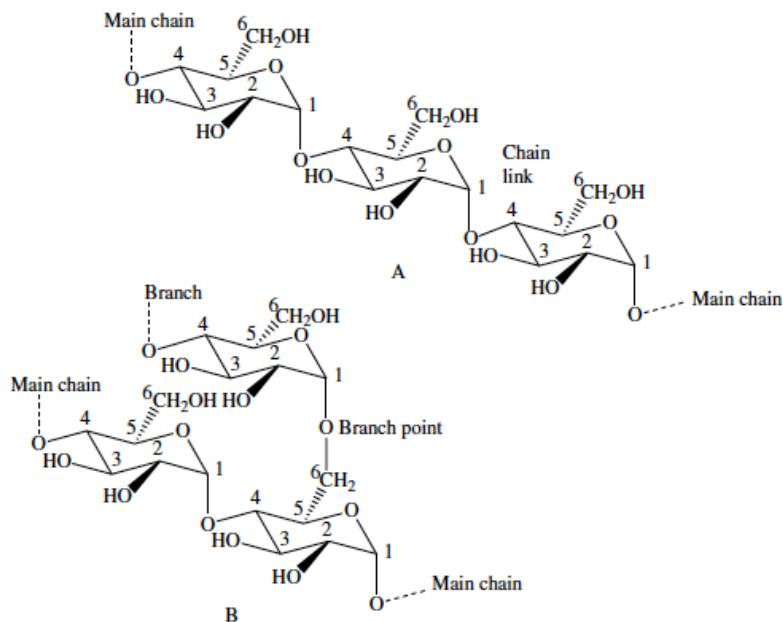


Fig. 11-3 Structures of (A) amylose and (B) amylopectin as chair projections of glucose residues.

because the open-chain form is a free aldehyde (see Sec. 11.2). Although a blood glucose concentration above 7 mM that is referred to as *hyperglycemia* is not dangerous in the short term, there are severe health consequences associated with hyperglycemia that lasts over periods of more than a few weeks.

**EXAMPLE 11.7** What is the nature of the chemical reactivity of glucose?

The aldehyde group of the open-chain form of glucose, which is in chemical exchange with the  $\alpha$ - and  $\beta$ -closed-ring forms, reacts with free amino groups on proteins, resulting in the covalent attachment of glucose residues to the protein. The product is called a Schiff base (Chap. 1). The extent of the reaction, that is spontaneous and not enzyme-catalyzed, depends on the concentration of glucose and the time of exposure to elevated concentrations of glucose.

After a carbohydrate-rich meal, glucose is distributed into tissues and then subsequently converted to the fuel stores of glycogen (glycogenesis) and triglyceride (lipogenesis), or catabolized by oxidation to carbon dioxide (via glycolysis and the Krebs cycle).

The liver is the first organ to receive blood that contains glucose absorbed from the intestine. The portal vein drains from the small intestine into the hepatic sinuses that are surrounded in a neat *columnar* fashion by hepatocytes. The arrival of glucose in the blood is detected by the  $\beta$ -cells in the pancreas, and they respond by secreting the peptide hormone *insulin*. Insulin facilitates glucose uptake by many cell types, from skeletal muscle to white blood cells (Fig. 11-4), but it has no effect on glucose uptake by the brain or red blood cells that rely on GLUT-1.

**Glycemic Index (GI)**

Because the maintenance of euglycemia is important for health, both the *disposal time* and the *amplitude* of the postmeal (*postprandial*) blood glucose concentration are useful measures when investigating patients with possible glucose-handling problems. Carbohydrates that are rapidly digested and absorbed lead to higher, and more prolonged, postprandial blood glucose concentrations. It is noted that the two starches amylose and amylopectin are digested at different rates, and as expected, they cause quite different effects on the time course of blood glucose concentration.

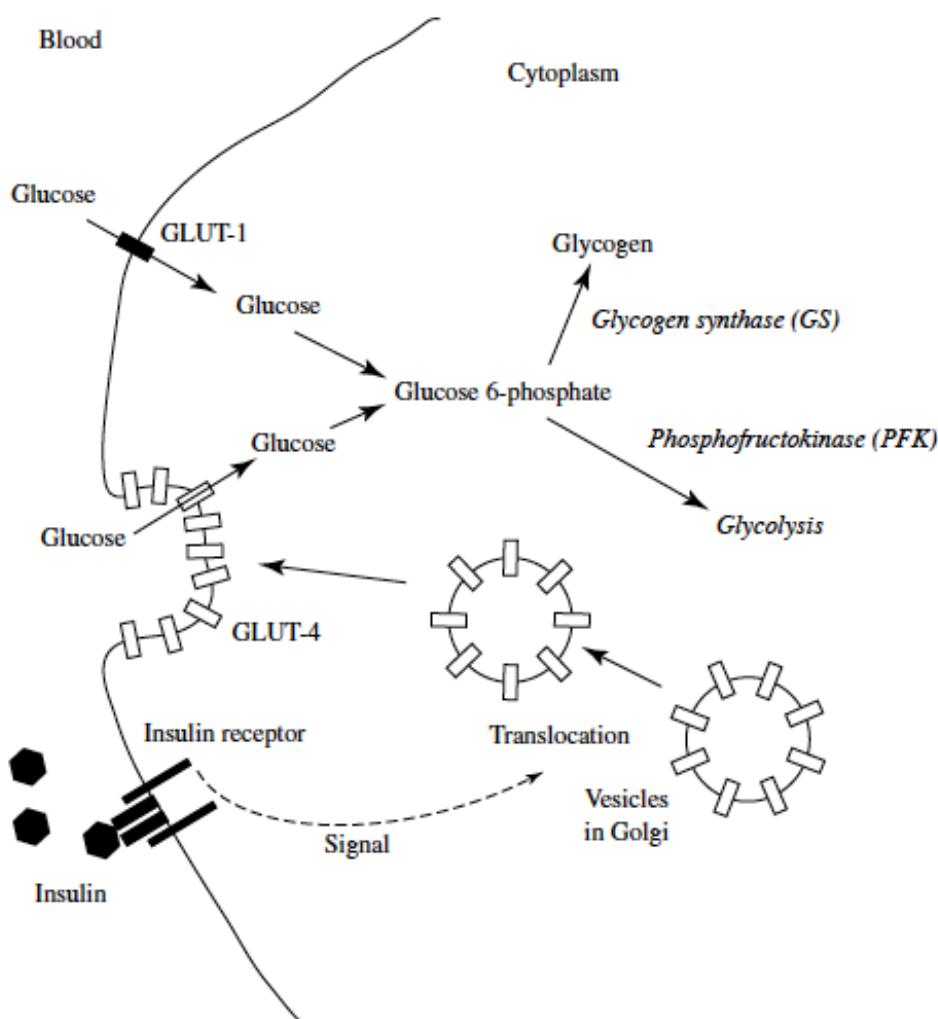


Fig. 11-4 Overview of glucose uptake and disposal in skeletal myocytes and adipocytes. Glucose enters the cell via GLUT-1 and via the insulin-stimulated insertion of GLUT-4 carriers into the plasma membrane. Glucose 6-phosphate is a point of *flux divergence* into glycolysis and glycogen synthesis.

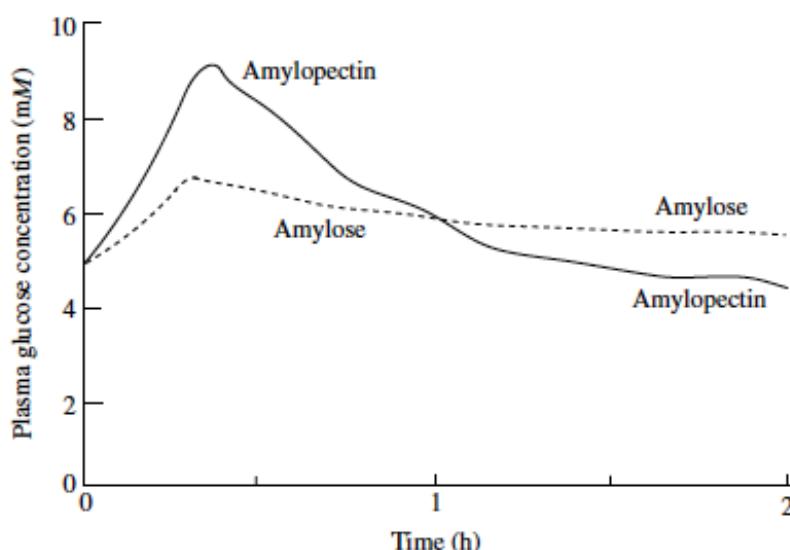
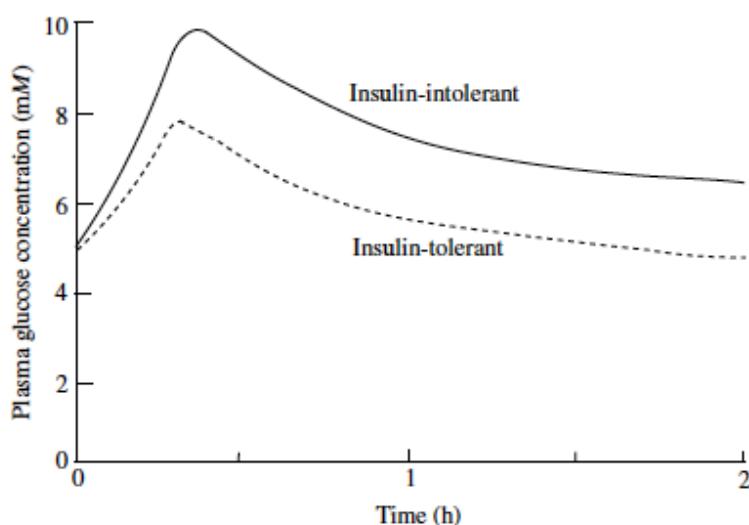


Fig. 11-5 Comparison of the postprandial glucose responses of amylose and amylopectin.

The different concentration maximum and overall time course of blood glucose that occur in a normal person in response to a standard dose of a particular food can be quantified and given a number; it is called the *glycemic index* (GI) of the food. The GI is calculated from the integral (area) under the curve, up to 120 min, that describes the postprandial blood glucose concentration. Each GI value is standardized and expressed as the ratio of the integral relative to that of a reference food, usually pure glucose. Healthy volunteers are fed a known amount of the test food containing 50 g of absorbable carbohydrate, on one occasion, and then an identical amount of pure glucose on another occasion.



**Fig. 11-6** Different plasma glucose response in two different patients after a meal; one who is insulin tolerant and one who is insulin intolerant, for example, as occurs in non-insulin dependent diabetes mellitus (NIDDM).

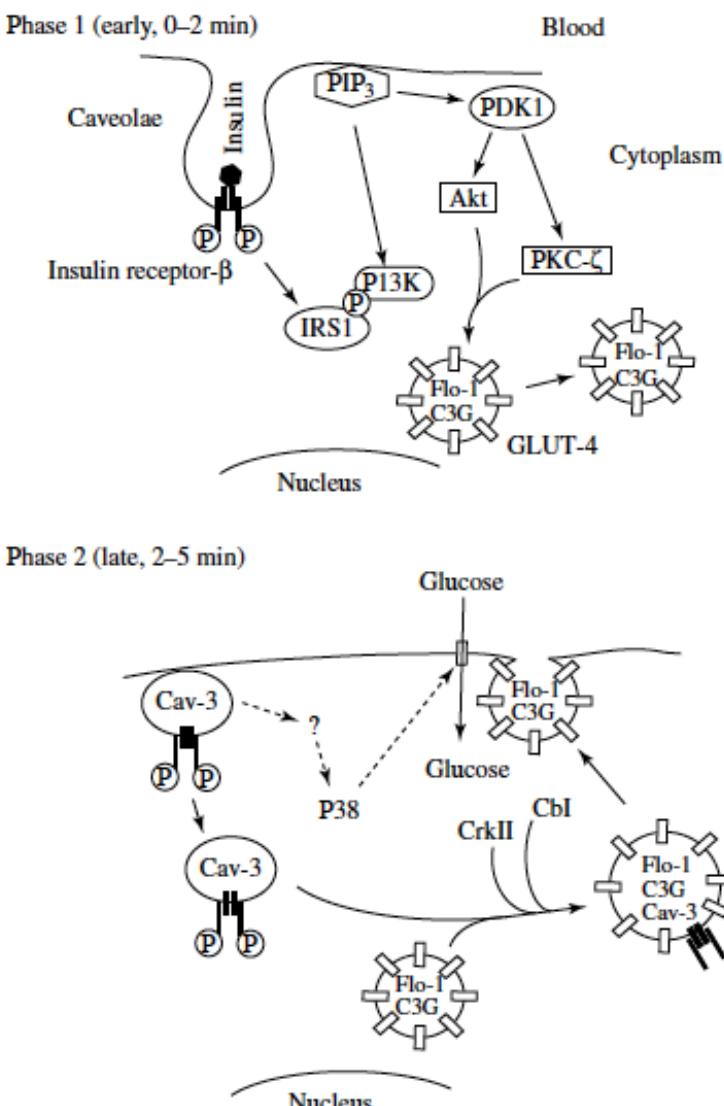
#### EXAMPLE 11.8 Which foods are expected to have a high GI?

Foods containing amylopectin or free glucose have high GI values. Examples are highly processed starch-rich foods that use modern grains. Some potato and rice products have GI values greater than 100; they give a postprandial glucose concentration that is much higher earlier after ingestion than pure glucose! This is due to the very rapid digestion of amylopectin starches and the fact that the high osmolality of a pure-glucose test solution delays stomach emptying and thus slows intestinal absorption.

#### Glucose Absorption into Cells

Although the metabolic fate of blood glucose varies from tissue to tissue, it commences with the transport of glucose from the blood across the plasma membrane of a cell. The family of integral membrane proteins, the GLUTs, mediates this uptake (see Prob. 10.32). GLUT-1 is present in all tissues where it provides a continuous, baseline flux of glucose; however, it does not give a sufficiently high rate of glucose transport to handle the sudden arrival of a postprandial glucose load. Some tissues have additional specialized GLUTs that are more responsive to the insulin signal (Chap. 13) which heralds a higher blood-glucose concentration.

**EXAMPLE 11.9** Muscle and adipose tissue contain a member of the GLUT family of glucose transporters (GLUT-4) that normally resides in a *holding zone* in vesicles that are associated with the endoplasmic reticulum. On the binding of insulin to an insulin receptor on the cell surface, an intracellular signal is transmitted to the vesicles, which causes the transporters to be translocated to, and fuse with, the plasma membrane. This delivery of GLUT-4 transporters to the plasma membrane allows a potentially great increase in the flux of glucose into the cell. Interestingly, exercise is even more effective in stimulating GLUT-4 translocation than is insulin.



**Fig. 11-7** Insulin stimulation of GLUT-4 translocation. This is rather complex, so to capture the current understanding of the system the diagram also needs to be complex: In the first stage of activation of glucose uptake (phase 1), the signaling protein, insulin receptor substrate 1 (IRS1), is phosphorylated by the insulin receptor. IRS1 then phosphorylates and activates phosphoinositol-3-kinase (PI3K) that phosphorylates phosphoinositol 4,5-bisphosphate and 4-phosphate to form phosphoinositol 3,4,5-trisphosphate ( $\text{PIP}_3$ ) and phosphoinositol 3,4-bisphosphate ( $\text{PIP}_2$ ), respectively, in the cell membrane (e.g., Fig. 6-6). These phosphoinositides are allosteric activators of phosphoinositide-dependent kinase-1 (PDK-1) that in turn phosphorylates and activates protein kinase B (also called Akt) as well as atypical-protein-kinase C (PKC-ζ). These two enzymes promote the translocation of perinuclear membrane domains that contain the protein flotillin and GLUT-4 to the plasma membrane. Phase 2 sees caveolin-3 (Cav-3)-containing domains move from the sarcolemma (endoplasmic reticulum) to the cytoplasm where they interact with flotillin/GLUT-4 containing domains. Insulin receptors now move from the plasma membrane attached to vesicles that contain Cav-3, which in the presence of the regulatory proteins CrkII and Cbl, and with the GDP/GTP exchange activator protein C3G, finalizes the movement of GLUT-4 to the plasma membrane. Cavolin-3 also acts via p38 MAP kinase to make GLUT-4 function in glucose transport.

### Locking Glucose inside Cells

Once inside the cell, glucose is phosphorylated to produce glucose 6-phosphate; this has a net negative charge, rendering it unable to bind to the GLUT transporters. Thus the plasma membrane becomes impermeable to the modified glucose that is now locked within the cytoplasm (Prob. 10.9). Phosphorylation of a glucose molecule consumes a molecule of ATP. This constitutes an investment of energy in the metabolism of the molecule, so the step is often referred to as a *priming* or *sparking* reaction for the subsequent metabolic steps (Example 10.12).

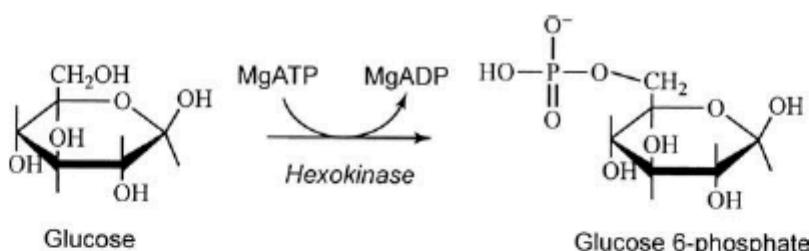


Fig. 11-8 Phosphorylation of glucose by hexokinase.

Hexokinase catalyzes the phosphorylation of glucose in most tissues; but hepatocytes use an additional form of this enzyme, *glucokinase*. The ways in which the two enzymes are regulated are quite different, and this strongly influences the tissue-specific manner in which glucose is metabolized.

#### EXAMPLE 11.10

The key differences between hexokinase and glucokinase are as follows.

Hexokinase, in all cells, can phosphorylate other 6-carbon monosaccharides such as fructose and galactose, whereas glucokinase is specific for glucose and is only found in the liver. Hexokinase is inhibited by its product, glucose 6-phosphate, but glucokinase is not. Hence hexokinase will slow down if glucose 6-phosphate accumulates, whereas glucokinase will continue unabated; consequently glucose 6-phosphate concentrations do not become very high in peripheral tissues such as skeletal muscle, but can rise significantly in the liver. There it can be incorporated into large amounts of glycogen.

Another difference is that glucokinase has a high  $K_m$  for glucose ( $\sim 10 \text{ mM}$ ), but that for hexokinase is  $\sim 100$  times less ( $\sim 0.1 \text{ mM}$ ). Enzymes operate near their maximal rate ( $V_{max}$ ) when their substrate concentration is  $>10$  times  $K_m$ ; hence hexokinase is nearly always operating near its maximum rate. Conversely, the flux of glucose through glucokinase varies almost linearly within the normal blood glucose concentration range.

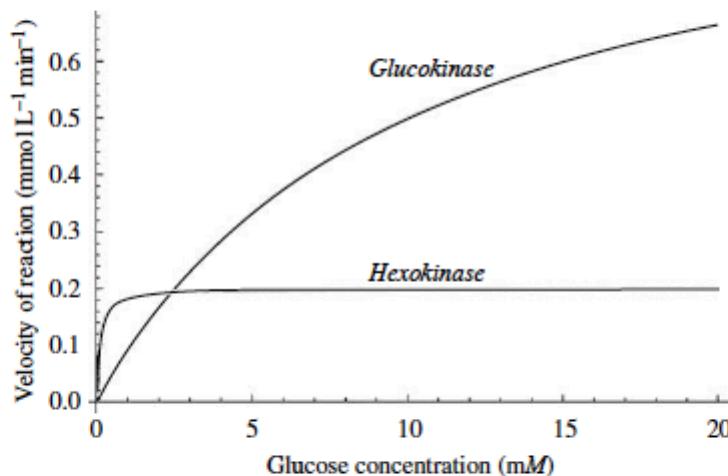


Fig. 11-9 Enzyme kinetics of glucokinase and hexokinase. This is a schematic only and shows glucokinase having a maximal velocity that is 10 times greater than that of hexokinase, while the  $K_m$  of hexokinase for glucose ( $0.1 \text{ mM}$ ) is 1/100th of that for glucokinase ( $10 \text{ mM}$ ).

### Fate of Glucose 6-Phosphate

Glucose 6-phosphate has one of two major fates: either *glycogenesis* or *glycolysis*. The particular fate is determined by regulation of the fluxes via the enzymes in the two pathways. Even though both of these pathways contain multiple enzyme-catalyzed steps, each pathway has specific controls on the constituent enzymes.

#### EXAMPLE 11.11 What is the major flux-controlling step of glycogen synthesis?

Synthesis of glycogen from glucose 6-phosphate involves its conversion into glucose 1-phosphate and the transfer of this glucose residue onto the nucleotide UDP. Thus the glucose residue is *activated* for transfer onto a growing glycogen polymer. The protein *glycogenin* is at the core of glycogen molecules. It has the unusual property of catalyzing its own glycosylation, attaching C-1 of a UDP-glucose molecule to the -OH of a tyrosine residue. Therefore glycogenin serves as a primer for the synthesis of glycogen. After ~6 rounds of attachment of glucose residues via  $\alpha$ -(1 $\rightarrow$ 4) linkages from UDP-glucose, glycogen synthase takes over the reactions. While glycogen synthase catalyzes the formation of the new glycosidic bonds, it is the slowest of the enzymes in the glycogenesis pathway. This step is effectively irreversible, and glycogen synthase is subjected to many regulatory effectors. Branching of the growing linear chains of glucose takes place at periodic intervals via the *branching enzyme*, as shown in Fig. 11-10.

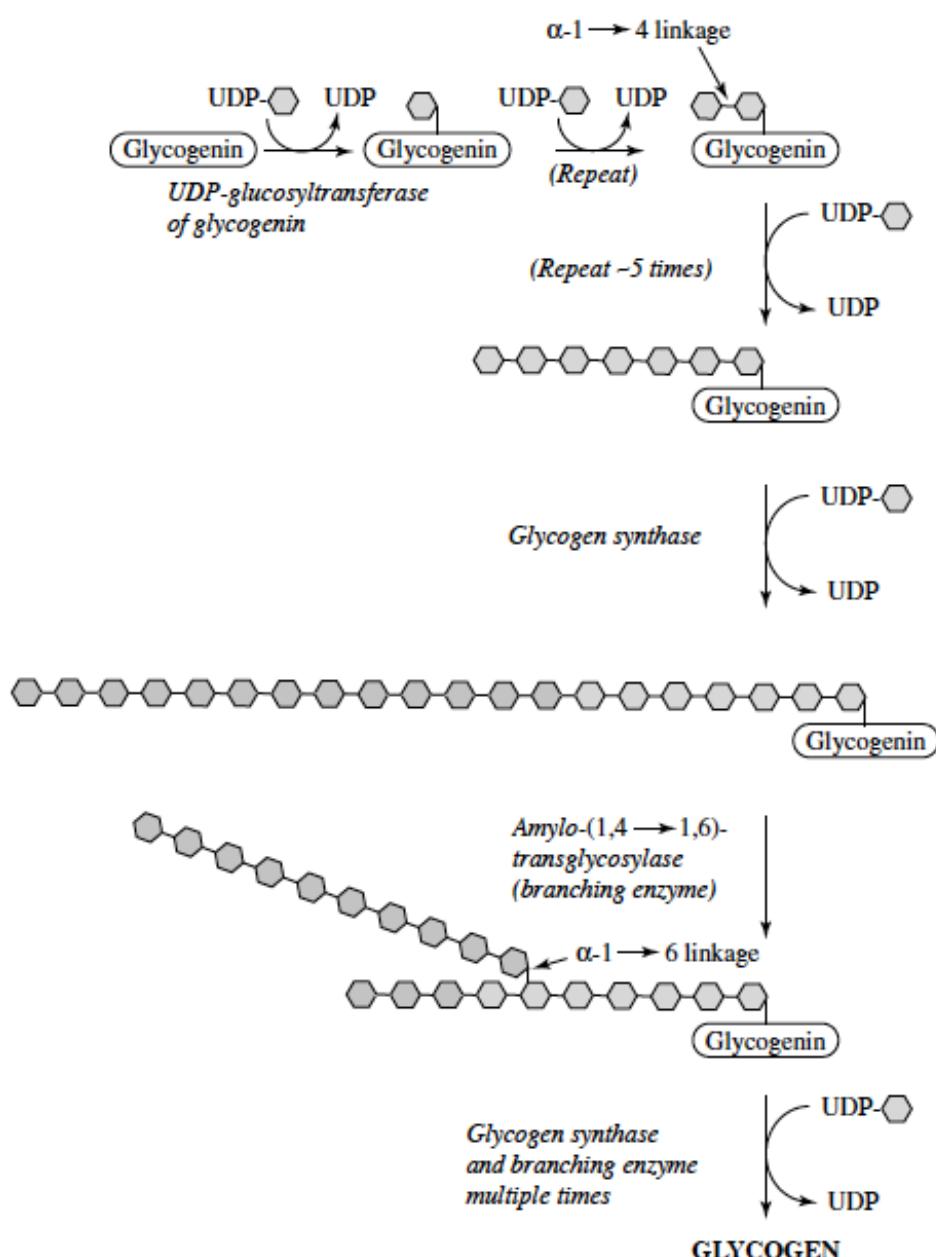


Fig. 11-10 Overview of glycogen synthesis.

**EXAMPLE 11.12** What is the mechanism of insertion of branch points into a growing glycogen polymer?

An enzyme called *branching enzyme* catalyzes the hydrolysis of small sections, ~10–12 glucose residues in length, from the end of a growing glycogen chain. It then transfers this section to a region of the chain nearer the origin of the polymer and catalyzes the formation of an  $\alpha$ -1 $\rightarrow$ 6 bond to another glucose residue. Branch points in glycogen occur once every ~10 residues.

**Control of Glycogen Synthase and Phosphofructokinase**

Because the fate of glucose 6-phosphate depends largely on the relative activities of *glycogen synthase* and *phosphofructokinase*, to understand the direction of carbon flow from glucose, it is necessary to understand how these enzymes are regulated.

Glycogen synthase is inactivated (*switched off*) when specific serine residues are *phosphorylated*. This reaction is catalyzed by *glycogen synthase kinase* that uses ATP as a cosubstrate. Glycogen synthase is reactivated (*switched on*) by *protein phosphatase 1* which catalyzes the hydrolysis of the phosphate groups from the enzyme. Protein phosphatase 1 in turn is *stimulated by insulin*. The binding of *insulin* to insulin receptors in the plasma membrane of the cell triggers a *signaling cascade* that stimulates protein phosphatase 1.

The total amount of glycogen synthase in the cell does not change in a few minutes or hours, but the proportion of the inactive and active forms does, and this is under the control of insulin. Insulin determines the relative activities of *glycogen synthase kinase* and *protein phosphatase 1*. When protein phosphatase 1 is more active than glycogen synthase kinase, such as will be the case with insulin stimulation of the cell, glycogen synthase becomes dephosphorylated and hence is activated. Overall, glycogen synthase is stimulated by insulin.

In addition to being switched fully *on* or *off* by reversible phosphorylation, glycogen synthase is subject to more subtle allosteric regulation. It is activated by glucose 6-phosphate, so if the cytosolic concentration of this metabolite rises sufficiently, the *otherwise inhibited* phosphorylated glycogen synthase is stimulated into activity.

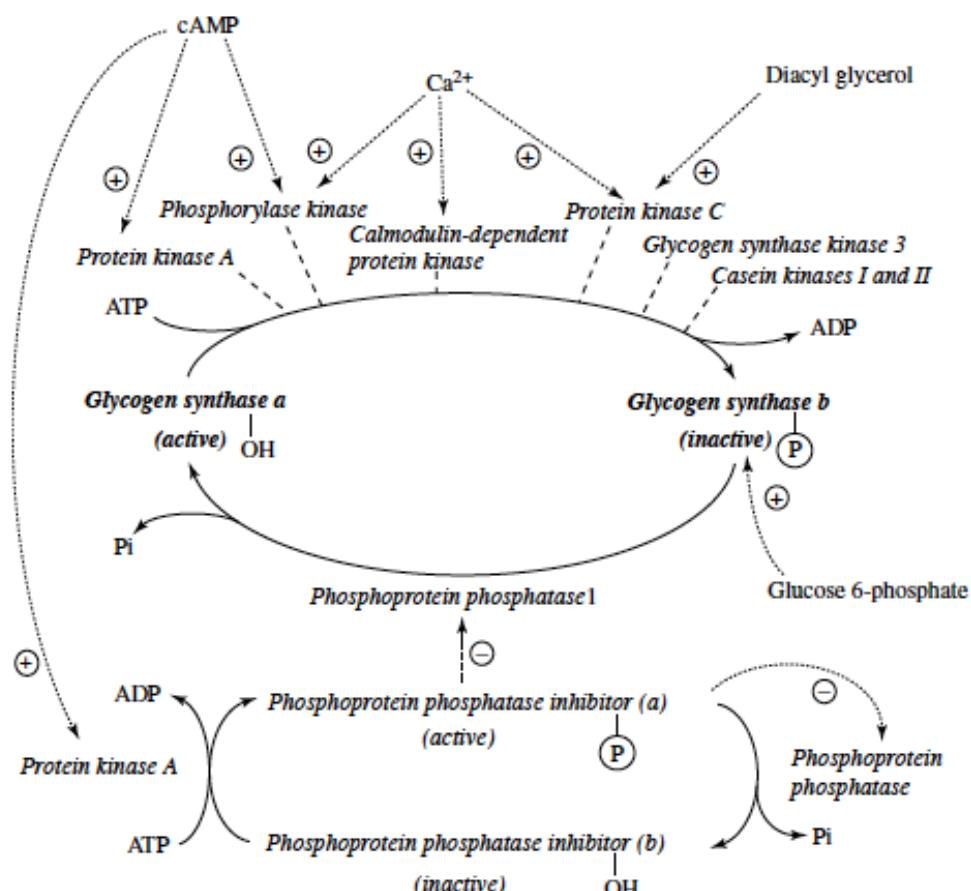


Fig. 11-11 Regulation of glycogen synthase by phosphorylation, dephosphorylation, and glucose 6-phosphate. Phosphorylation of glycogen synthase takes place via at least seven different protein kinases as indicated. cAMP denotes cyclic AMP. The dashed arrows with adjacent + and - signs denote activation or inhibition, respectively.

**EXAMPLE 11.13** Phosphofructokinase is regulated allosterically.

In contrast to glycogen synthase, phosphofructokinase is *not* regulated by phosphorylation and dephosphorylation; instead it is *regulated allosterically* (Chap. 5). Phosphofructokinase contains several different allosteric sites that bind a variety of different metabolites. Most importantly it is very sensitive to changes in metabolite concentrations that reflect the *energy charge* of the cell. Energy charge is a convenient way of describing the availability of ATP in the cell and is defined as  $([ATP] + 0.5[ADP])/([ATP] + [ADP] + [AMP])$ .

Binding of ADP or AMP allosterically *activates* phosphofructokinase, but high concentrations of ATP are *inhibitory*. At first sight this seems to be a contradictory response elicited by ATP since it is a substrate for the enzyme. However, careful analysis of the relationship between the activity of phosphofructokinase and ATP concentration shows that there is always sufficient ATP in the normal cell for the reaction to proceed, but that ATP inhibition becomes significant only when its concentration is very high. *Fructose 2,6-bisphosphate* is an *inhibitor*, as is *citrate*. The regulation of the concentration of fructose 2,6-bisphosphate is via a phosphorylation-dephosphorylation process as shown in Fig. 11-12.

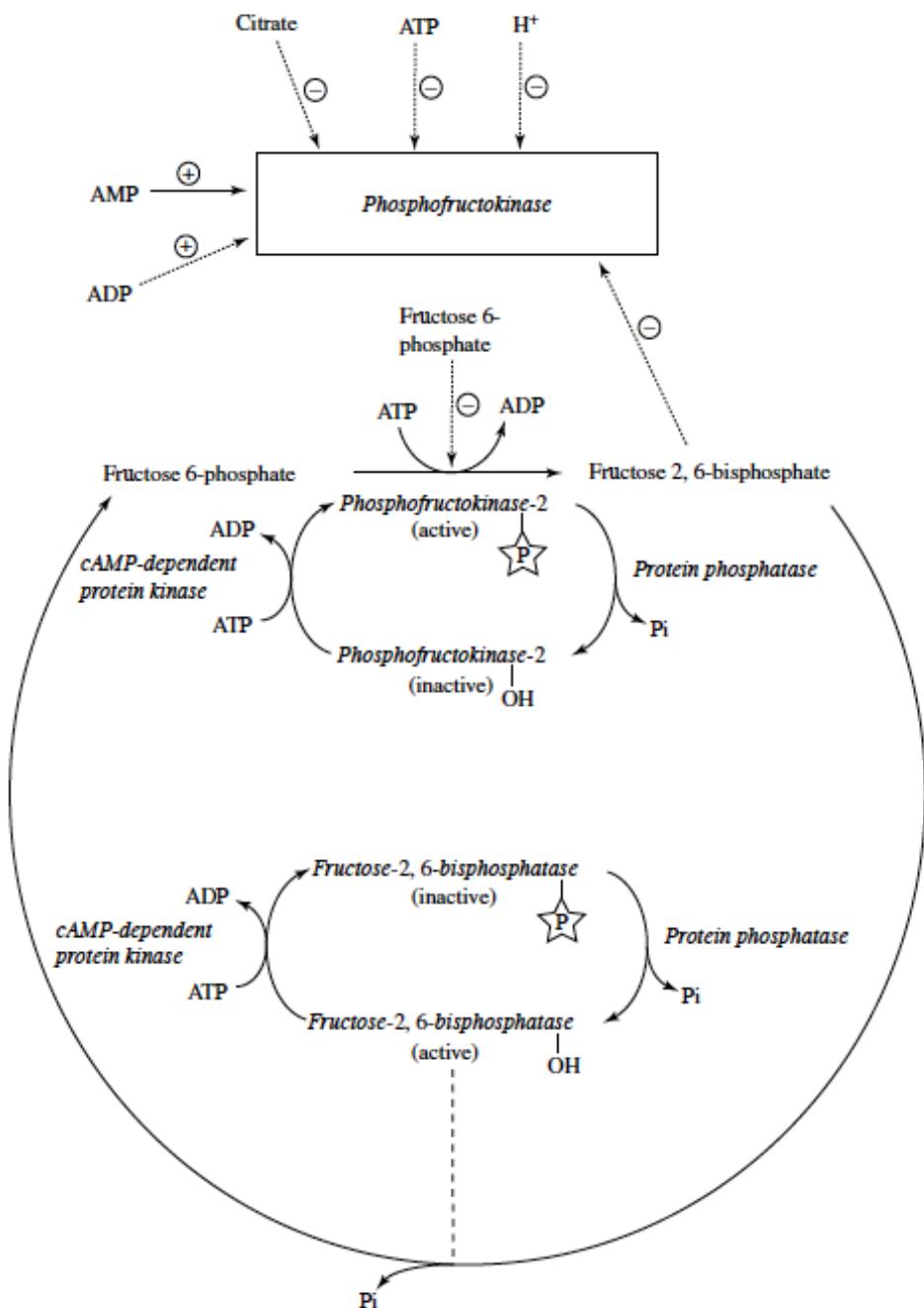


Fig. 11-12 Regulation of phosphofructokinase by allosteric effectors.

**EXAMPLE 11.14** Phosphofructokinase is also inhibited by H<sup>+</sup>.

Phosphofructokinase rarely operates at maximum velocity which only occurs at about pH 8.5. This is a safety valve for the preservation of skeletal muscle because when the muscle is active, it devours a lot of glucose and produces heat and lactate. The formation of lactate (and H<sup>+</sup>) inhibits phosphofructokinase which causes glycolysis to slow down, thereby protecting the muscle from overheating.

Overall, *glycogen synthase* is stimulated by insulin, and a falling cellular energy charge stimulates *phosphofructokinase*. Appreciation of the fact that glycogen synthesis is an anabolic process and consumes ATP allows us to see that if insulin stimulates glycogen synthesis, then it must also decrease the concentration of ATP, which will stimulate phosphofructokinase. Therefore, insulin stimulates glycogen accumulation directly but also indirectly stimulates glycolysis. This is an excellent example of how flux through an anabolic pathway is linked by regulatory processes to flux via a catabolic pathway.

## 11.5 Regulation of Glycogen Production

### Tissue Differences in the Regulation of Glycogen Synthesis

Glucose uptake into skeletal myocytes is insulin-dependent (Sec. 11.4), and glucose 6-phosphate concentrations do not rise greatly because of its inhibition of hexokinase; this *product inhibition* is a form of *negative feedback control* of glucose flux via hexokinase. Even if there are high rates of GLUT-4 mediated glucose uptake into cells, if glucose 6-phosphate accumulates, then hexokinase will be inhibited and glucose will exchange back out of the cells. However, insulin also activates glycogen synthase that places demand (a *sink*) on glucose 6-phosphate and releases the inhibition of hexokinase. Thus the stimulation of glycogen synthase by insulin diverts glucose to glycogen in skeletal myocytes.

In contrast, in hepatocytes the concentration of glucose 6-phosphate rises rapidly in response to an increase in blood glucose concentration. Elevation of glucose 6-phosphate is sufficient to allosterically activate glycogen synthase, even in the absence of any insulin-induced dephosphorylation of the enzyme. The high intracellular glucose concentration exerts a stimulatory effect on protein phosphatase 1, via an increased release of it from an inactive complex with another glycogen-metabolizing enzyme, *glycogen phosphorylase* (see more in Chap. 13). Thus, a high blood glucose concentration promotes glucose into glycogen synthesis. In principle, increased synthesis of glycogen could occur just in response to hyperglycemia, but the additional stimulation of glycogen synthase by insulin provides a further boost to the rate of glycogen synthesis.

### Other Factors Affecting Glycogen Synthesis

Figure 11-13 shows more details (compared with Fig. 11-10) of the pathway of glycogen synthesis. The reaction between glucose 1-phosphate and UTP that generates the activated precursor UDP-glucose is a *priming* reaction that involves the formation of pyrophosphate (PPi). Pyrophosphate is rapidly hydrolyzed via *pyrophosphatase*, to generate two orthophosphate ions (Pi); since this reaction is effectively irreversible, the hydrolysis drives the formation of UDP-glucose.

The formation of a glycosidic bond occurs between the 4-OH group on the glucose moiety at the end of the glycogen molecule and the activated 1-position of the glucose residue in UDP-glucose. As in nucleic acid and protein synthesis, glycogen synthesis has a fixed direction of propagation.

**EXAMPLE 11.15** There are basic similarities between glycogen synthesis and the synthesis of some other key macromolecules.

The reactions of glycogen, DNA, RNA, and protein synthesis all use activated precursors, UDP-glucose, dNTPs, NTPs, and amino-acyl tRNAs, respectively. However, unlike the others, glycogen synthesis does not use a template in its propagation. And during the formation of glycogen, branch points are introduced into the emerging polymer (Fig. 11-10).

For a tissue to synthesize glycogen, *glycogen synthase* must be present. White adipose tissue has little of this enzyme, so even though glucose uptake into white adipose tissue is responsive to insulin, little glycogen is made there.

## 11.6 Glycolysis

### Fate of the Glucose That Enters Glycolysis

The reactions of glycolysis lead to extracting some of the energy in the bonds of glucose and storing it in the high-energy terminal *phospho-anhydride bond* of ATP. For each molecule of glucose that passes through

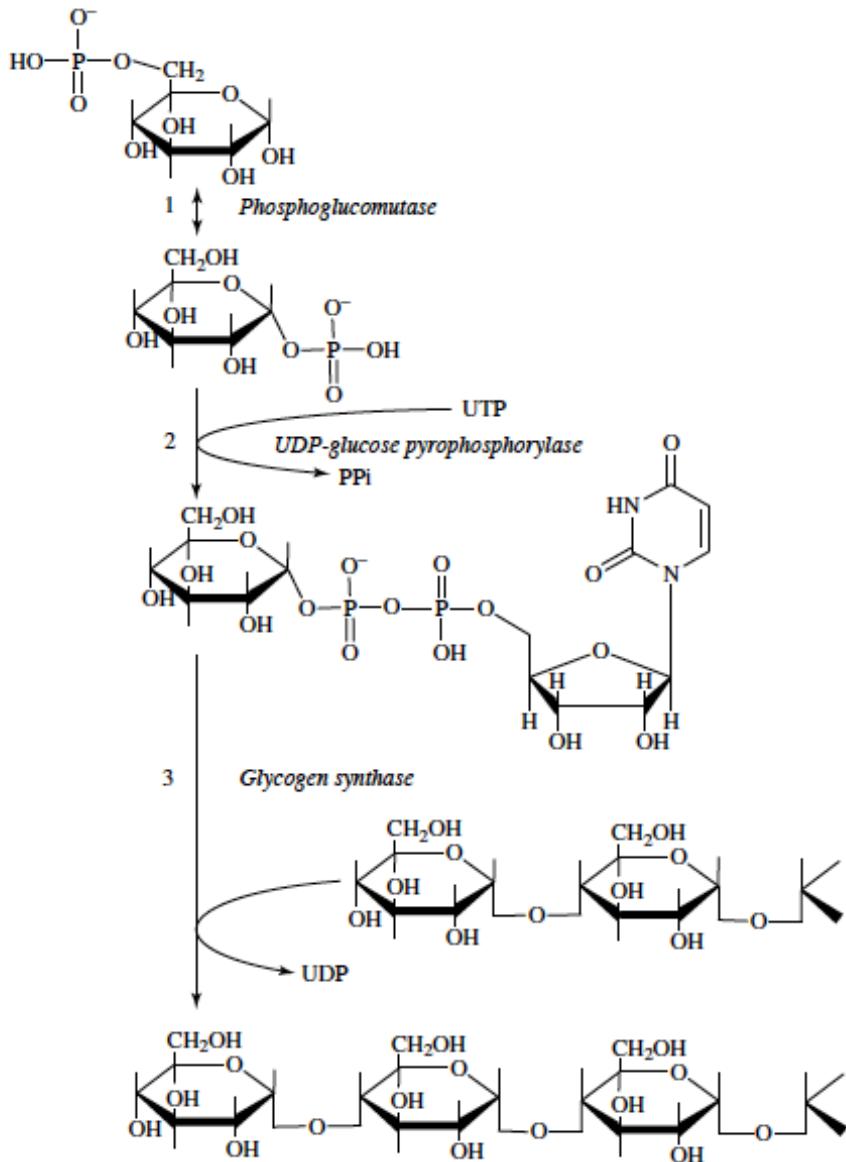


Fig. 11-13 Chemical details of glycogen synthesis. The process of chain branching is shown in Fig. 11-10.

glycolysis, a net 2 molecules of ADP are phosphorylated (with the special exception of the red blood cell; see Prob. 10.35). The glycolytic pathway resides in the cytoplasm, but the full ATP yield from glucose is only obtained when pyruvate passes into the mitochondria and is fully oxidized to  $\text{CO}_2$  and water. In addition, glycolysis via its product pyruvate provides the key building block, acetyl-CoA, for fatty acid synthesis. In the liver, and adipose tissue, conversion of acetyl-CoA to fatty acids and then to *triglycerides* occurs at a high rate.

### PFK and the Second Stage of Glycolysis

The molecular details of glycolysis are shown in Fig. 10-8. From one molecule of glucose, the reactions leading to the formation of fructose 1,6-bisphosphate use 2 molecules of ATP; fructose-1,6-bisphosphate undergoes *aldol cleavage* to form two 3-carbon sugar phosphates, the *triosephosphates*, that are rapidly interconverted by the relatively high concentration of *triosephosphate isomerase* that exists in the cytoplasm. In the next reaction, one of the triosephosphates, *glyceraldehyde 3-phosphate*, is oxidized by  $\text{NAD}^+$  to yield NADH and a doubly phosphorylated carboxylic acid. Then one of these phosphate moieties is transferred to a molecule of ADP to give ATP, leaving a monophosphorylated carboxylic acid. In a second dephosphorylation reaction, another conversion of ADP to ATP takes place, and the resulting nonphosphorylated compound is pyruvate.

The mechanism of phosphorylation of ADP during glycolysis is quite different from that which takes place in the mitochondria. In glycolysis, the phosphate transfer occurs by *direct interaction* between a metabolite and ADP and is called *substrate-level phosphorylation*; on the other hand, in mitochondria ATP synthase mediates the reaction in the process of *oxidative phosphorylation*. This reaction is closely linked to the consumption of oxygen in mitochondria (see Sec. 10.8).

**EXAMPLE 11.16** Is the overall flux of carbon atoms through glycolysis markedly influenced by the three-carbon stages of the pathway?

With the exception of *pyruvate kinase*, the reactions involving the three-carbon phosphates are freely reversible, so this part of glycolysis can undergo net flux in the reverse direction from phosphoenol pyruvate to fructose-1,6-bisphosphate (Fig. 10-8). Reversal of the pyruvate kinase reaction does not take place to any significant extent, and yet carbon flux from pyruvate back to phosphoenol pyruvate does occur in some tissues. This requires a group of *specialized enzymes* that are found only in those tissues that can make glucose from pyruvate; this is restricted to those cells and organs capable of gluconeogenesis, notably the liver, kidney, and neurons.

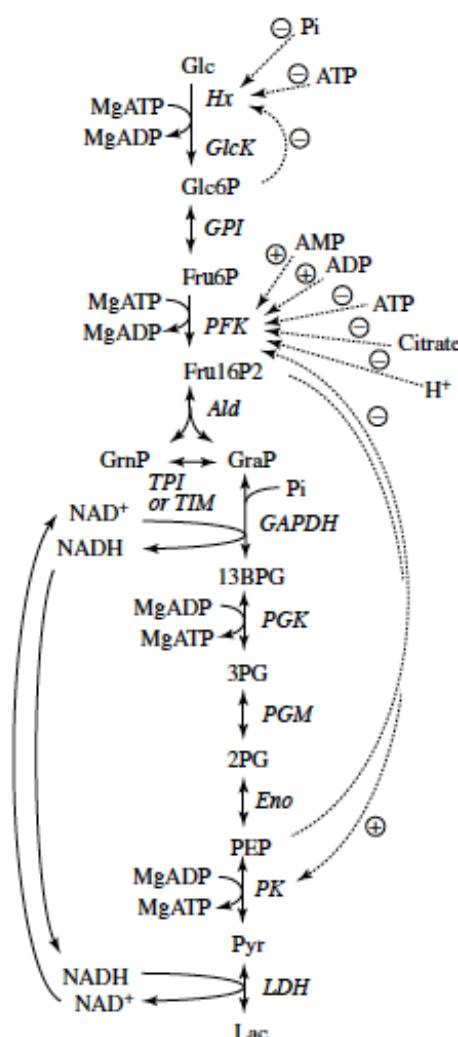


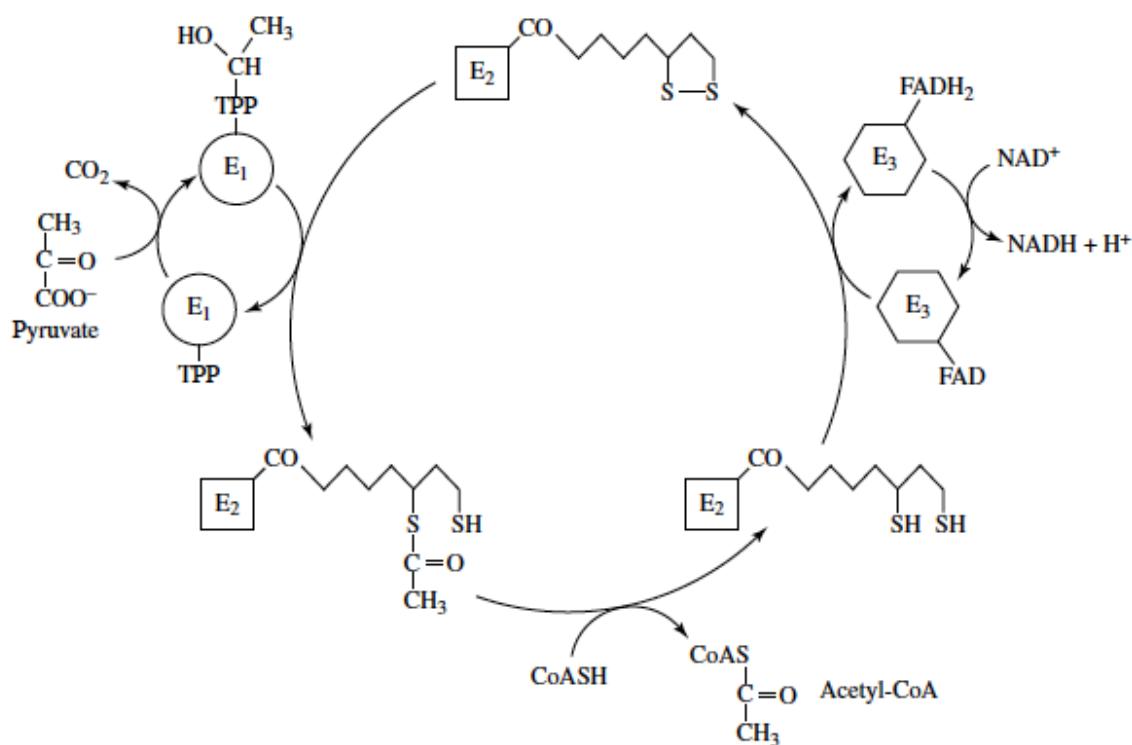
Fig. 11-14 Control of glycolysis. The metabolites and enzymes are denoted by their standard abbreviations (see Fig. 10-8 for the full names). The dashed arrows specify regulation of the enzymes to which they point, and the + and - signs denote activation and inhibition, respectively.

Pyruvate produced in glycolysis undergoes chemical transformations along several different routes. A notable one is transport into the mitochondria where it is oxidized by  $\text{NAD}^+$  via the *pyruvate dehydrogenase* complex. Another route involves its reduction via *lactate dehydrogenase*, with cytosolic NADH produced by glyceraldehyde-3-phosphate dehydrogenase in glycolysis, to yield lactate. This reaction is generally a feature of cells undergoing very rapid glycolytic flux, or in those that lack oxygen and therefore have diminished means of oxidizing NADH and hence recycle their limited supply of  $\text{NAD}^+$  (as in red blood cells that have no mitochondria; see Prob. 10.35). A third route is via transamination with glutamate to make alanine (see Sec. 14.1).

In the fully oxygenated postprandial state in hepatocytes, pyruvate takes the mitochondrial route. The stimulation by insulin of the *pyruvate dehydrogenase complex* (Sec. 11.7), via dephosphorylation mediated by pyruvate dehydrogenase phosphatase, opens up the pathway to pyruvate metabolism in mitochondria.

## 11.7 The Pyruvate Dehydrogenase Complex

This enzyme complex constitutes a *point of no return* for carbon atoms derived from glucose in humans and most other higher organisms; there is no pathway that enables the synthesis of carbohydrate from the 2-carbon precursor acetate. This important feature of pyruvate dehydrogenase is crucial to an understanding of fuel selection in exercise and starvation, and is discussed further in Chap. 13.



**Fig. 11-15** The pyruvate dehydrogenase reaction takes place via *three enzymes* in a complex: Pyruvate is decarboxylated by the E<sub>1</sub> (*pyruvate decarboxylase*) component of the enzyme complex; a key cofactor is *thiamine pyrophosphate* (TPP) that transfers the hydroxyethyl moiety to one of the sulfur atoms on oxidized lipoamide that is covalently bound to E<sub>2</sub> (*dihydrolipoil transacetylase*). When this transfer takes place, the 2-carbon moiety is oxidized to an acetyl moiety; and then the acetyl moiety is transferred to CoA to yield acetyl-CoA which is then released from the active site of E<sub>2</sub>. The reduced lipoamide moiety is recycled back to its oxidized form by donating hydrogen atoms to FAD in E<sub>3</sub> (*lipoamide dehydrogenase*), and the reaction cycle begins over again.

**EXAMPLE 11.17** The process of converting pyruvate to acetyl-CoA is highly regulated. Metabolic regulation is almost invariably synonymous with *multimeric proteins*, and this is true of pyruvate dehydrogenase. The three enzymes (Fig. 11-15) exist in a large complex consisting of 24 copies of E<sub>1</sub> (pyruvate decarboxylase), 24 copies of E<sub>2</sub> (dihydrolipoil transacetylase), and 12 copies of E<sub>3</sub> (lipoamide dehydrogenase). Regulation of flux through the overall reaction is achieved by reversible phosphorylation, as shown in Fig. 11-16.

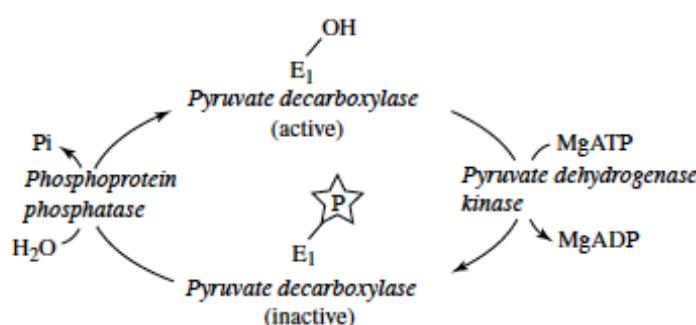


Fig. 11-16 Regulation of the pyruvate dehydrogenase reaction occurs by reversible phosphorylation of the pyruvate decarboxylase ( $E_1$ ) part of the multimeric complex.

## 11.8 Krebs Cycle Flux

Acetyl-CoA has many metabolic fates, but there are two that begin with the *condensation* of acetyl-CoA with *oxaloacetate* to give citrate.

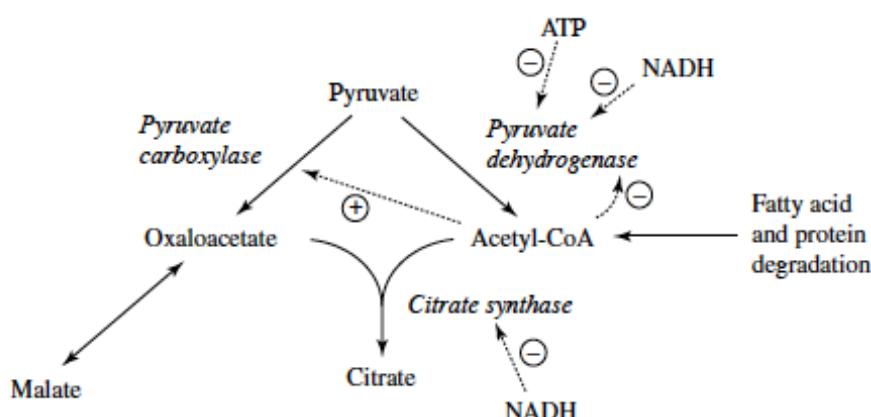


Fig. 11-17 Fate of pyruvate in the mitochondrial matrix. Note the control exerted by acetyl-CoA: elevation of its concentration inhibits its own synthesis and stimulates its own conversion to oxaloacetate.

### EXAMPLE 11.18 What is the molecular mechanism of citrate formation?

Citrate synthase catalyzes the transfer of the acetyl moiety from acetyl-CoA to oxaloacetate to yield citrate. In the process a negative charge is conferred on the methyl carbon of the acetyl moiety that facilitates its attack on the carbonyl carbon of oxaloacetate. The reaction releases CoA, thus regenerating the mitochondrial pool of this vital carrier.

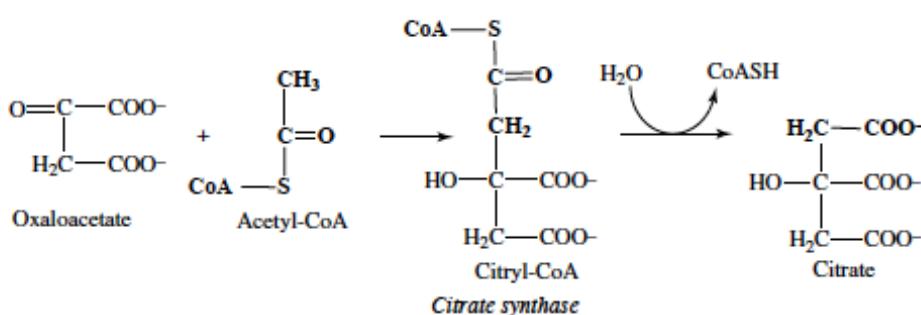


Fig. 11-18 Molecular mechanism of citrate synthase.

The Krebs cycle completes the disassembly of glucose to six molecules of  $\text{CO}_2$  by combining the two carbon atoms from acetyl-CoA with oxaloacetate to make citrate; this is then successively transformed to release two molecules of  $\text{CO}_2$  and to regenerate oxaloacetate. This reformation of a carrier molecule in a cyclic manner led Hans Krebs to the concept of a *metabolic cycle*; he first observed this with the urea cycle (Sec. 14.8).

The oxidation of the two carbon atoms is accomplished by  $\text{NAD}^+$  and FAD, yielding three molecules of NADH and one  $\text{FADH}_2$ . Substrate-level phosphorylation also takes place in which GDP is phosphorylated to GTP. Note that the two carbon atoms that are lost as  $\text{CO}_2$  molecules in one turn of the cycle are not both of those present in the original acetate moiety; one is derived from the initial molecule of oxaloacetate. In a subtle turn of events, while chemically this molecule is recycled, structurally it is resynthesized (Prob. 10.17).

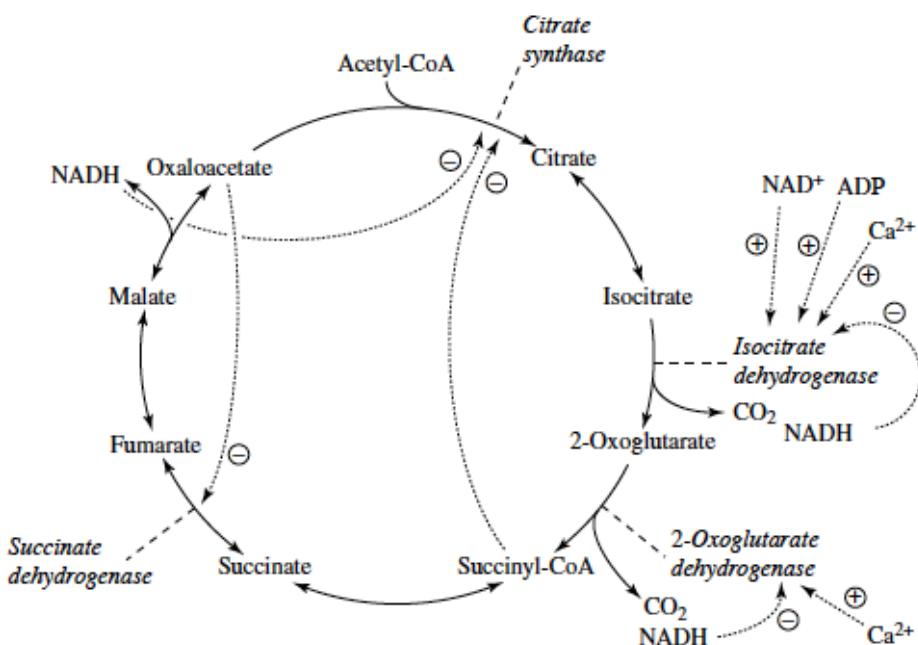


Fig. 11-19 Regulation of flux through the Krebs cycle. Details of the chemical structures and a complete list of enzyme names are given in Fig. 10-9.

The two decarboxylation steps in the Krebs cycle are catalyzed by *2-oxoglutarate dehydrogenase* and *isocitrate dehydrogenase*, and both reactions produce NADH. The rapid diffusion away of  $\text{CO}_2$  in water renders the dehydrogenase reactions effectively irreversible. Both enzymes are sensitive to the ratios of NADH to  $\text{NAD}^+$  and ATP to ADP. When either ATP or NADH concentrations are low, they are stimulated. Thus these enzymes respond to both the *energy charge* in the mitochondria and the amount of *reductant* available. The cycle is further regulated by feedback inhibition of succinate dehydrogenase by oxaloacetate.

In addition, both 2-oxoglutarate dehydrogenase and isocitrate dehydrogenase are stimulated by a rise in mitochondrial  $\text{Ca}^{2+}$  concentration; this is important in stimulating the rate of the Krebs cycle during exercise.

#### EXAMPLE 11.19 Citrate transport into the cytoplasm.

Citrate that is not oxidized by isocitrate dehydrogenase can be transported from the mitochondrial matrix into the cytoplasm. In the cytoplasm of adipocytes and hepatocytes, oxaloacetate and acetyl-CoA are formed from citrate, not by the reversal of the citrate synthase-catalyzed reaction, but by *ATP-dependent citrate lyase*. As the name indicates, the free energy of ATP hydrolysis drives this reaction in the degradative direction.

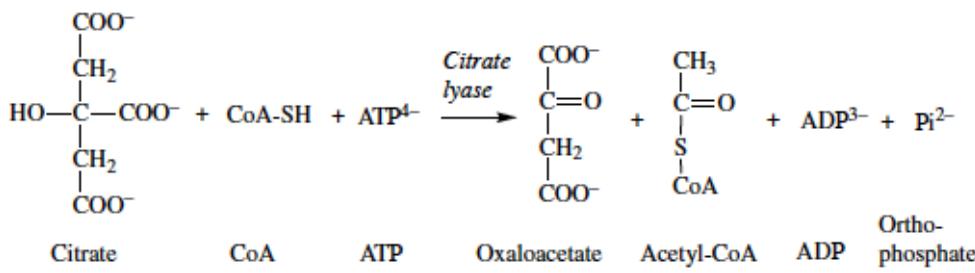


Fig. 11-20 ATP-dependent citrate lyase.

### 11.9 Metabolic Shuttles

In the cytoplasm, oxaloacetate is reduced by NADH to malate via *cytoplasmic malate dehydrogenase*. Malate can pass into the mitochondrial matrix via special *dicarboxylic acid transporters* that are in the inner mitochondrial membrane. Once in the matrix, oxaloacetate is reoxidized to malate by *mitochondrial malate dehydrogenase*. Thus this series of enzyme and transporter reactions has effectively shuttled reducing equivalents in NADH from the cytoplasm to the matrix. This shuttle constitutes the most efficient way of transferring hydrogen atoms from NADH in the cytoplasm to the mitochondrial matrix, and hence into the electron transport chain (see Sec. 10.12 for the glycerol 3-phosphate shuttle).

An additional shuttle involves cytosolic *malate* which is oxidized by *NADP<sup>+</sup>*, a phosphorylated variant of NAD<sup>+</sup>, via the *malic enzyme*. Malate is decarboxylated, releasing CO<sub>2</sub> that diffuses away, rendering the reaction effectively irreversible. The other products are pyruvate and NADPH. Pyruvate can be transported back into the mitochondrial matrix where pyruvate dehydrogenase produces NADH from it. Thus hydrogen atoms are shuttled from the cytoplasm to the mitochondrial matrix. However, energy is expended to reconvert the pyruvate back into oxaloacetate for further metabolism. This reaction is catalyzed by *pyruvate carboxylase* that is described in greater detail in Chap. 13 (see Fig. 13-9).

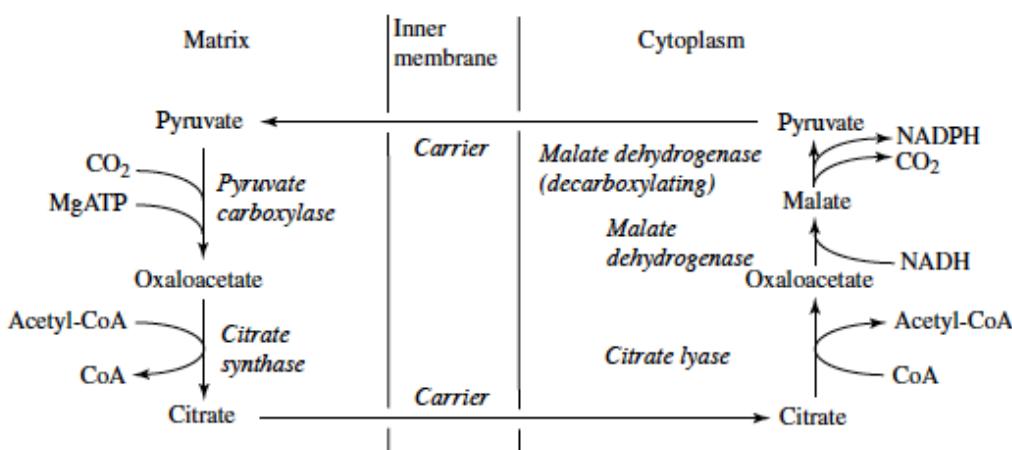


Fig. 11-21 Oxaloacetate transport, as citrate, into the mitochondrial matrix.

#### EXAMPLE 11.20 How do NADP<sup>+</sup> and NADPH differ from NAD<sup>+</sup> and NADH?

These pairs of molecules are identical except for the presence of a phosphate group at the 2' position on the ribose moiety. This is not a high-energy phosphate but rather a molecular *tag* that enables enzymes to discriminate between the two forms of redox compound. In higher animals there do not appear to be any *NADH transferase* enzymes that catalyze direct transfer of hydrogen atoms from NADH to NADP<sup>+</sup> or from NADPH to NAD<sup>+</sup>.

NADH and NADPH are equivalent in terms of their *standard redox potentials*, but because redox enzymes are usually selective for one or the other of them, two distinct pools of reductants exist. NADH is used as a source of reducing equivalents for the electron transport chain (ETC) while NADPH provides reducing equivalents for many *biosynthetic reactions*. Hence, even within a single spatial compartment such as the cytoplasm, the NADH to NAD<sup>+</sup> ratio can be very low, favoring oxidation of fuels, while simultaneously the NADPH to NADP<sup>+</sup> ratio can be very high, facilitating biosynthesis.

### 11.10 Lipogenesis

In the cytoplasm, the acetyl moiety of acetyl-CoA can enter the reactions of fatty acid synthesis or lipogenesis. As with other biosynthetic processes, this pathway involves the energy-dependent sequential addition of activated precursors to a growing molecule; but in contrast to nucleic acid, protein, and glycogen synthesis, lipogenesis also requires reducing equivalents.

The first step of the pathway involves the ATP-dependent carboxylation of acetyl-CoA to give malonyl-CoA. The reaction is catalyzed by *acetyl-CoA carboxylase* that contains *biotin* as an essential cofactor. The reaction mechanism is remarkable because the intermediates are ferried from one active site to another by means of a long, flexible arm linked to the biotin.

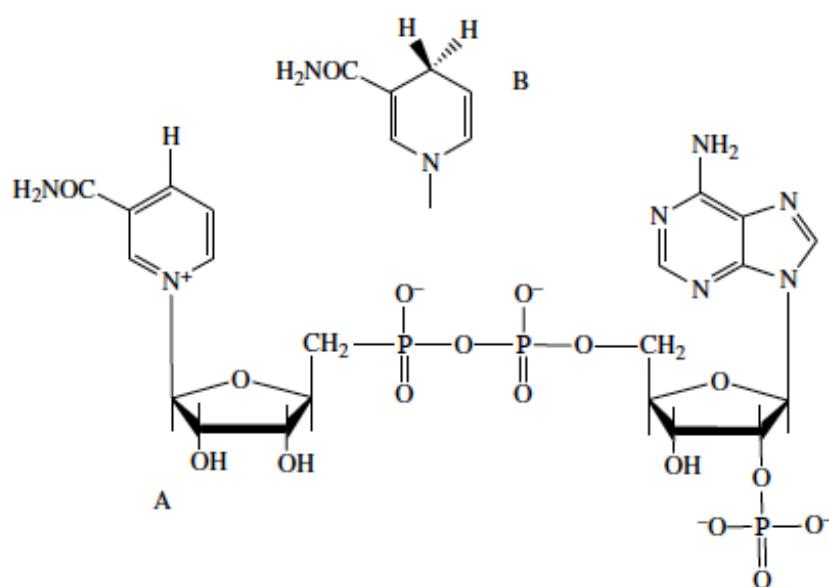


Fig. 11-22 NADP<sup>+</sup> (A) and the reduced nicotinyl moiety (B) of NADPH.

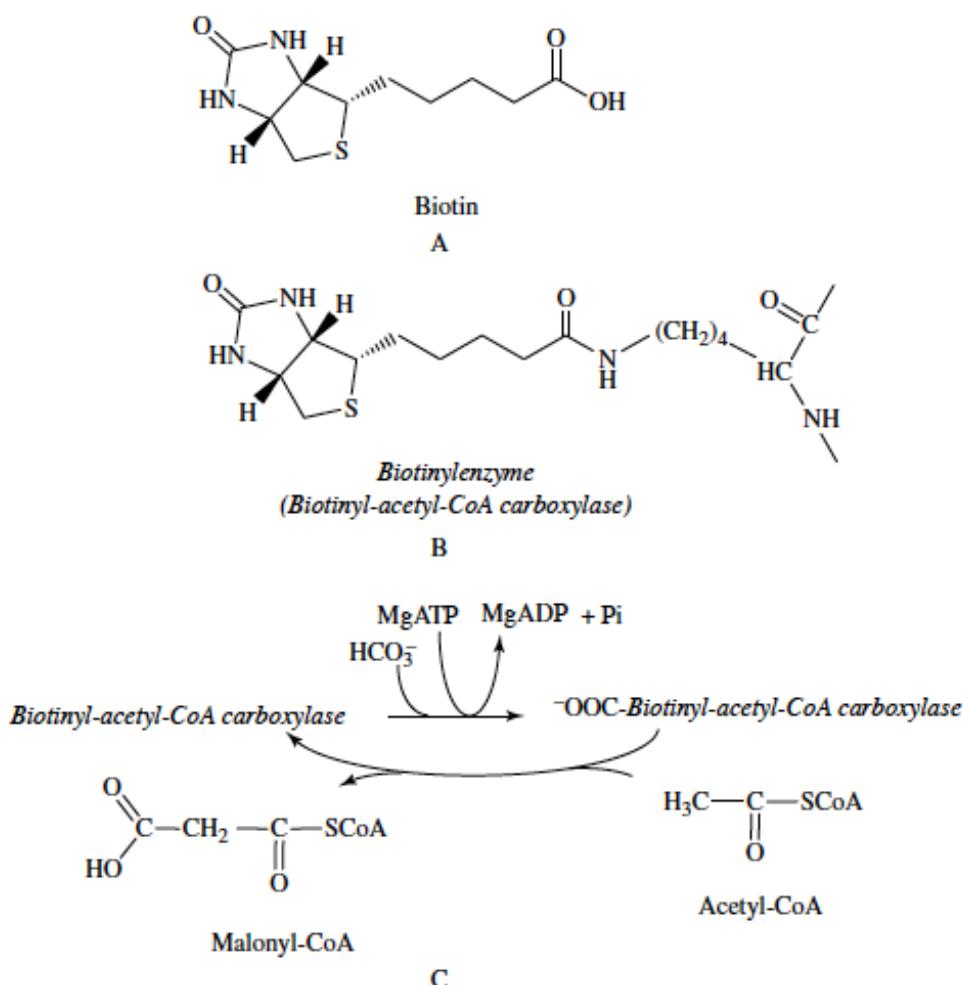


Fig. 11-23 Mechanism of acetyl-CoA carboxylase. Note the central role of (A) biotin that is attached (B) to the apoenzyme by a Schiff base linkage to the ε-amino group of a lysine side chain. (C) The overall reaction requires energy from the hydrolysis of the Mg<sup>2+</sup> complex of ATP, and the fixation of CO<sub>2</sub> as HCO<sub>3</sub><sup>-</sup>.

### Control of Acetyl-CoA Carboxylase

In animals acetyl-CoA carboxylase is a filamentous polymer of  $M_r$  4–8 MDa, made from 230-kDa monomers. Insulin is the most potent regulator of acetyl-CoA carboxylase. When *insulin* binds to an insulin receptor in the plasma membrane of the cell, this triggers a series of protein-kinase reactions that lead to the enzyme becoming phosphorylated. It also has *alternative* phosphorylation sites that are inhibitory; and counterregulatory hormones such as *glucagon* and *epinephrine* decrease its activity by stimulating kinases that phosphorylate these sites.

Acetyl-CoA carboxylase is also stimulated by elevated cytoplasmic citrate concentrations; the citrate effect is an *allosteric* one that leads to the *polymerization* of the enzyme as part of the activation process. Elevation of citrate concentration indicates a situation in which acetyl-CoA is in excess of the capacity of the Krebs cycle to handle it. Hence diversion of acetyl-CoA from energy transduction to energy storage, in the form of fatty acids, is favored.

Malonyl-CoA is converted into new fatty acids by a remarkable enzyme system, the fatty acyl synthase complex. This consists of several individual active sites on the same polypeptide chain, with the intermediates that are involved in the process being positioned sequentially in the correct temporal order in these different sites.

### Mechanism of Fatty Acid Synthase

Fatty acid synthase assembles acetyl groups derived from the malonyl portion of malonyl-CoA onto a growing fatty-acyl chain (Fig. 11-24). The growing chain remains attached to the enzyme during the whole process. After each 2-carbon addition, a sequence of *reduction/dehydration/reduction* reactions occurs that reduces the carbonyl carbon to a fully saturated —CH<sub>2</sub>—. The processing leads finally to the release of fatty-acyl-CoA.

Fatty acid synthase has two free sulphydryl (—SH) groups on one of its subunits, called the *acyl-carrier protein* (ACP). It is a 4-phosphopantetheine group (as in CoA; Fig. 10-6) attached to a seryl hydroxyl that bears the —SH group. This group is linked to the growing fatty-acyl chain and the incoming malonyl group, respectively, and it positions the growing molecules in exactly the right way for group interaction and transfer to occur optimally.

The reaction sequence begins with the binding of an acetyl moiety from acetyl-CoA, at one sulphydryl group termed the XXX site, and a malonyl moiety at the other, the YYY site. Decarboxylation of the end of the malonyl residue leaves a very reactive intermediate that attacks the carbonyl carbon in the acetate at the XXX site. This *nucleophilic attack* results in the transfer of the acetyl group onto the end of the former malonyl moiety. Reduction of the acetyl-derived carbonyl group is achieved by NADPH, leaving an alcohol (—OH) group that is removed in a dehydration step. The resulting double bonded —CH=CH— portion is further reduced by NADPH to give a 4-carbon fatty acid that is still attached to the YYY site. Finally, the nascent fatty acid moves to the XXX site, ready for the arrival of another malonyl group into the XXX site and, then a repeat of the cycle.

## 11.11 Pentose Phosphate Pathway (PPP)

The major source of NADPH used in fatty acid synthesis is the pentose phosphate pathway (PPP).

The transport of citrate from the mitochondrial matrix into the cytoplasm is linked to the transfer of reducing equivalents yielding NADPH via malate decarboxylation (Fig. 11-21). However, the amount of NADPH produced in this process is insufficient to provide all that is required for fatty acid synthesis.

The additional pathway diverts glucose 6-phosphate from the first step of glycolysis and reduces NADP<sup>+</sup> in two redox reactions (Fig. 11-25). This reaction sequence is called the *oxidative part* of the pathway or the *oxidative hexose phosphate shunt*. It is especially active in *lipogenic* cells such as hepatocytes and adipocytes.

### Oxidative PPP

The first enzyme in the pathway, *glucose-6-phosphate dehydrogenase* is strongly product-inhibited by NADPH. It oxidizes glucose 6-phosphate using NADP<sup>+</sup> and generates NADPH with the other product being 6-phosphoglucono- $\delta$ -lactone. The latter is hydrolyzed via  $\delta$ -lactonase and the resulting 6-phosphogluconate is oxidized by NADP<sup>+</sup> via *6-phosphogluconate dehydrogenase* with the concomitant release of CO<sub>2</sub>, and the production of a ketopentose phosphate, ribulose 5-phosphate.

### Nonoxidative PPP

The remainder of the pathway consists of a series of *isomerase* and *group transfer* reactions (Fig. 11-26). These produce sugar phosphates ranging in size from the 3-carbon glyceraldehyde 3-phosphate to the 7-carbon sedoheptulose 7-phosphate. Also derived from ribulose 5-phosphate is ribose 5-phosphate, an essential component of *ribonucleosides* and *ribonucleotides*. The group exchange reactions are catalyzed by *transaldolase*.

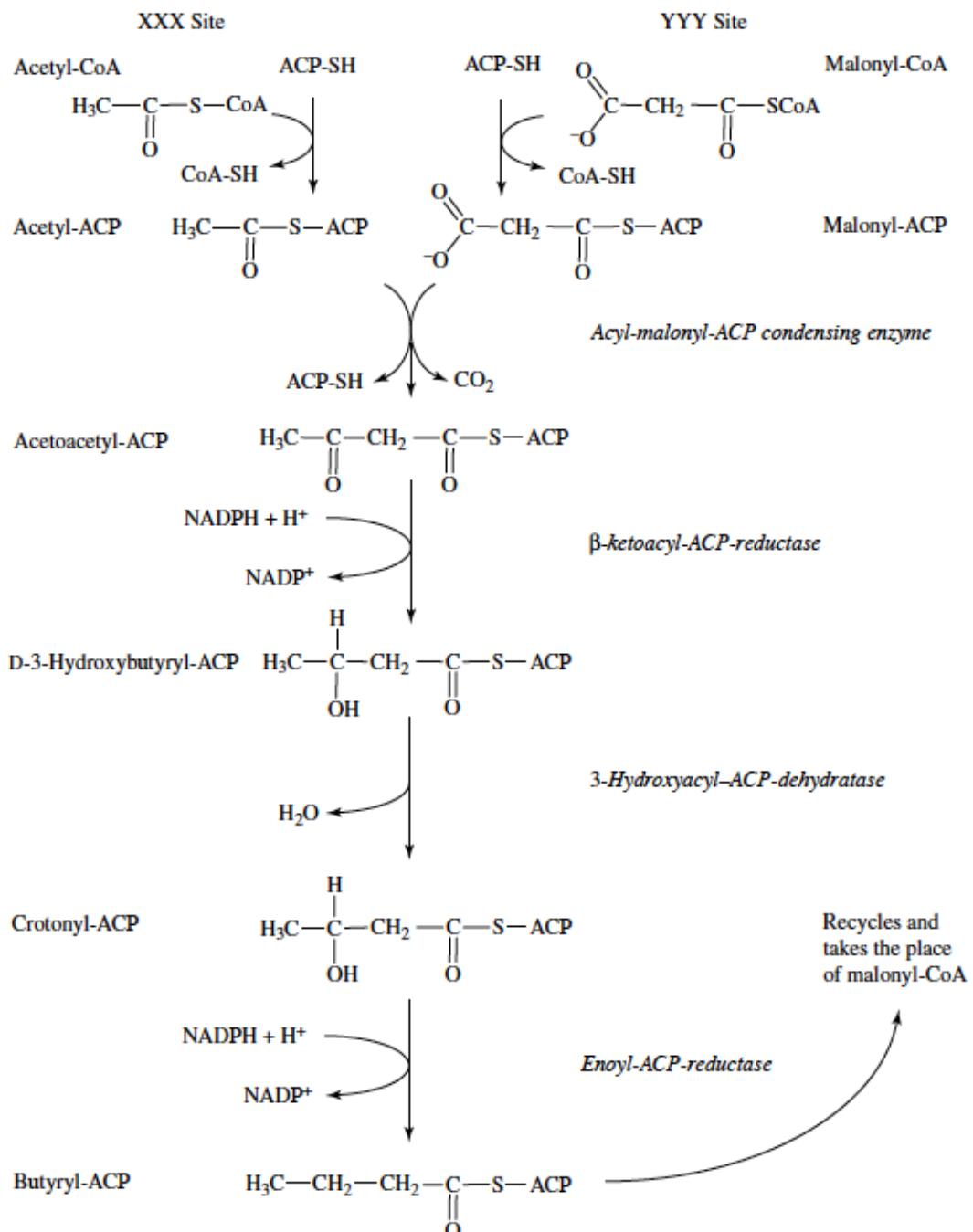


Fig. 11-24 Mechanism of fatty-acyl-CoA synthesis.

and transketolase; the latter interestingly contains the prosthetic group thiamine pyrophosphate (TPP). Transketolase transfers a 2-carbon *glycolaldehyde* moiety from a ketose phosphate donor to an aldose phosphate acceptor. Transaldolase transfers a 3-carbon *glyceraldehyde* moiety from a ketose phosphate donor to an aldose phosphate acceptor. Residual flux of carbon atoms back into glycolysis occurs via glyceraldehyde 3-phosphate and fructose 6-phosphate.

### 11.12 Metabolism of Two Other Monosaccharides

The two major monosaccharides, other than glucose, that enter the bloodstream from the diet are *fructose* from sucrose and *galactose* from lactose. In both cases these hexoses are metabolically transformed and enter the early stages of the *glycolytic pathway*.

### Fructose

After absorption from the gut, fructose is taken up into tissues via GLUT-5 transporters in the plasma membranes of most cells. In the muscle (and other peripheral tissues) fructose is converted to fructose 6-phosphate which is simply an early intermediate in glycolysis. The liver, via its portal vein, is the first tissue to encounter fructose as it is absorbed from the intestine; but it does not contain sufficiently high hexokinase activity to handle all the fructose load, although it does phosphorylate some of it. Instead the liver has the fructose-specific enzyme *fructokinase* that yields fructose 1-phosphate in the presence of ATP. From here, aldolase B, an *isozyme* of the main aldolase in glycolysis, cleaves fructose 1-phosphate to *glyceraldehyde* and *dihydroxyacetone phosphate*. The glyceraldehyde enters glycolysis after phosphorylation by ATP via the enzyme *trikinase*.

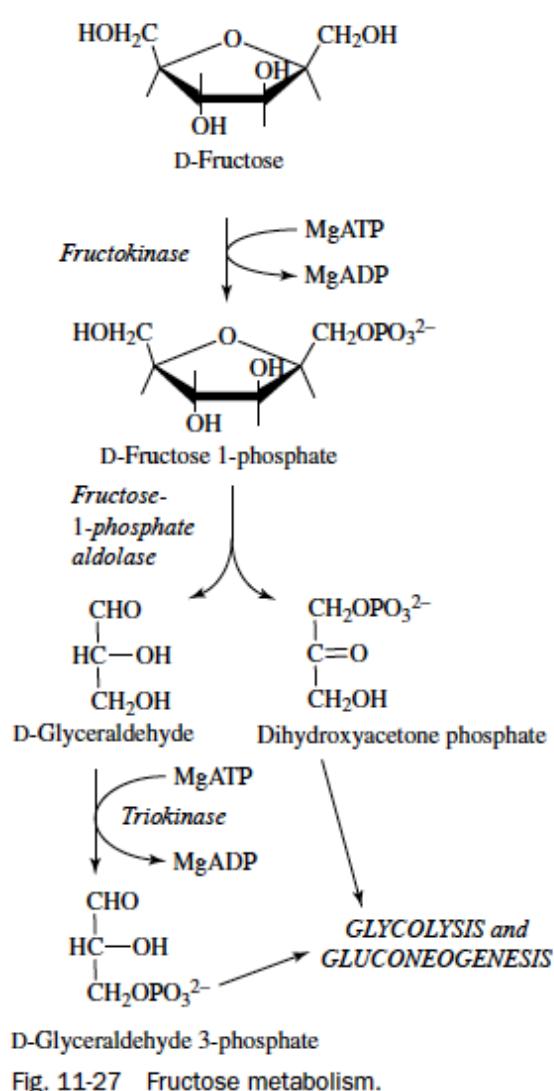


Fig. 11-27 Fructose metabolism.

### Galactose

In contrast to fructose, galactose is metabolized primarily in the liver. The liver contains both the enzyme galactokinase (which catalyzes the conversion of galactose into galactose 6-phosphate) and a suite of enzymes that are necessary to convert galactose 6-phosphate into glucose 6-phosphate. The latter enzymes belong to a class called the *epimerases*. Galactose and glucose are epimers of each other, differing in absolute configuration only around the carbon atom in position 4 (Sec. 11-2, Fig. 11-1). The epimerization takes place while the monosaccharide is conjugated to UDP, thus galactose is substituted for glucose on UDP-glucose.

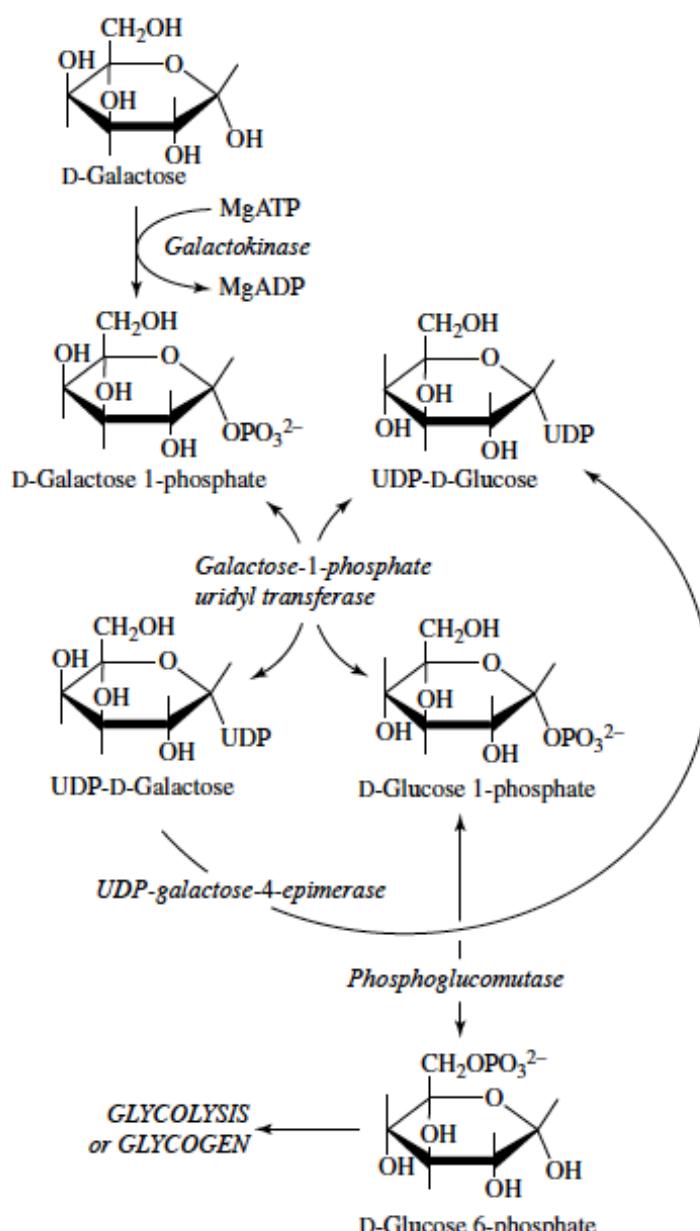


Fig. 11-28 Galactose metabolism in the liver.

In a manner similar to fructose, some individuals have a *genetic defect* in one of the enzymes involved in galactose metabolism; and consumption of galactose by these individuals can be detrimental to their health. The most common defect in galactose metabolism is with *galactokinase*, and not with the epimerase.

### 11.13 Food Partitioning

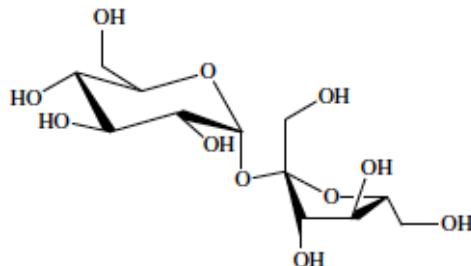
After a meal, there are several possible fates of the ingested carbohydrate. The regulatory influences dictate whether carbohydrate will be stored as glycogen or triglyceride, or oxidized to carbon dioxide.

Given the limit on the size of the glycogen molecule, it is reasonable to assume that, after a meal, glycogen stores would be maximized first with the excess carbon atoms from glucose being diverted to lipogenesis. However, leading nutrition scientists contend that lipogenesis is not very active in people eating a Western diet. This is so because the expression of lipogenic enzymes is down-regulated by consumption of triglycerides. Thus most of the triglyceride in white adipose tissue comes from the diet and is not synthesized de novo. Recent research indicates that glycogen concentrations can, temporarily, become very high in the liver after a high-carbohydrate meal.

**11.7.** Why does pure sucrose, a disaccharide, not have reducing properties?

**SOLUTION**

Sucrose, the main sugar, in cane and beet is a disaccharide in which the anomeric hydroxyl group of  $\alpha$ -D-glucose is condensed with the anomeric hydroxyl group of  $\beta$ -D-fructose. It is therefore both an  $\alpha$ -glucoside and a  $\beta$ -fructoside. Neither unit possesses an anomeric hydroxyl, and neither ring can open to give an aldehyde group.

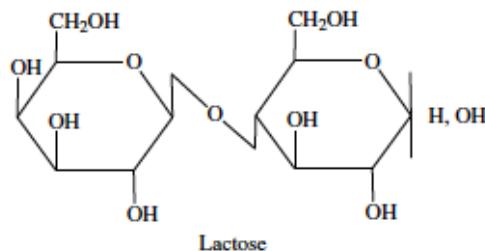


**11.8.** A solution of D-glucose contains predominantly the  $\alpha$ - and  $\beta$ - anomers of D-glucopyranose; in fact the ratio is ~36:64. Both forms are not reducing so why is a solution of glucose a strong reducing agent?

**SOLUTION**

Because there is some open-chain glucose present with reducing properties. As this reacts, the equilibria between it and the nonreducing forms are disturbed, causing more of the open-chain form to appear. Ultimately, all the glucose will have reacted with an oxidant via the open-chain form.

**11.9.** The disaccharide shown is lactose, the main carbohydrate of mammalian milk. Give (a) its full name and (b) its abbreviated name.



**SOLUTION**

- (a)  $\beta$ -Galactopyranosyl-(1 $\rightarrow$ 4)-glucopyranoside.
- (b)  $\beta$ -Gal-(1 $\rightarrow$ 4)-Glc.

## DIGESTION AND ABSORPTION OF CARBOHYDRATES

**11.10.** How are the different solubilities of sucrose, glucose, and fructose employed in making confectionary?

**SOLUTION**

A solution of equal parts of glucose and fructose is more soluble than an equal mass of sucrose. A paste of sucrose can, when hydrolyzed into glucose and fructose, become a solution. Soft-centered chocolates are created by coating a semisolid sucrose-treated sucrose paste, and over several weeks, the sucrose becomes hydrolyzed to give a liquid center.

**11.11.** What happens to amylose starch that is not fully hydrolyzed in the small intestine? What might be the clinical consequences?

**SOLUTION**

Undigested amylose passes into the large intestine (colon) where it is fermented by bacteria that produce short-chain fatty acids, hydrogen and methane. These compounds cause intestinal discomfort and intestinal bloating; and their production explains the well-known association between flatulence and the consumption of baked

beans. Baked beans also contain complex oligosaccharides, some of which are not hydrolyzed in the small intestine and hence enter the colon and lead to the same symptoms as with undigested starch.

- 11.12.** Sucrase deficiency comes about either by profound damage to the small intestine or more usually from an inherited defect. The gene for *sucrase* resides on chromosome 3, and homozygous individuals for the defective gene have *sucrose intolerance*. The total world incidence of the inherited intolerance is ~1100, so it is very rare. What do you think would be the signs and symptoms of this enzyme defect?

**SOLUTION**

Sucrase is associated with the brush border of the intestinal mucosal cells. It catalyzes the hydrolysis of sucrose to D-glucose and D-fructose. Its absence means that sucrose passes through the small intestine where it enters the large intestine. There it interferes with water absorption because it is osmotically active and leads to the retention of water in the bowel lumen. This gives rise to abdominal cramps and watery diarrhea. Treatment involves limiting sucrose in the diet and more recently the ingesting of a proprietary enzyme, Sucraid.

## BLOOD GLUCOSE HOMEOSTASIS

- 11.13.** What are the molecular and clinical consequences of glycation of proteins?

**SOLUTION**

The presence of covalently attached glucose residues generally causes a protein to malfunction. This has serious effects on the eye and the kidney. In the eye some of the proteins have slow turnover, such as crystallins in the lens and structural proteins in the retina. In the kidney, structural proteins in cells and in the extracellular matrix are exposed to high concentrations of glucose from the blood and the urinary filtrate. Damage to these tissues leads to blindness, vascular disease with high blood pressure, and nephropathy, respectively.

- 11.14.** Which foods have a low GI?

**SOLUTION**

Amylose-containing legumes are the *classical* low-GI foods. In a seemingly paradoxical way, foods that contain sucrose and lactose are often seen to have low or medium GI values. This arises because one-half of the carbohydrate in these disaccharides is not glucose but, respectively, fructose and galactose; and these are not detected by the glucose assay. Nevertheless they are rapidly absorbed and potentially exacerbate hyperglycemia. More scientific work is needed in this area.

Dairy foods also have a low GI because they elicit an early extra insulin (blood-glucose lowering) response via the amino acids that are released by digestion of proteins; the amino acids enter the bloodstream with glucose and, like it, stimulate insulin release from the  $\beta$ -cells of the pancreas.

- 11.15.** What is the merit of knowing the GI of a food?

**SOLUTION**

Maintenance of euglycemia is not achieved in patients with insulin resistance and Type I diabetes. For these individuals, eating low-GI foods obviates large surges in blood glucose concentration after a meal. Knowing the GI of food helps in planning a diet with an appealing range of different foods, which leads to better control over blood glucose concentration without necessarily the recourse to drug and hormone treatment to control hyperglycemia.

- 11.16.** Predict what might happen to GLUT-4 once the insulin stimulus for its incorporation into the plasma membrane of a cell has been removed.

**SOLUTION**

GLUT-4 transporters reenter the cytoplasm by invagination of the plasma membrane and the formation of vesicles via endocytosis. Thus the number of GLUT-4 molecules on the cell surface is reduced, leaving only GLUT-1 to mediate a basal rate of glucose transport.

- 11.17.** Glucose uptake into the liver is not dependent on insulin, but glucose concentration inside a hepatocyte rises after a carbohydrate meal. Are GLUT-4 transporters likely to be involved?

**SOLUTION**

In the liver (and pancreas) a high rate of glucose uptake occurs after a meal. But instead of using the insulin-responsive GLUT-4 system, hepatocytes have a relatively large number of GLUT-2 transporters that are always active in the plasma membrane. Thus glucose transport into the hepatocytes is always rapid, and the concentration in the cytoplasm closely mirrors that in the bloodstream (i.e., plasma and red blood cells).

- 11.18.** In what way do the properties of GLUT-2 and glucokinase complement each other in the metabolism of glucose by the liver?

**SOLUTION**

GLUT-2 is not regulated by insulin, and it mediates the rapid uptake of glucose into the liver from the bloodstream; thus the glucose concentration in the cytoplasm of hepatocytes is virtually equal to that in the portal vein. The high  $K_m$  of glucokinase means that the flux through it, when intracellular glucose concentration rises, responds in direct proportion to the increase. Thus GLUT-2 and glucokinase work together to bring about rapid assimilation of glucose into hepatocytes.

- 11.19.** Since the three kinase enzymes of glycolysis are effectively irreversible (Fig. 11-32), speculate on which the main flux control enzymes in glycolysis are.

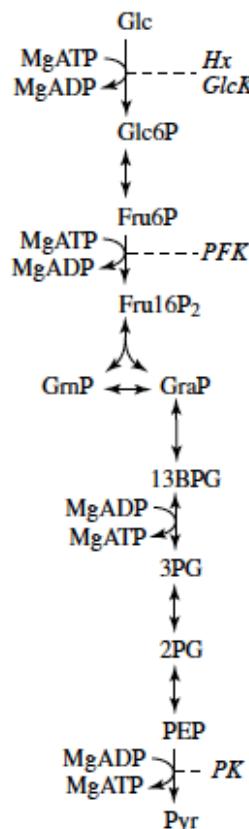


Fig. 11-32 Location of the flux-controlling kinases in glycolysis.

**SOLUTION**

The glycolytic pathway down to pyruvate involves 10 enzyme-catalyzed steps with three kinases that use ATP (Fig. 11-32). The reaction catalyzed by phosphofructokinase (PFK) plays the major part in controlling the overall flux of the whole pathway. It catalyzes the phosphorylation of fructose 6-phosphate (Fru6P) to fructose 1,6-bisphosphate (Fru16P<sub>2</sub>). Hexokinase and pyruvate kinase (PK) have relatively higher maximal velocities. PFK is the target for more effector molecules than the other two kinases (see Fig. 11-14).

- 11.20.** What are the general properties of enzymes that have high flux control?

**SOLUTION**

Reactions that have a lot of control over the overall flux through a metabolic pathway are of course relatively slow, but they also tend to be effectively irreversible, such as the kinases. Another feature is that they are often saturated by their substrates; in other words, their substrate concentrations under normal steady-state conditions are at 10 times their  $K_m$ , so they are unresponsive to minor changes in substrate concentration. Flux through the enzyme is then controlled by altering its intrinsic properties via effector molecules.

- 11.21. Use the metaphor of passenger movement, or flow in a busy railway station, to illustrate flux control in a metabolic pathway.

**SOLUTION**

Suppose that the city railway station has many tracks but a single set of five automatic, ticket-operated exit gates. The gates constitute a single step in the “pathway” that commuters must take from their homes to work. At morning peak hours many trains arrive, bringing thousands of passengers, all of whom must pass through the exit gates. It is clear that, at this time, these gates are likely to become saturated with commuters. At this point, increasing the number of commuters trying to leave the platforms will not increase the flux of commuters via the exits. In this saturated state, flux through the gates is less dependent on the absolute number of commuters and much more dependent on the intrinsic activity of the gates themselves. It is also clear that the movement of commuters through the gates is effectively irreversible. Unless an alternative set of gates is provided for passengers wishing to enter the station, the prevailing pressure of commuters, that is akin to concentration in a metabolic pathway, will overwhelmingly drive the reaction in the “exit” direction. Of course the metaphor with irreversibility works even better if the gates formally allow movement in only one direction.

There are several ways of regulating flow through the gates. First, the station could have a larger complement of gates and open a larger number of them during peak times. This is analogous to a metabolic step in which an enzyme is switched from being fully inactive to fully active, something that is often achieved metabolically by reversible phosphorylation of the enzyme. The total amount of enzymes (gates) does not change, just the proportion of enzymes (gates) in the active form does.

Second, each individual gate could be made to work faster. For the enzyme, this might be achieved by a change in the conformation of the active site, such as might be induced by the binding of stimulatory effectors to allosteric sites (Chap. 5). Third, each morning, construction staff could erect a completely new set of gates for the peak periods, dismantling them just a few hours later, making the space available for flower sellers and buskers. While this latter approach may seem inefficient, cells often respond to situations by increasing the transcription and translation of genes and thus make more enzymes that are relevant to a new situation. Just such a course of action would generally only be taken in the station in response to alternative needs for space; and regulation of concentrations of flux-controlling enzymes via changes in gene expression constitutes such a longer-term response.

- 11.22. Use the metaphor in Prob. 11.21 to illustrate whether an enzyme with high flux control always catalyzes the major point of flux control in a metabolic pathway.

**SOLUTION**

Outside of peak hours, the exit gates will no longer be saturated with people. Therefore the main flux-controlling step of the pathway will have shifted to some other process such as the rate of arrival of trains, or even the rate at which people are leaving their homes. This illustrates the point that different steps in a complex pathway can become more important in controlling flux under different steady-state conditions.

Because no single enzyme in any known pathway has absolute control over the flux through it, the old notion of a single “rate-limiting step” has given way to the more refined and mathematically rigorous notion of *flux control coefficients* in metabolic control theory or analysis (MCA). For example, in the station metaphor at peak hour, the flux of patrons through the exit gates could be *equally limited* by the width of the passageways leading to the gates as to the gates themselves.

- 11.23. What are the kinetic characteristics of an enzyme that has low flux control in a metabolic pathway?

**SOLUTION**

Such an enzyme under normal steady-state conditions tends to have the concentrations of its substrates and products at or near to their equilibrium values. Any enzyme enhances equally both the forward and the reverse rates of its catalyzed reaction. Those enzymes that catalyze seemingly irreversible reactions usually involve hydrolysis; and since the water concentration in cells and tissues is thousands of times greater than metabolites, the equilibrium position of these reactions is poised in favor of the forward or hydrolytic process. In reactions not involving water, the net direction of the reactions depends on the relative concentration of the substrates and products. If the activity of the enzyme is sufficiently high, relative to others in a metabolic pathway, altering the activity of the enzyme simply makes both the forward and reverse reactions go faster, thus maintaining a *pseudo-equilibrium*.

**REGULATION OF GLYCOGEN PRODUCTION**

- 11.24.** What is the likely limit to the size of a glycogen molecule?

**SOLUTION**

The best way to visualize the situation is via the construction of a 3D model of a growing glycogen molecule; as the molecule becomes larger, the branches begin to interfere with one another. The extent of steric hindrance is sufficient to impair the activities of glycogen synthase and the branching enzyme, which are buried within the growing polymer. Glycogen synthase also must be in close association with glycogenin possibly via *adaptor proteins* to catalyze the addition of new glucose molecules; as the polymer becomes larger, this protein-protein interaction is weakened. Inspection of any treelike structure reveals that toward the extremities packing constraints dictate that the branches become shorter; when the chains become too short, the branching enzyme ceases to function.

- 11.25.** How many molecules of ATP are required to store one glucose molecule as glycogen?

**SOLUTION**

Figures 11-10 and 11-13 appear to indicate that as the glucose molecule is incorporated into glycogen, one molecule of UTP is converted to UDP and pyrophosphate (PPi). Phosphorylation of a molecule of UDP uses one molecule of ATP; the reaction is simply  $\text{UDP} + \text{ATP} \rightleftharpoons \text{UTP} + \text{ADP}$ . However, one molecule of ATP is used in the synthesis of glucose 6-phosphate. Therefore two molecules of ATP are required for the addition of each glucose molecule that is stored as glycogen. Another way of viewing this is to note that PPi has a high-energy phosphate bond, and it takes a molecule of ATP to reinstate the energy loss that occurs when PPi is hydrolyzed by *pyrophosphatase*.

**GLYCOLYSIS**

- 11.26.** What is the fate of cytosolic pyruvate when it is reduced by cytosolic NADH?

**SOLUTION**

Reduction of pyruvate by NADH via *lactate dehydrogenase* yields lactic acid. The carboxylic proton readily and reversibly dissociates in a reaction with a  $pK_a$  of ~4; therefore at pH 7 the molecule exists mostly as the lactate anion. Lactate undergoes facilitated diffusion into and out of cells via an integral membrane protein, the *monocarboxylate transporter*. When there is net loss of lactate from a cell, it is mostly taken up by the liver and the heart. In the liver it is turned back into pyruvate and then via the gluconeogenic pathway (Sec. 13.7) to glucose. In the heart the conversion to pyruvate is followed by oxidation via pyruvate dehydrogenase, the Krebs cycle, and oxidative phosphorylation in mitochondria.

Some investigators believe that the major route for liver glycogen synthesis involves this pathway. Thus glucose that enters muscle cells after a meal proceeds through glycolysis to lactate; this is released from the muscle and is taken up by the liver where gluconeogenesis leads to glycogen synthesis. This is referred to as the *indirect route* of postprandial glycogen synthesis.

**KREBS CYCLE FLUX**

- 11.27.** What factors influence the metabolic fate of citrate in the mitochondria?

**SOLUTION**

If there is demand for ATP in a cell, citrate enters the Krebs cycle. If there is a stimulus for fatty acid synthesis, citrate is exported from the mitochondria to the cytoplasm where lipogenesis takes place. As occurs with the reciprocal relationship between glycolysis and glycogen synthesis, the two fates of citrate are complementary.

- 11.28.** Why is ATP required for the ATP-citrate lyase (ACL) reaction but not for the citrate synthase reaction?

**SOLUTION**

This difference illustrates the fact that the thioester bond in compounds that contain CoA (like acetyl-CoA) has a high free energy. Similarly, when fatty-acyl CoA molecules are produced during the priming/trapping phase of fatty acid oxidation, ATP bond energy is also supplied to drive the reaction forward (Sec. 10-5).

## METABOLIC SHUTTLES

- 11.29. In the redirection of citrate carbon atoms in the mitochondrial matrix back to acetyl-CoA for fatty acid synthesis in the cytoplasm, why is acetyl-CoA itself not transported out of the mitochondria?

**SOLUTION**

The large CoA molecule renders complexes containing it unable to cross normal membranes. In Chap. 10, it was noted that fatty acids conjugated to CoA could not pass into the mitochondria; they require transfer to carnitine before transport from the cytoplasm can take place.

- 11.30. Are there two separate metabolic pools of CoA in most cells, one in the mitochondria and the other in the cytoplasm?

**SOLUTION**

Yes. Because CoA cannot freely cross membranes, not only do the cytoplasmic and mitochondrial compartments contain separate pools of CoA, but also the acetyl CoA-to-CoA ratio can be quite different in each compartment. Similarly, mitochondrial and cytosolic NAD<sup>+</sup> and NADH pools are wholly separate; and the NADH to NAD<sup>+</sup> ratio on one side of the mitochondrial membrane can be different from that on the other side.

- 11.31. How does oxaloacetate that is generated from citrate released from the mitochondrial matrix return there?

**SOLUTION**

A temporary deficiency of oxaloacetate in the matrix reduces the rate of disposal of acetyl-CoA, but cytosolic oxaloacetate produced via ATP-dependent citrate lyase returns to the matrix, thus overcoming the deficiency. Oxaloacetate itself does not cross the inner mitochondrial membrane; it is converted to compounds for which there are specific transporters.

## LIPOGENESIS

- 11.32. Malonyl-CoA inhibits the transport of fatty acids into the mitochondria. How might this assist insulin in promoting glucose oxidation?

**SOLUTION**

Although the balance between glucose and fatty acid oxidation is described in Chap. 13, it is relevant to note here that malonyl-CoA inhibits carnitine acyl transferase I (CAT-I), the enzyme that catalyzes the exchange of fatty acids for carnitine as part of the cytosol-to-matrix fatty acid transport system. Inhibition of CAT-I occurs when acetyl-CoA carboxylase is activated by insulin. By inhibiting the uptake of fatty acids into mitochondria, malonyl CoA favors the oxidation of glucose and prevents fatty acids from being oxidized at the same time as they are being synthesized.

- 11.33. How many malonyl-CoA and NADPH molecules are required to produce a 16-carbon fatty acid molecule?

**SOLUTION**

Refer to Fig. 11-24: Starting with one acetyl-CoA molecule to fill the XXX site (left side of the diagram) and a malonyl-CoA to fill the YYY site (right side of the diagram), a 4-carbon fatty acyl chain will be formed by also using two molecules of NADPH. Each subsequent addition of two carbon atoms requires a molecule of malonyl-CoA and two more molecules of NADPH. Thus a 16-carbon fatty acid molecule, *palmitic acid*, requires 1 acetyl-CoA, 7 malonyl-CoA, and 16 NADPH molecules for its synthesis.

- 11.34. What is the mechanism of release of fatty acids from fatty acid synthase?

**SOLUTION**

When the growing fatty-acyl chain reaches 16 carbon atoms long, it is transferred onto a molecule of CoA. This produces *palmitoyl-CoA*, or what is more generically called *fatty-acyl-CoA*.

- 11.35. What is the metabolic fate of fatty-acyl-CoA?

**SOLUTION**

Because carnitine acyl transfertase I (CAT-I) is inhibited by fatty-acyl-CoA, these molecules are not normally oxidized in the mitochondria. Instead, fatty-acyl-CoA donates its fatty acyl moiety to the 3-carbon

backbone of a glycerol 3-phosphate molecule, producing mono-, di-, and then triacyl glyceride. The latter resides in the cytoplasm of many cell-types as lipid droplets.

Precursors of membrane phospholipids are diglycerides formed from glycerol 3-phosphate. These molecules are made by reducing dihydroxyacetone phosphate from glycolysis with NADH in the cytoplasm. In the liver, glycerol 3-phosphate is also produced from glycerol itself by ATP-dependent phosphorylation via *glycerol kinase*. This enzyme is notably absent from other tissues.

- 11.36.** How many molecules of ATP are hydrolyzed during the synthesis of one molecule of palmitoyl-CoA?

**SOLUTION**

Refer to Fig. 11-24: None. However, recall that one molecule of ATP is hydrolyzed in the formation of malonyl-CoA, one of the substrates of the reaction pathway. For this reason malonyl-CoA can be viewed as an activated acetyl-CoA. Hence 7 molecules of ATP are used in the formation of 1 molecule of palmitoyl-CoA.

- 11.37.** Does the carbon atom in carbon dioxide that condenses onto acetyl-CoA in forming malonyl-CoA, via acetyl-CoA carboxylase, reside in the final fatty acid?

**SOLUTION**

Refer to Figs. 11-23 and 24. No. The same carbon atom that was condensed onto acetyl-CoA is released during the chain elongation phase.

- 11.38.** What is the process involved in forming unsaturated fatty acids?

**SOLUTION**

These fatty acids are formed by desaturation of saturated fatty acids. The process occurs after the release of the saturated fatty acid from fatty acid synthase, and it requires a special enzyme, *fatty-acid desaturase*, which uses FAD as a cofactor. The enzyme only creates —C=C— double bonds between carbon atoms less than nine away from the carboxyl end. Thus many nutritionally important unsaturated fatty acids that contain double bonds at higher positions along their carbon chain, such as linolenic acid, which is unsaturated at the C9, C12, and C15, must be derived from the diet. Such fatty acids are called *essential*, as is the case for those amino acids for which there is an obligatory requirement in the diet.

Omitting the second reduction phase in a particular cycle of fatty acid synthesis would produce unsaturated fatty acids, but this seems not to occur in nature. Furthermore, in such a process double bonds would be placed at every second carbon atom in a fatty acid molecule, and yet another means would be required for additional desaturation steps between the intervening carbon atoms.

## PENTOSE PHOSPHATE PATHWAY

- 11.39.** What regulates carbon flux through the pentose phosphate pathway?

**SOLUTION**

The main control of flux in the oxidative pentose phosphate pathway under normal conditions is the profound product inhibition by NADPH on the first enzyme, glucose-6-phosphate dehydrogenase (G6PDH). NADPH concentrations can become low due to active lipogenesis. Then G6PDH will increase in activity.

Note that NADPH is also a substrate for glutathione reductase, regenerating the powerful antioxidant glutathione in conditions of oxidative stress.

- 11.40.** How might the PPP, lipogenesis, and the Krebs cycle interact and influence flux through one another's reactions?

**SOLUTION**

The insulin-stimulated drive to increase lipogenesis and triacylglycerol synthesis creates a demand for ATP, NADPH, and glycerol 3-phosphate. ATP demand is satisfied by an increased activity of the Krebs cycle that occurs in response to a fall in ADP concentration in the mitochondrial matrix. A fall in the concentration of NADPH in the cytoplasm stimulates flux via the oxidative PPP. The use of glycerol 3-phosphate in esterification of fatty acids to make phospholipids stimulates glycolytic flux. Thus, by directly stimulating lipogenesis, insulin indirectly stimulates the PPP, the Krebs cycle, and the first four reactions of glycolysis.

- 11.41.** Beriberi is a neurological and cardiovascular disorder that is caused by a *deficiency of thiamine* (also called vitamin B<sub>1</sub>). It has been a serious health problem in Asia and continues to be in those places where *rice* is the main staple food. The problem is exacerbated if the rice is dehusked (*polished*) because only the outer layers of the seeds contain appreciable amounts of thiamine. Beriberi also occurs in some malnourished chronic alcoholics, so to avoid this problem in some countries certain alcohol-containing drinks are fortified with thiamine. Beriberi is characterized by pain in the limbs, weak muscles, abnormal skin sensation, and an enlarged heart with inadequate cardiac output. Which biochemical processes are affected by thiamine deficiency?

**SOLUTION**

Thiamine pyrophosphate (TPP) is the prosthetic group in three main enzymes in humans: pyruvate dehydrogenase; 2-oxoglutarate dehydrogenase; and transketolase in the pentose phosphate pathway. The common feature of the three enzymes is the transfer of a 2-carbon activated aldehyde unit. In beriberi the plasma concentrations of pyruvate and 2-oxoglutarate are elevated; the pyruvate concentrations are especially high after glucose ingestion. The low activity of transketolase in the red blood cells is diagnostic of beriberi.

**METABOLISM OF TWO OTHER MONOSACCHARIDES**

- 11.42.** An inborn error of liver fructose-1-phosphate aldolase (aldolase B) leads to a condition known as *fructose intolerance*. The condition is characterized by life-threatening liver damage that can occur after consuming fructose in the diet. Why is it life-threatening?

**SOLUTION**

Fructokinase catalyzes the formation of fructose 1-phosphate from fructose and ATP. It is a very rapid reaction that has minimal product inhibition by fructose 1-phosphate. Normally, fructose 1-phosphate enters glycolysis after aldolase B has catalyzed its scission to yield glyceraldehyde and dihydroxyacetone phosphate; then dihydroxyacetone phosphate passes via triosephosphate isomerase to glyceraldehyde 3-phosphate and then into the final part of glycolysis, generating pyruvate. This pyruvate leads to regeneration of ATP. If aldolase B is inactive, then phosphate becomes trapped in fructose 1-phosphate which seriously compromises ATP regeneration. As a result, ATP concentrations in the liver fall dramatically, leading to a failure of the many ATP-dependent reactions including those that pump ions across the plasma membrane. This leads to irreversible damage of the hepatocytes. Even in normal individuals, the activity of aldolase B is sometimes a little lower than that of fructokinase so, in general, ingesting large doses of fructose is not advisable.

- 11.43.** What are the metabolic and likely clinical consequences of a deficiency of galactokinase?

**SOLUTION**

This deficiency causes an inability to trap galactose inside the hepatocytes. Thus galactose is not efficiently cleared from the bloodstream. The accumulation of galactose is especially damaging to sensitive tissues such as the eye lens and the retina, because the open-chain aldehydic form, which is present in dynamic equilibrium with the closed pyranose forms, reacts with exposed amino groups. This causes *glycation* of many different proteins including those in the lens, leading to cataracts and retinal impairment. Thus an end-stage complication of galactosemia is blindness.

**SUPPLEMENTARY PROBLEMS**

- 11.44.** Restorers of old machinery sometimes place rusty items in a large barrel of molasses, the syrup from cane sugar, for several months. Why do they do this?
- 11.45.** Most evolutionarily higher plants produce nectar that is secreted in their flowers. This attracts bees that collect it and in the process transfer pollen from the male part of the flower to the female part. The bees transfer the nectar back to the hive where it is stored and concentrated by airflow from their buzzing wings. This increases the osmolality of the solution rendering it uninhabitable by most microorganisms. But another biochemical process rapidly doubles the osmolality. What is it?
- 11.46.** A standard test for amylose is to add a solution containing potassium iodide and iodine. If amylose is present, the solution turns a deep blue. What is the explanation for this effect?





























