Electrone transport chain and oxidative phosphorylation

Bioenergetics describes the transfer and utilization of energy in biologic systems.

Free energy ΔG : predict the direction of a reaction.

- If negative, there is a net loss of energy, and the reaction goes spontenously.
- If positive, there is a net gain of energy, and the reaction does not go spontenously.
- If zero, the reactants are in equilibrium.

A. ATP as an energy carrier:

Reactions or processes that have a large positive free energy, are made possible by coupling with a second spontaneous reaction with a large negative free energy, such as the exergonic hydrolysis of adenosine triphosphate (ATP).

ATP consists of a molecule of adenosine (adenine+ribose) to which three phosphate groups are attached (figure 6.5). If one phosphate group is removed ADP is produced. If two phosphate groups are removed AMP is produced.

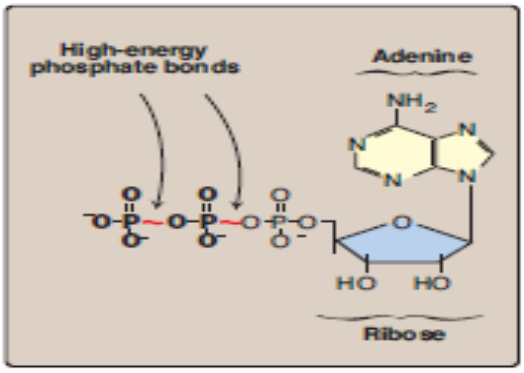


Figure 6.5
Adenosine triphosphate.

B. Electron transport chain

Energy-rich molecules, such as glucose, are metab olized by a series of oxidation reactions ultimately yielding CO_2 and water (Figure 6.6). The metabolic intermediates of these reactions donate electrons to specific coenzymes—**nicotinamide adenine dinucleotide (NAD^+) and flavin adenine dinucleotide (FAD)—**to form the energy-rich reduced coenzymes, NADH and $FADH_2$. These reduced coenzymes can, in turn, each donate a pair of electrons to a specialized set of electron carriers, collectively called the electron transport chain, described in this section.

As electrons are passed down the electron transport chain, they lose much of their free energy. Part of this energy can be captured and **stored by the production of ATP** from ADP and inorganic phosphate (P_i). This process is called oxidative phosphorylation. **The remainder of the free energy** not trapped as ATP **is used to drive ancillary reactions** such as Ca^{2+} transport into mitochondria, and **to generate heat.**

Organization of the electron transport chain

The inner mitochondrial membrane can be disrupted into five separate protein complexes, called Complexes I, II, III, IV, and V. Complexes I–IV each contain part of the electron transport chain (Figure 6.8). Each complex accepts or donates electrons to relatively **mobile electron carriers**, such as **coenzyme Q and cytochrome c.**

Each carrier in the electron transport chain can receive electrons from an electron donor, and can subsequently donate electrons to the next carrier in the chain. The electrons ultimately combine with oxygen and protons to form water. This requirement for oxygen makes the electron transport process the respiratory chain, which accounts for the greatest portion of the body's use of oxygen.

Complex V is a protein complex that contains a domain (Fo) that spans the inner mitochondrial membrane, and a domain (F1) that appears as a sphere that protrudes into the mitochondrial matrix. Complex V catalyzes ATP synthesis and so is referred to as ATP synthase.

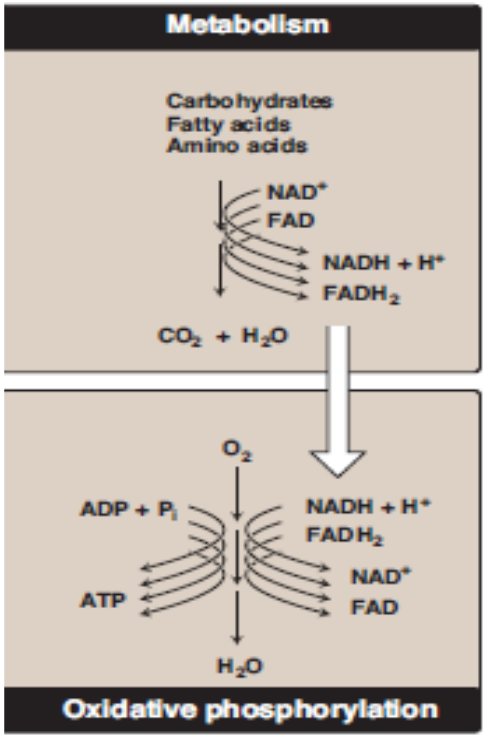


Figure 6.6
The metabolic breakdown of energy-yielding molecules.

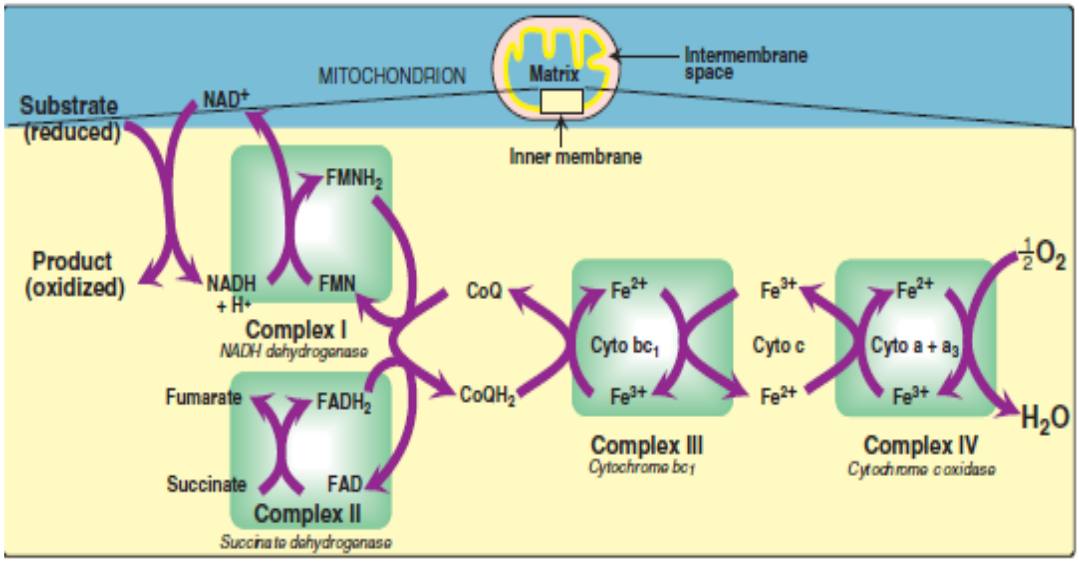


Figure 6.8
Electron transport chain. [Note: NADH, produced from a variety of oxidative (catabolic) processes, is the substrate for Complex I. Succinate, an intermediate of the TCA cycle, is the substrate for Complex II.]

Reactions of the electron transport chain:

- With the exception of coenzyme Q, all members of this chain are proteins.
- These proteins may function as enzymes as is the case with dehydrogenases.
- May contain iron as part of an iron-sulfur center as in cytochromes.
- May contain copper as does the cytochrome a+a3 complex.

1- **NADH is formed (NAD+ is reduced to NADH)**
Two electrons and one proton (hydride ion) are transferred to NAD+, forming NADH plus a free proton, H+.

2- **NADH dehydrogenase (complex I)**
Free proton plus the hydride ion carried by NADH are next transferred to NADH dehydrogenase. Contain iron-sulfur center that is necessary for the transfer of the hydrogen atoms to the next member of chain CoQ.

3- **Coenzyme Q**
Is a mobile carrier and can accept hydrogen atoms from both complex I and complex II to complex III.

The remaining members of the electron transport chain are cytochromes. Each contains a heme group. electrons are passed along the chain from CoQ to cytochromes bc1, c, a+a3.
Cytochrome c-----is a mobile carrier.

4- **Cytochrome a+a3----**
This complex has a heme iron can react directly with O2, and also is called cytochrome oxidase. At this site, the transpoted electrons, O2, and free protons are brought together and O2 is reduced to water. Cytochrome oxidase contains copper atoms that are reqiurd for this complex reaction to occur.

Site specific inhibitors:

These compounds prevent the passage of electrons by binding to a component of the chain, blocking oxidation/reduction reaction.

Oxidative phosphorylation: The flow of electrons from NADH to O2 does not directly result in ATP synthesis.

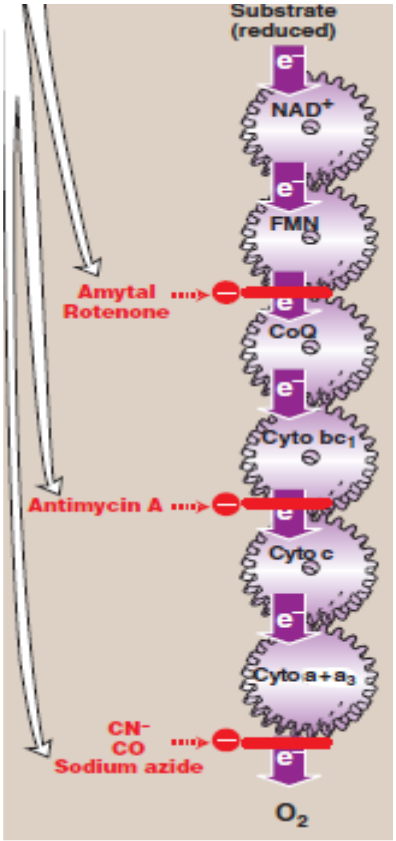
Chemiosmotic hypothesis:

This hypothesis explains how the free energy generated by the transport of electrons by the electron transport chain (ETC) is used to produce ATP from ADP +Pi. Electron transport is coupled to the phosphorylation of ADP by the transfer "pumping" of protons across the inner mitochondrial membrane from matrix to the intermembrane space at complexes I,III, and IV. This process creates an electrical ingredient (with more positive charges on the outside of the membrane than on the inside) and a PH ingredient (the outside of the membrane is at a lower PH than the inside) the energy generated by this proton gradient is sufficient to drive ATP synthesis. Thus, the proton gradient serves as the common intermediate that couples oxidation to phosphorylation.

ATP synthase:

The enzyme ATP synthase(complex V) synthesizes ATP using the energy of the proton gradient generated by ETC.

The chemiosmotic hypothesis proposes that after protons have been pumped they reenter the matrix by passing through the channel in the membrane spanning domain (F0) of complex V driving rotation of this domain. This rotation causes conformational changes in the extra-membranous F1 domain that allow it to bind ADP+Pi phosphorylates ADP to ATP and release ATP



Introduction to Carbohydrates

I. OVERVIEW

Carbohydrates are the most abundant organic molecules in nature. They have a wide range of functions, including providing a significant fraction of the dietary calories for most organisms, acting as a storage form of energy in the body, and serving as cell membrane components that mediate some forms of intercellular communication.

Carbohydrates also serve as a structural component of many organisms, including the cell walls of bacteria, the exoskeleton of many insects, and the fibrous cellulose of plants.The empiric formula for many of the simpler carbohydrates is (CH2O)n, hence the name “hydrate of carbon.

II. CLASSIFICATION AND STRUCTURE OF CARBOHYDRATES

Classification:

1- Monosaccharides.(mono=one)
2- Disaccharides.(Di= Two)
3- Oligosaccharides.(oligo= few)3-10 carbons.
4- Polysaccharides. (poly = many) more than ten.

Monosaccharides (simple sugars) can be classified according to the number of carbon atoms they contain. Examples of some monosaccharides commonly found in humans are listed in Figure 7.1. Carbohydrates with an aldehyde group are called aldoses, whereas those with a keto group are called ketoses (Figure 7.2). Mono saccharides can be linked by **glycosidic bonds** to create larger structures (Figure 7.3).

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

Figure 7.1
Examples of monosaccharides found in humans, classified according to the number of carbons they contain.

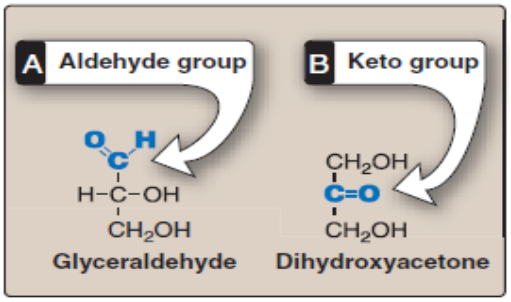


Figure 7.2
Examples of an aldose (A) and a ketose (B) sugar.

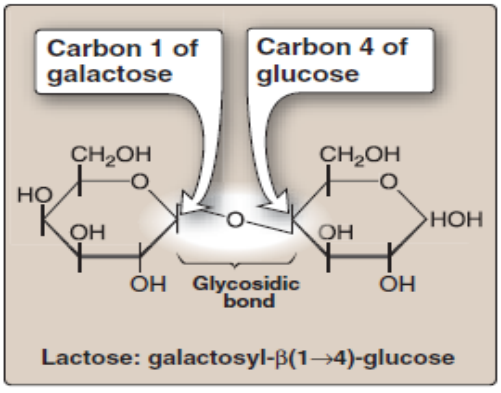


Figure 7.3
A glycosidic bond between two hexoses producing a disaccharide.

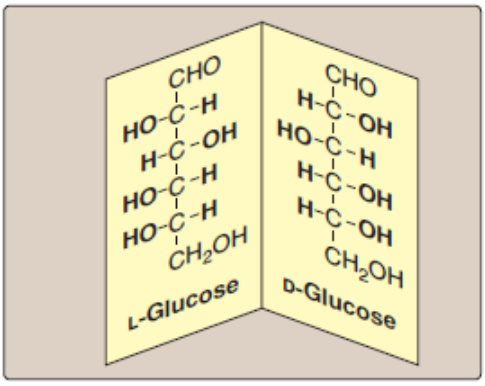


Figure 7.5
Enantiomers (mirror images) of glucose.

A. Isomers and epimers

Compounds that have the same chemical formula but have different structures are called **isomers**. For example, fructose, glucose, and galactose are all isomers of each other, having the same chemical formula, $C_6H_{12}O_6$. Carbohydrate isomers that differ in configuration around only one specific carbon atom are defined as **epimers** of each other.

B. Enantiomers

A special type of isomerism is found in the pairs of structures that are mirror images of each other. These mirror images are called **enantiomers**, and the two members of the pair are designated as a D- and an L-sugar (Figure 7.5). The vast majority of the sugars in humans are D-sugars. In the D isomeric form, the –OH group on the asymmetric carbon is on the right, whereas in the L-isomer it is on the left.

C. Cyclization of monosaccharides

Less than 1% of each of the monosaccharides with five or more carbons exists in the open-chain (acyclic) form. Rather, they are predominantly found in a ring (cyclic) form. [Note: Pyranose refers to a six-membered ring consisting of five carbons and one oxygen, whereas furanose denotes a five-membered ring with four carbons and one oxygen.]

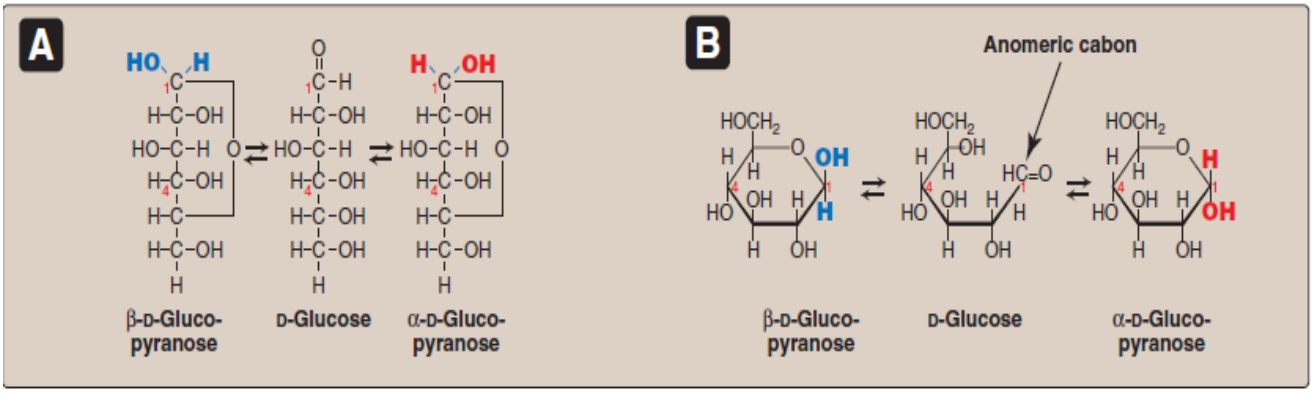


Figure 7.6

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

1. **Anomeric carbon:**

Cyclization creates an anomeric carbon (the former carbonyl carbon), generating the α□and β□configurations of the sugar, for example, α-D-glucopyranose and β-D-glucopryanose (see Figure 7.6). These two sugars are both glucose but are **anomers** of each other.

D. Joining of monosaccharides

Monosaccharides can be joined to form disaccharides, oligosaccharides, and polysaccharides. **Important disaccharides include lactose (galactose + glucose), sucrose (glucose + fructose), and maltose (glucose + glucose).**

Important polysaccharides include branched glycogen (from animal sources) and starch (plant sources) and unbranched cellulose (plant sources); each is a polymer of glucose. The bonds that link sugars are called glycosidic bonds. These are formed by enzymes that use nucleotide sugars such as UDP-glucose as substrates.

Naming glycosidic bonds: Glycosidic bonds between sugars are named according to the numbers of the connected carbons, and with regard to the position of the anomeric hydroxyl group of the sugar involved in the bond. If this anomeric hydroxyl is in the α□configuration, the linkage is an α-bond. If it is in the β□configuration, the linkage is a β-bond. Figure (7.3)

Dietary carbohydrates:

The plant starches amylopectin and amylose, which are present in grains, tubers, and vegetables. These starches are polysaccharides, containing 10,000 to 1 million glucosyl units.

The other major sugar found in fruits and vegetables is sucrose, a disaccharide of glucose and fructose. Sucrose and small amounts of the monosaccharides glucose and fructose are the major natural sweeteners found in fruit, honey, and vegetables.

Dietary fiber, that portion of the diet that cannot be digested by human enzymes of the intestinal tract, is also composed principally of plant polysaccharides and a polymer called lignan.

Most foods derived from animals, such as meat or fish, contain very little carbohydrate except for small amounts of glycogen (which has a structure similar to amylopectin) and glycolipids. The major dietary carbohydrate of animal origin is lactose, a disaccharide composed of glucose and galactose found exclusively in milk and milk products.

III. DIGESTION OF DIETARY CARBOHYDRATES

The principal sites of dietary carbohydrate digestion are the mouth and intestinal lumen. This digestion is rapid and is catalyzed by enzymes known as glycoside hydrolases (glycosidases) that hydrolyze glycosidic bonds. Because there is little monosaccharide present in diets of mixed animal and plant origin, the enzymes are primarily endoglycosidases that hydrolyze polysaccharides and oliosaccharides, and disaccharidases that hydrolyse tri- and disaccharides into their reducing sugar components (Figure 7.8). The final products of carbohydrate digestion are the monosaccharides, glucose, galactose and fructose, which are absorbed by cells of the small intestine.

A. Digestion of carbohydrates begins in the mouth

The major dietary polysaccharides are of plant (starch, composed of amylose and amylopectin) and animal (glycogen) origin. During mastication, salivary α-amylase acts briefly on dietary starch and glycogen, hydrolyzing random □(1-4) bonds. Because branched amylopectin and glycogen also contain α(1-6) bonds, which α-amylase cannot hydrolyze, the digest resulting from its action contains a mixture of short, branched and unbranched oligosaccharides kown as dextrins (Figure 7.9)

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

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

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

The final digestive processes occur primarily at the mucosal lining of the upper jejunum, and include the action of several disaccharidases (Figure 7.10).

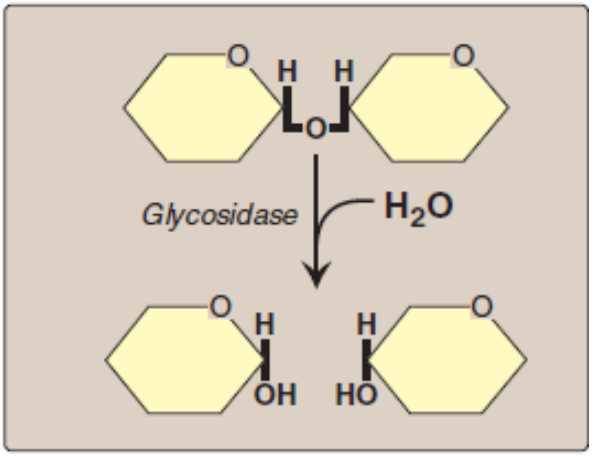


Figure 7.8
Hydrolysis of a glycosidic bond.

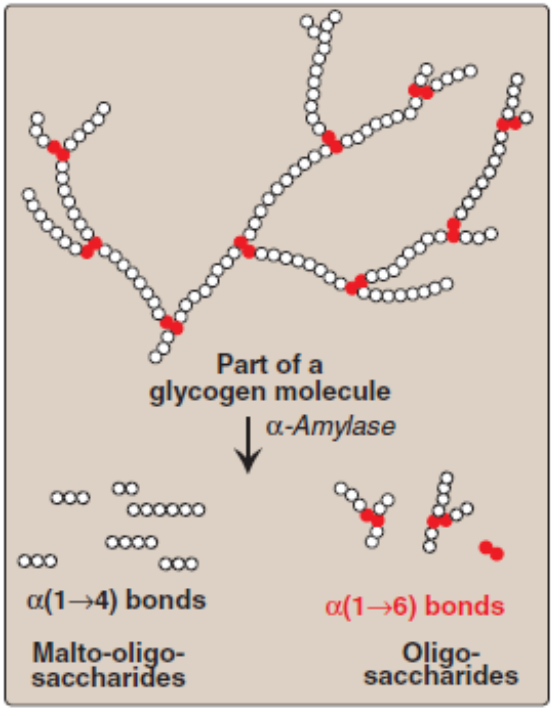


Figure 7.9
Degradation of dietary glycogen by salivary or pancreatic α-*amylase*.

D. Absorption of monosaccharides by intestinal mucosal cells:

The duodenum and upper jejunum absorb the bulk of the dietary sugars. However different sugars have different mechanisms of absorption.

Lactose Intolerance
Lactose is hydrolyzed to galactose and glucose by lactase in humans (by β- Galactosidase in Bacteria).Some adults do not have lactase.Such adults cannot digest the sugar.It remains in the intestines and gets fermented by the bacteria. The condition is called as Lactose intolerance. Such patients suffer from watery diarrhea, abnormal intestinal flow and chloeic pain. They are advised to avoid the consumption of Lactose containing foods like Milk.

Glycolysis.

I. INTRODUCTION TO METABOLISM

Biochemical reactions in cell are organized into multistep sequences called **pathways**. In a pathway, the product of one reaction serves as the substrate of the subsequent reaction. These are collectively called **metabolism**, which is the sum of all the chemical changes occurring in a cell, a tissue, or the body. Most pathways can be classified as either **catabolic** (degradative) or **anabolic** (synthetic). **Catabolic** reactions break down complex molecules, such as proteins, polysaccharides, and lipids, to a few simple molecules. **Anabolic** pathways form complex end products from simple precursors.

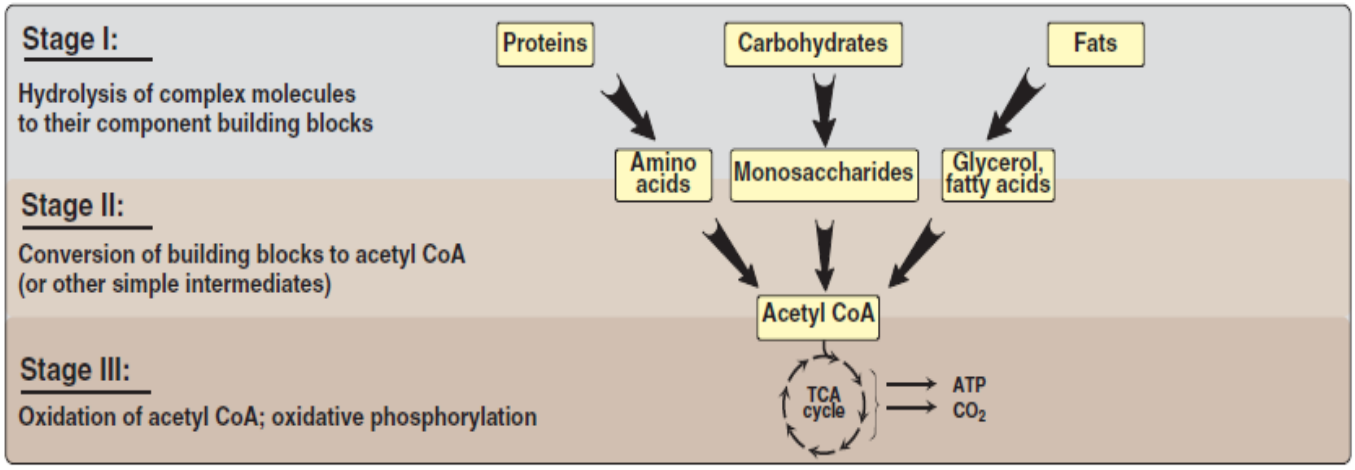


Figure 8.3
Three stages of catabolism.

II. TRANSPORT OF GLUCOSE INTO CELLS

Glucose cannot diffuse directly into cells, but enters by one of two transport mechanisms:

1- Na+-independent, facilitated diffusion transport system.
2- Na+-monosaccharide co-transporter system.

A. Na+-independent facilitated diffusion transport

This system is mediated by a family of 14 glucose transporters in cell membranes. They are designated GLUT-1 to GLUT-14 (glucose transporter isoforms 1–14). Extra cellular glucose binds to the transporter, which then alters its conformation, transporting glucose across the cell membrane.

Specialized functions of GLUT isoforms:

In facilitated diffusion, glucose movement follows a concentration gradient, that is, from a high glucose concentration to a lower one.

- **GLUT-1, GLUT -3, and GLUT-4** are primarily involved in glucose uptake from the blood.
- **GLUT-2**, which is found in the **liver** and **kidney**, can either transport glucose into these cells when blood glucose levels are high, or transport glucose from these cells when blood glucose levels are low (for example, during fasting).
- **GLUT-5** is unusual in that it is the primary transporter for fructose (instead of glucose) in the small intestine and the testes.

B. Na+-monosaccharide co-transporter system
This is an energy-requiring process that transports glucose "against" a concentration gradient—that is, from low glucose concentrations outside the cell to higher concentrations within the cell. This system is a carrier-mediated process in which the movement of glucose is coupled to the concentration gradient of Na+, which is transported into the cell at the same time. The carrier is a sodium-dependent–glucose transporter or SGLT. This type of transport occurs in the epithelial cells of the intestine and renal tubules

IV. OVERVIEW OF GLYCOLYSIS

The glycolytic pathway is employed by all tissues for the breakdown of glucose to provide energy (in the form of ATP) and intermediates for other metabolic pathways

Pyruvate is the end product of **glycolysis** in cells with mitochondria and an adequate supply of oxygen. This series of ten reactions is called aerobic glycolysis. **Aerobic glycolysis** sets the stage for the oxidative decarboxylation of pyruvate to acetyl CoA, a major fuel of the TCA (or citric acid) cycle. Alternatively, pyruvate is reduced to lactate. This conversion of glucose to lactate is called **anaerobic glycolysis** because it can occur without the participation of oxygen.

V. REACTIONS OF GLYCOLYSIS
The conversion of glucose to pyruvate occurs in two stages. The first five reactions of glycolysis correspond to an energy investment phase in which the phosphorylated forms of intermediates are synthesized at the expense of ATP. The subsequent reactions of glycolysis constitute an energy generation phase in which **a net of two molecules of ATP** are formed per glucose molecule metabolized.

Summary of reactions:

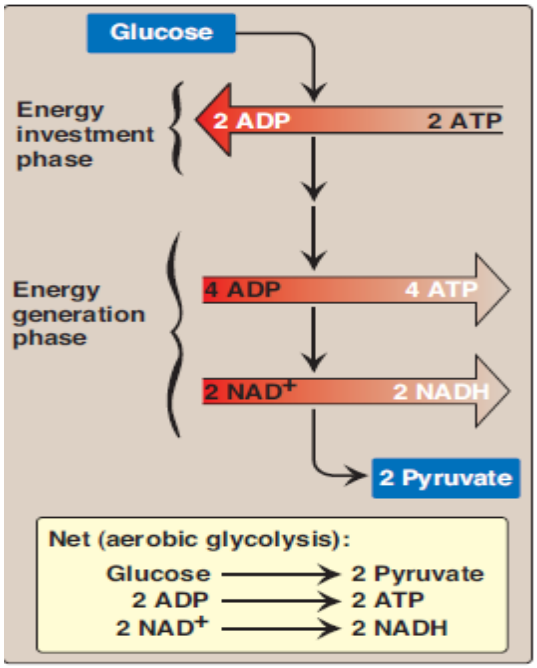
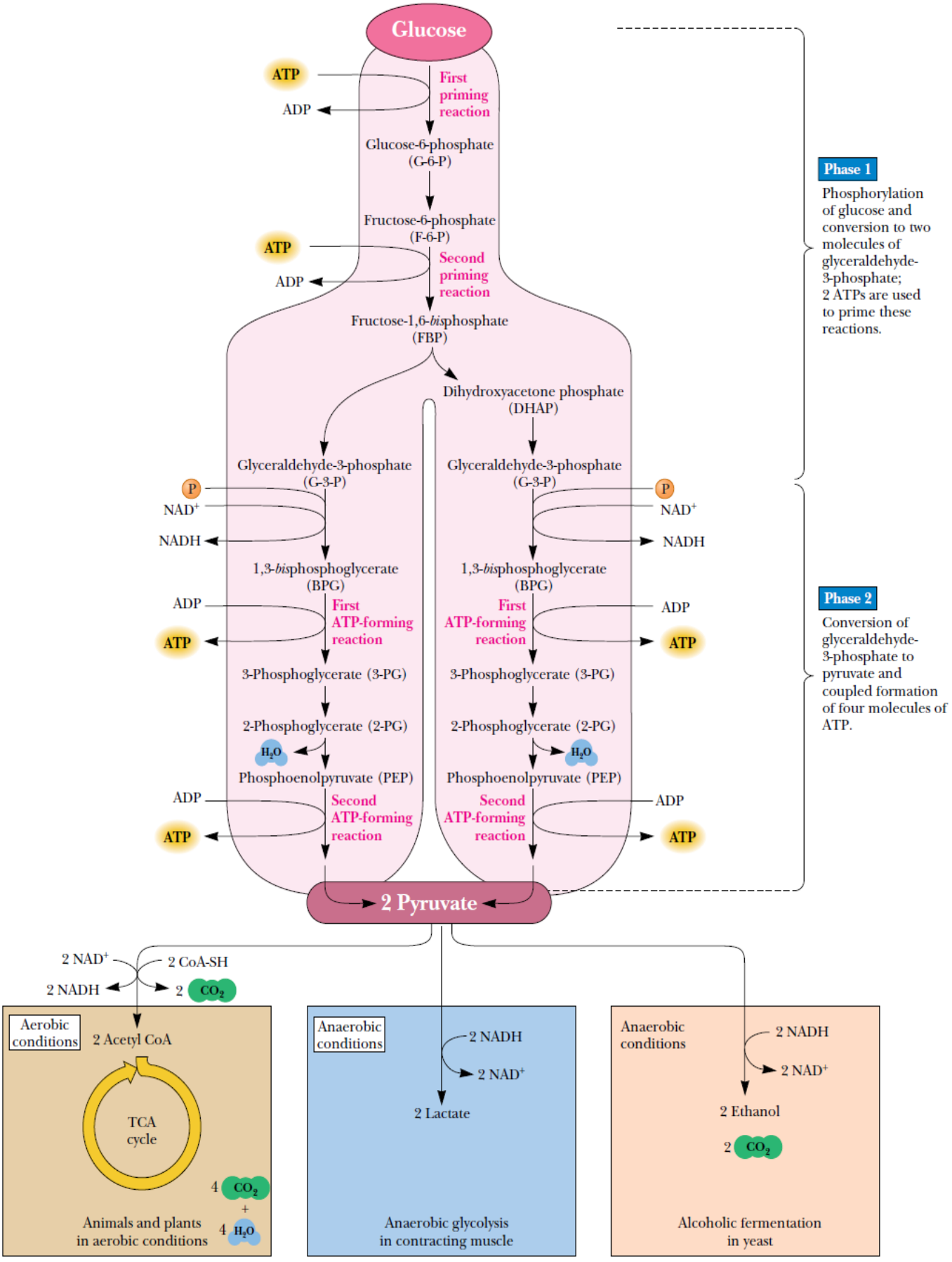


Figure 8.11
Two phases of aerobic glycolysis.


Glucose
ATP
ADP
First priming reaction
Glucose-6-phosphate (G-6-P)
Fructose-6-phosphate (F-6-P)
ATP
ADP
Second priming reaction
Fructose-1,6-bisphosphate (FBP)
Dihydroxyacetone phosphate (DHAP)
Glyceraldehyde-3-phosphate (G-3-P)
Glyceraldehyde-3-phosphate (G-3-P)
P
NAD+
NADH
P
NAD+
NADH
1,3-bisphosphoglycerate (BPG)
1,3-bisphosphoglycerate (BPG)
ADP
ATP
First ATP-forming reaction
ADP
ATP
First ATP-forming reaction
3-Phosphoglycerate (3-PG)
3-Phosphoglycerate (3-PG)
2-Phosphoglycerate (2-PG)
2-Phosphoglycerate (2-PG)
H2O
H2O
Phosphoenolpyruvate (PEP)
Phosphoenolpyruvate (PEP)
ADP
ATP
Second ATP-forming reaction
ADP
ATP
Second ATP-forming reaction
2 Pyruvate
Phase 1
Phosphorylation of glucose and conversion to two molecules of glyceraldehyde-3-phosphate; 2 ATPs are used to prime these reactions.
Phase 2
Conversion of glyceraldehyde-3-phosphate to pyruvate and coupled formation of four molecules of ATP.
2 NAD+
2 NADH
2 CoA-SH
2 CO2
Aerobic conditions
2 Acetyl CoA
TCA cycle
4 CO2
+
4 H2O
Animals and plants in aerobic conditions
Anaerobic conditions
2 NADH
2 NAD+
2 Lactate
Anaerobic glycolysis in contracting muscle
Anaerobic conditions
2 NADH
2 NAD+
2 Ethanol
2 CO2
Alcoholic fermentation in yeast

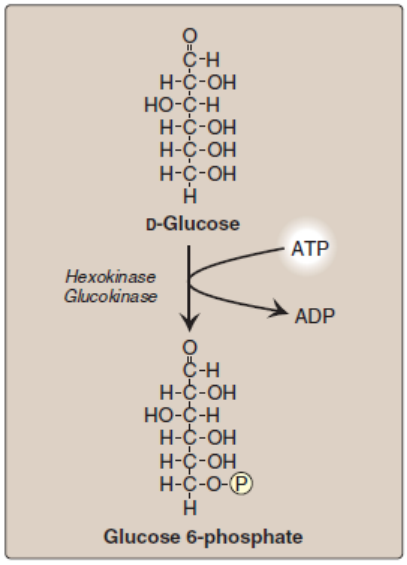


Figure 8.12
Energy investment phase: phosphorylation of glucose.

STEP 1. Phosphorylation of glucose
Phosphorylated sugar molecules do not readily penetrate cell membranes,
because there are no specific transmembrane carriers for these compounds, and because they are too polar to diffuse through the lipid core of membranes.

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

1. **Hexokinase:**

In most tissues, the phosphorylation of glucose is catalyzed by hexokinase, one of three regulatory enzymes of glycolysis. Hexokinase has broad substrate specificity and is able to phosphorylate several hexoses in addition to glucose. Hexokinase is **inhibited** by the reaction product, **glucose 6-phosphate.** Hexokinase has a **low K_m** for glucose. This permits the efficient phosphorylation and subsequent metabolism of glucose even when tissue concentrations of glucose are low. Hexokinase, however, has a low V_{max} for glucose and, therefore, cannot phosphorylate more sugars than the cell can use.

2. **Glucokinase:**

In liver parenchymal cells and β□cells of the pancreas.In β□cells, glucokinase functions as the glucose sensor, determining the threshold for insulin secretion. In the liver, the enzyme facilitates glucose phosphorylation during hyperglycemia. Despite the popular but misleading name glucokinase, the sugar specificity of the enzyme is similar to that of other hexokinase isozymes.

Glucokinase differs from hexokinase in several important properties. For example, it has a **much higher K_m**, requiring a higher glucose concentration for half-saturation. Thus, glucokinase functions only when the intracellular concentration of glucose in the hepatocyte is elevated, such as during the brief period following consumption of a carbohydrate-rich meal, when high levels of glucose are delivered to the liver via the portal vein.
Glucokinase has a **high V_{max}**, allowing the liver to effectively remove the flood of glucose delivered by the portal blood. This prevents large amounts of glucose from entering the systemic circulation following a carbohydrate rich meal, and thus minimizes hyperglycemia during the absorptive period.

Glucokinase activity is not directly inhibited by glucose 6-phosphate as are the other hexokinases, but rather is **indirectly inhibited by fructose 6-phosphate** (which is in equilibrium with glucose 6-phosphate, a product of glucokinase), and is **stimulated** by glucose (a substrate of glucokinase).

STEP 2. Isomerization of glucose 6-phosphate
The isomerization of glucose 6-phosphate to fructose 6-phosphate is catalyzed by **phosphoglucose isomerase** (Figure 8.15). The reaction is readily reversible and is not a rate-limiting or regulated step.

STEP 3. Phosphorylation of fructose 6-phosphate
The irreversible phosphorylation reaction catalyzed by **phosphofructokinase-1 (PFK-1)** is the most important **control point** and the **rate-limiting** and **committed step** of glycolysis (Figure 8.16). PFK-1 is controlled by the available concentrations of the substrates ATP and fructose-6-phosphate, and by the following regulatory substances.

1- **Regulation by energy levels within the cell:**
- PFK-1 is **inhibited** allosterically by elevated levels of ATP, which act as an "energy-rich" signal.
- PFK-1 is **inhibited** by elevated levels of citrate, an intermediate in the TCA cycle.
- PFK-1 is **activated** allosterically by high concentrations of AMP, which signal that the cell's energy stores are depleted.

2- Regulation by fructose 2,6-bisphosphate:
Fructose 2,6-bisphosphate is the most potent activator of PFK-1 and is able to activate the enzyme even when ATP levels are high. Fructose 2,6-bisphosphate is formed by phosphofructokinase-2.

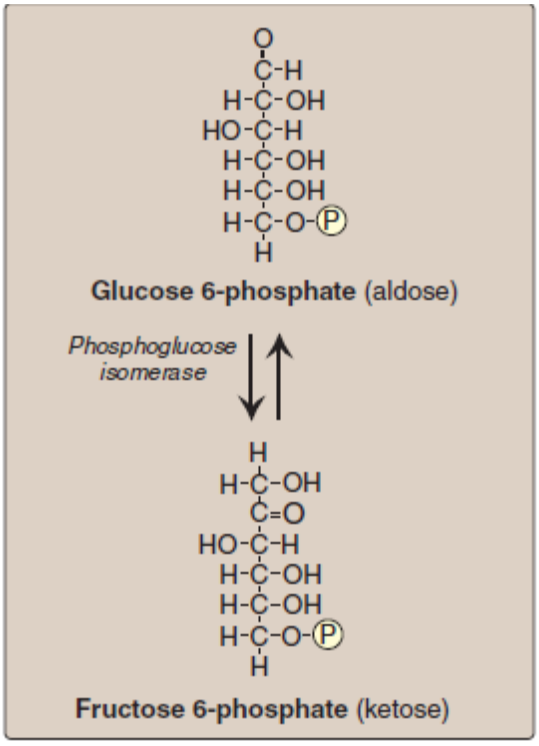


Figure 8.15
Aldose-ketose isomerization of glucose 6-phosphate to fructose 6-phosphate.

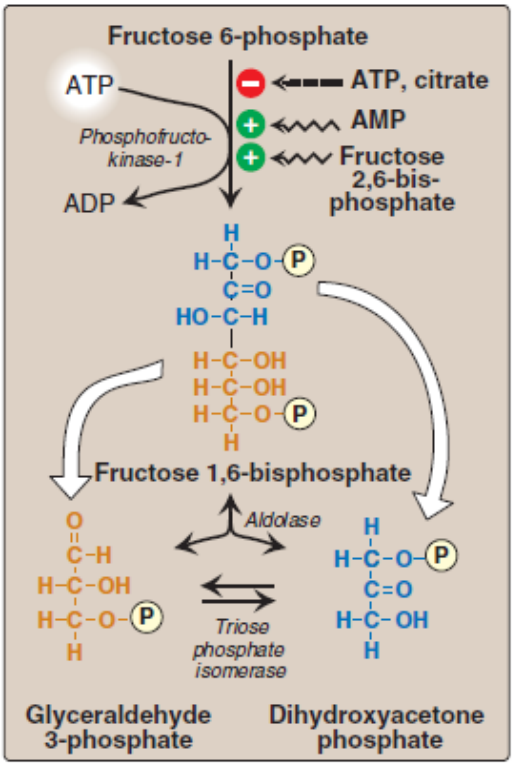


Figure 8.16
Energy investment phase (continued): Conversion of fructose 6-phosphate to triose phosphates.

STEP 4. Cleavage of fructose 1,6-bisphosphate
Aldolase cleaves fructose 1,6-bisphosphate to dihydroxy acetone phosphate and glyceraldehyde 3-phosphate (see Figure 8.16). The **reaction is reversible and not regulated**.

STEP 5. Isomerization of dihydroxyacetone phosphate
Triose phosphate isomerase interconverts dihydroxyacetone phosphate and glyceraldehyde 3-phosphate (see Figure 8.16). Dihydroxyacetone phosphate must be isomerized to glyceraldehyde-3-phosphate for further metabolism by the glycolytic pathway. This isomerization results in the **net production of two molecules of**
glyceraldehyde 3-phosphate from the cleavage products of fructose 1,6-bisphosphate.

STEP 6. Oxidation of glyceraldehyde 3-phosphate
The conversion of glyceraldehyde 3-phosphate to 1,3-bisphosphoglycerate by **glyceraldehyde 3-phosphate dehydrogenase** is the first oxidation-reduction reaction of glycolysis (Figure 8.18).

1. Synthesis of 1,3-bisphosphoglycerate (1,3-BPG): The oxidation of the aldehyde group of glyceraldehyde 3-phosphate to a carboxyl group is coupled to the attachment of P_i to the carboxyl group. The high-energy phosphate group at carbon 1 of 1,3-BPG conserves much of the free energy produced by the oxidation of glyceraldehyde 3-phosphate. The energy of this high-energy phosphate drives the synthesis of ATP in the next reaction of glycolysis.

2. Synthesis of 2,3-bisphosphoglycerate (2,3-BPG) in red blood cells: Some of the 1,3-BPG is converted to 2,3-BPG by the action of bisphosphoglycerate mutase (see Figure 8.18). 2,3-BPG, which is found in only trace amounts in most cells, is present at high concentration in red blood cells (increases O_2 delivery). 2,3-

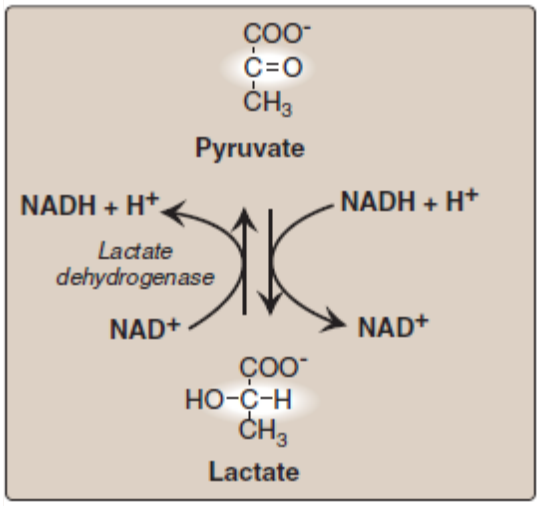


Figure 8.21
Interconversion of pyruvate and lactate.

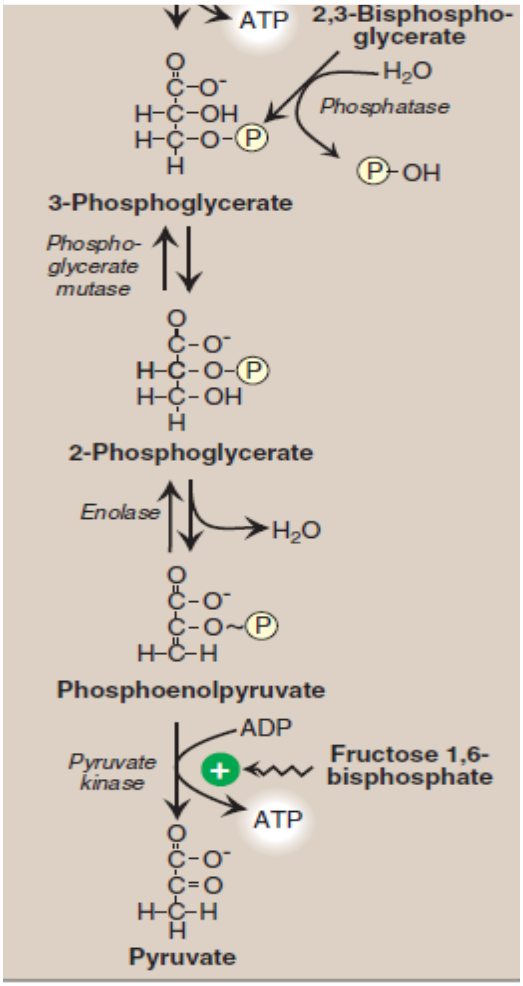


Figure 8.18
Energy generating phase: conversion of glyceraldehyde 3-phosphate to pyruvate.

BPG is hydrolyzed by a phosphatase to 3-phosphoglycerate, which is also an intermediate in glycolysis (see Figure 8.18).

STEP 7. Synthesis of 3-phosphoglycerate producing ATP
When 1,3-BPG is converted to 3-phosphoglycerate, the high-energy phosphate group of 1,3-BPG is used to synthesize ATP from ADP (see Figure 8.18). This reaction is catalyzed by **phosphoglycerate kinase**, which, unlike most other kinases, is physiologically **reversible**.

Because two molecules of 1,3-BPG are formed from each glucose molecule, this kinase reaction replaces the two ATP molecules consumed by the earlier formation of glucose 6-phosphate and fructose 1,6-bisphosphate.

STEP 8. Shift of the phosphate group from carbon 3 to carbon 2 The shift of the phosphate group from carbon 3 to carbon 2 of phosphoglycerate by **phosphoglycerate mutase** is freely reversible (see Figure 8.18).

STEP 9. Dehydration of 2-phosphoglycerate
The dehydration of 2-phosphoglycerate by **enolase** redistributes the energy within the 2-phosphoglycerate molecule, resulting in the formation of phosphoenolpyruvate (PEP), which contains a highenergy enol phosphate (see Figure 8.18). The reaction is **reversible**.

STEP 10. Formation of pyruvate producing ATP
The conversion of PEP to pyruvate is catalyzed by **pyruvate kinase,** the third **irreversible** reaction of glycolysis. The equilibrium of the pyruvate kinase reaction favors the formation of ATP (see Figure 8.18).

Reduction of pyruvate to lactate:

Lactate, formed by the action of lactate dehydrogenase, is the final product of anaerobic glycolysis in eukaryotic cells (Figure 8.21). The formation of lactate is the major fate for pyruvate in lens and cornea of the eye, kidney medulla, testes, leukocytes and red blood cells, because these are all poorly vascularized and/or lack mitochondria.

1. **Lactate formation in muscle:** In exercising skeletal muscle, NADH production exceeds the oxidative capacity of the respiratory chain. This results in an elevated NADH/NAD+ ratio, favoring reduction of pyruvate to lactate. Therefore, during intense exercise, lactate accumulates in muscle, causing a drop in the intracellular pH, potentially resulting in cramps. Much of this lactate eventually diffuses into the bloodstream, and can be used by the liver to make glucose.

2. Lactic acidosis: Elevated concentrations of lactate in the plasma, termed lactic acidosis, occur when there is a collapse of the circulatory system, such as in myocardial infarction, pulmonary embolism, and uncontrolled hemorrhage, or when an individual is in shock.

The failure to bring adequate amounts of oxygen to the tissues results in impaired oxidative phosphorylation and decreased ATP synthesis. To survive, the cells use anaerobic glycolysis as a backup system for generating ATP, producing lactic acid as the end product.

Energy yield from glycolysis:
Despite the production of some ATP during glycolysis, the end products, pyruvate or lactate, still contain most of the energy originally contained in glucose. The TCA cycle is required to release that energy.

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

2. Aerobic glycolysis: The direct consumption and formation of ATP is the same as in anaerobic glycolysis—that is, a net gain of two ATP per molecule of glucose. Two molecules of NADH are also produced per molecule of glucose. Ongoing aerobic glycolysis requires the oxidation of most of this NADH by the electron transport chain, producing approximately three ATP for each NADH molecule entering the chain.

VI. ALTERNATE FATES OF PYRUVATE

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

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

C. Reduction of pyruvate to ethanol (microorganisms)
The conversion of pyruvate to ethanol occurs by the two reactions summarized in Figure 8.24. The decarboxylation of pyruvate by pyruvate decarboxylase occurs in yeast and certain other microorganisms, but not in humans. The enzyme requires thiamine pyrophosphate as a coenzyme, and catalyzes a reaction similar to that described for pyruvate dehydrogen

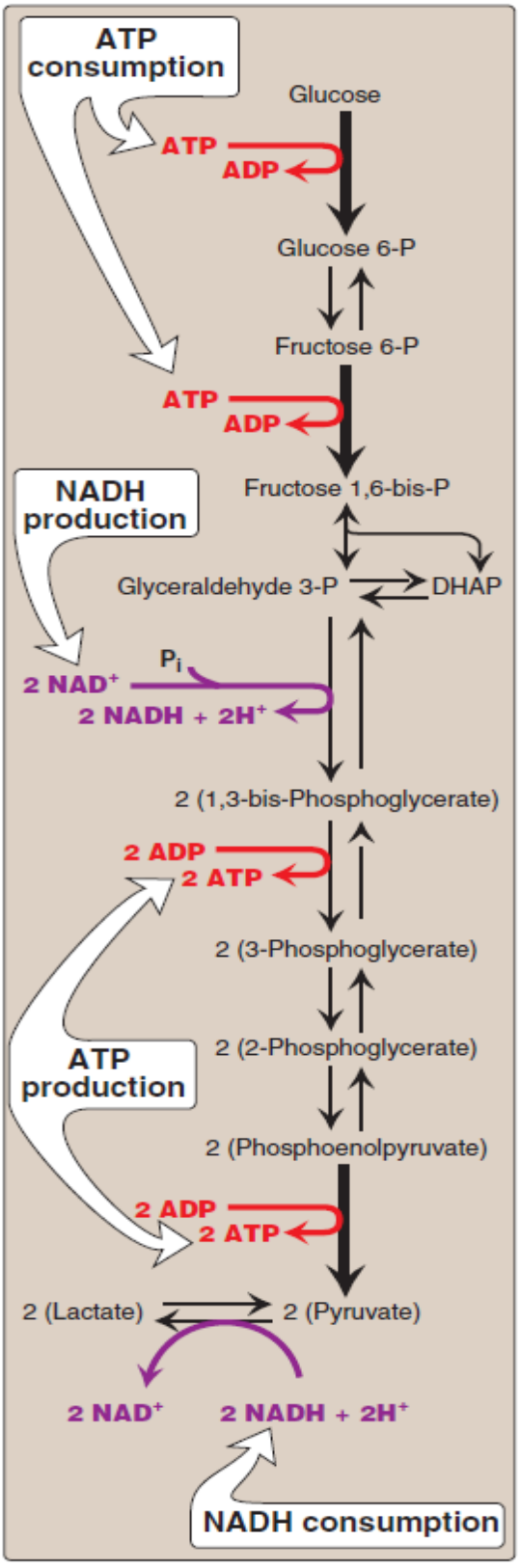


Figure 8.22
Summary of anaerobic glycolysis. Reactions involving the production or consumption of ATP or NADH are indicated. The three irreversible reactions of glycolysis are shown with thick arrows. DHAP = dihydroxyacetone phosphate.

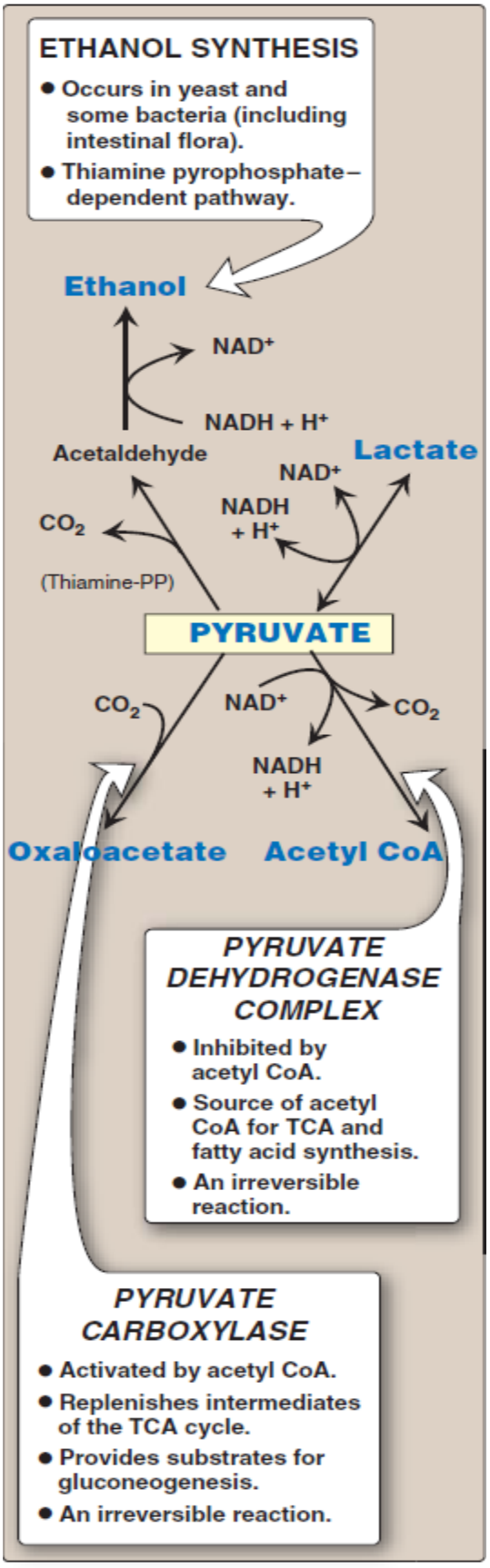
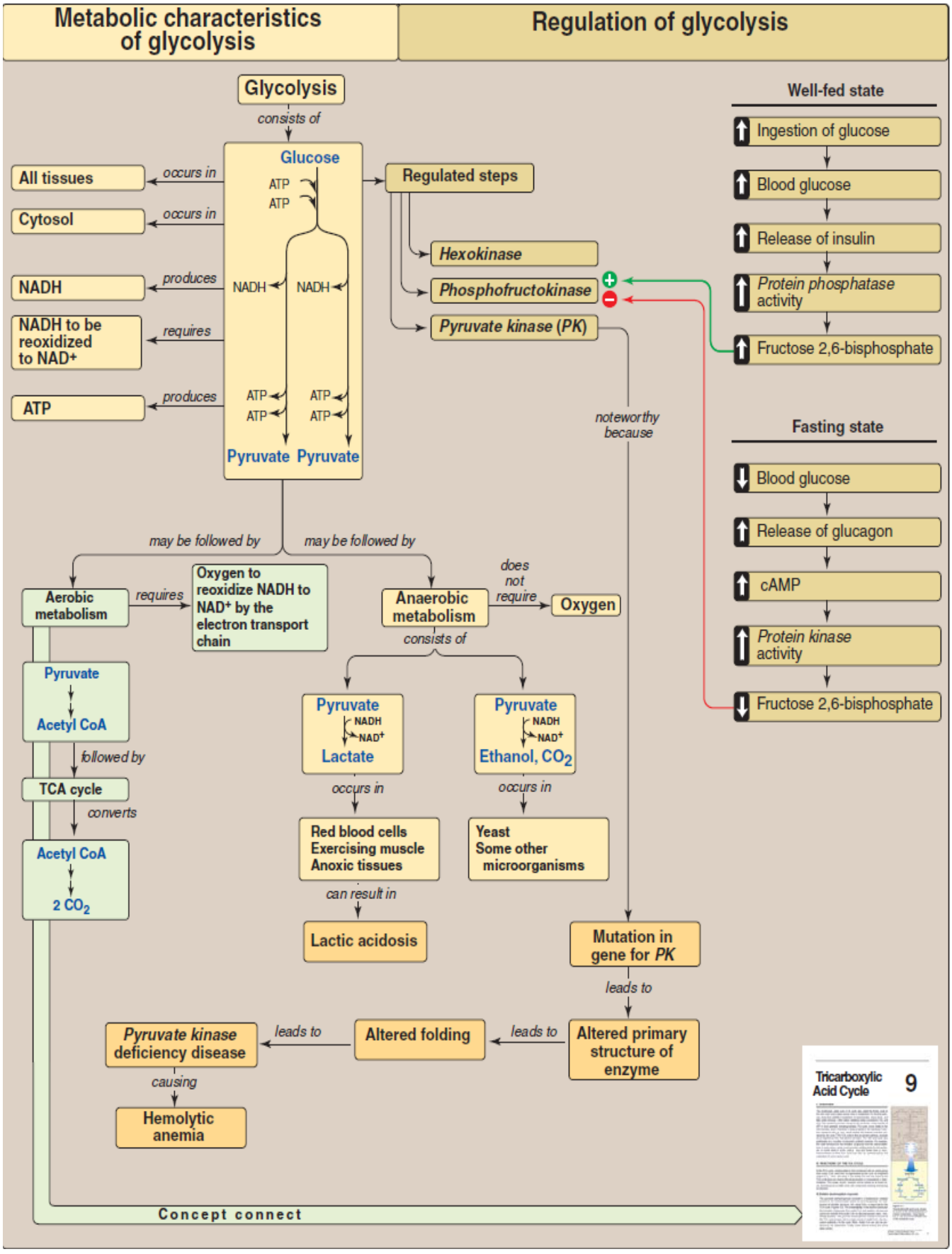


Figure 8.24
Summary of the metabolic fates of pyruvate.


Metabolic characteristics of glycolysis
Regulation of glycolysis
Glycolysis
consists of
Glucose
ATP
ATP
All tissues
occurs in
Cytosol
occurs in
NADH
produces
NADH to be reoxidized to NAD+
requires
ATP
produces
NADH
NADH
ATP
ATP
ATP
ATP
Pyruvate
Pyruvate
Regulated steps
Hexokinase
Phosphofructokinase
Pyruvate kinase (PK)
noteworthy because
Well-fed state
Ingestion of glucose
Blood glucose
Release of insulin
Protein phosphatase activity
Fructose 2,6-bisphosphate
Fasting state
Blood glucose
Release of glucagon
cAMP
Protein kinase activity
Fructose 2,6-bisphosphate
may be followed by
may be followed by
Aerobic metabolism
requires
Oxygen to reoxidize NADH to NAD+ by the electron transport chain
Anaerobic metabolism
does not require
Oxygen
consists of
Pyruvate
Acetyl CoA
followed by
TCA cycle
converts
Acetyl CoA
2 CO2
Pyruvate
NADH
NAD+
Lactate
occurs in
Red blood cells
Exercising muscle
Anoxic tissues
can result in
Lactic acidosis
Pyruvate
NADH
NAD+
Ethanol, CO2
occurs in
Yeast
Some other microorganisms
Mutation in gene for PK
leads to
Altered primary structure of enzyme
leads to
Altered folding
leads to
Pyruvate kinase deficiency disease
causing
Hemolytic anemia
Concept connect
Tricarboxylic Acid Cycle
9

Tricarboxylic Acid Cycle (TCA).

I. OVERVIEW

The tricarboxylic acid cycle (TCA cycle, also called the Krebs cycle or the citric acid cycle) plays several roles in metabolism. It is the final pathway where the oxidative metabolism of carbohydrates, amino acids, and fatty acids converge, their carbon skeletons being converted to CO2. This oxidation provides energy for the production of the majority of ATP in most animals, including humans.

The cycle occurs totally in the mitochondria and is, therefore, in close proximity to the reactions of electron transport, which oxidize the reduced coenzymes produced by the cycle. The TCA cycle is an aerobic pathway, because O2 is required as the final electron acceptor.

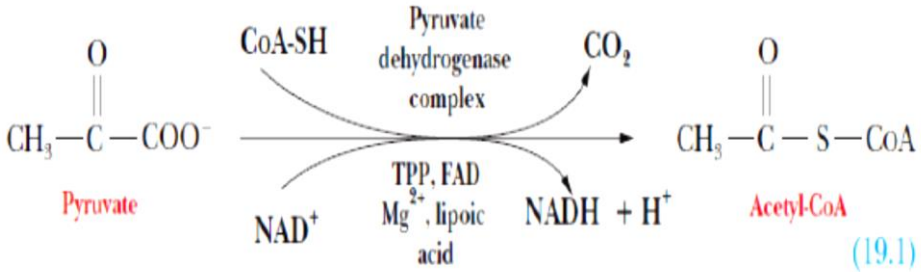
How is pyruvate converted to Acetyl-CoA?

Pyruvate, the endproduct of aerobic glycolysis, must be transported into the mitochondrion before it can enter the TCA cycle. This is accomplished by a specific pyruvate transporter that helps pyruvate cross the inner mitochondrial membrane. Once in the matrix, pyruvate is converted to acetyl CoA by the pyruvate dehydrogenase complex, which is a multienzyme complex (is a multimolecular aggregate of three catalytic enzymes and two tightly bound regulatory enzymes).

The pyruvate dehydrogenase complex contains five coenzymes that act as carriers for the intermediates of the reactions (thiamine pyrophosphate TPP, lipoic acid, CoA, FAD and NAD+).

Strictly speaking, the pyruvate dehydrogenase complex is not part of the TCA cycle proper, but is a major source of acetyl CoA—the two-carbon substrate for the cycle.

The overall reaction of the pyruvate dehydrogenase complex



(19.1)

II. REACTIONS OF THE TCA CYCLE:

Summary of TCA cycle reactions:

- ***Step 1: Formation of citrate:***
- ***Step 2: Isomerization of citrate to isocitrate.***
- ***Step 3: Formation of α-ketoglutrate and CO2 FIRST OXIDATION***
- ***Step 4: Formation of succinyl-CoA and CO2-SECOND OXIDATION.***
- ***Step 5: Formation of succinate.***
- ***Step 6: Formation of Fumarate-FAD linked oxidation.***
- ***Step 7: Formation of L-malate.***
- ***Step 8: Regenation of Oxaloacetate-FINAL OXIDATION STEP***

• ***Step 1: Formation of citrate:***
• **Synthesis of citrate from acetyl CoA and oxaloacetate**

The condensation of acetyl CoA and oxaloacetate to form citrate (a tricarboxylic acid) is catalyzed by citrate synthase (Figure 9.4). In humans, citrate synthase is inhibited by its product, citrate. Substrate availability is another means of regulation for citrate synthase. Citrate also inhibits phosphofructokinase, the rate-limiting enzyme of glycolysis.

• ***Step 2: Isomerization of citrate to isocitrate.***

Citrate is isomerized to isocitrate by aconitase, an Fe-S protein.

• ***Step 3: Formation of α-ketoglutrate and CO2 FIRST OXIDATION***
• **Oxidation and decarboxylation of isocitrate**

Isocitrate dehydrogenase catalyzes the irreversible oxidative decarboxylation of isocitrate, yielding the first of three NADH molecules produced by the cycle, and the first release of CO2 (see Figure 9.4). This is one of the rate-limiting steps of the TCA cycle. The enzyme is allosterically activated by ADP (a low-energy signal) and Ca2+, and is inhibited by ATP and NADH, whose levels are elevated when the cell has abundant energy stores.

• ***Step 4: Formation of succinyl-CoA and CO2-SECOND OXIDATION.***
• **Oxidative decarboxylation of –ketoglutarate**

The conversion of α-ketoglutarate to succinyl CoA is catalyzed by the α-ketoglutarate dehydrogenase complex, a multimolecular aggregate of three enzymes (Figure 9.5). The reaction releases the second CO2 and produces the second NADH of the cycle. The coenzymes required are thiamine pyrophosphate, lipoic acid, CoA, FAD, NAD+, and.
α-Ketoglutarate dehydrogenase complex is inhibited by its products, NADH and succinyl CoA, and activated by Ca2+.

• ***Step 5: Formation of succinate.***
• **Cleavage of succinyl CoA**

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

• ***Step 6: Formation of Fumarate-FAD linked oxidation.***
• **Oxidation of succinate**

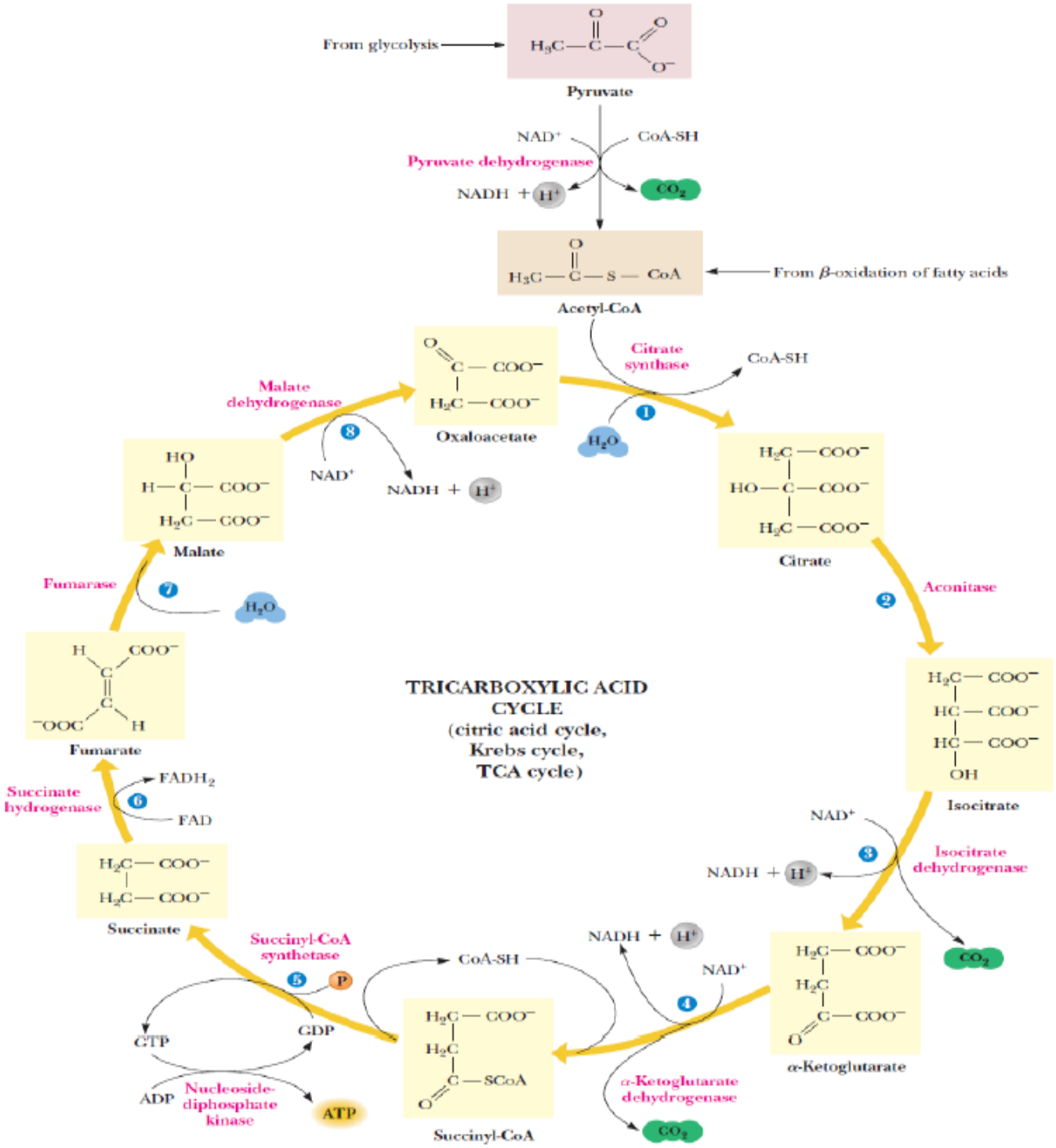
Succinate is oxidized to fumarate by succinate dehydrogenase, as FAD (its coenzyme) is reduced to FADH2 (see Figure 9.5). Succinate dehydrogenase is the only enzyme of the TCA cycle that is embedded in the inner mitochondrial membrane.

Step 7: Formation of L-malate.
Hydration of fumarate

Fumarate is hydrated to malate in a freely reversible reaction catalyzed by fumarase (also called fumarate hydratase, see Figure 9.5).

• ***Step 8: Regenation of Oxaloacetate-FINAL OXIDATION STEP***
• **Oxidation of malate**

Malate is oxidized to oxaloacetate by malate dehydrogenase (Figure 9.6). This reaction produces the third and final NADH of the cycle.



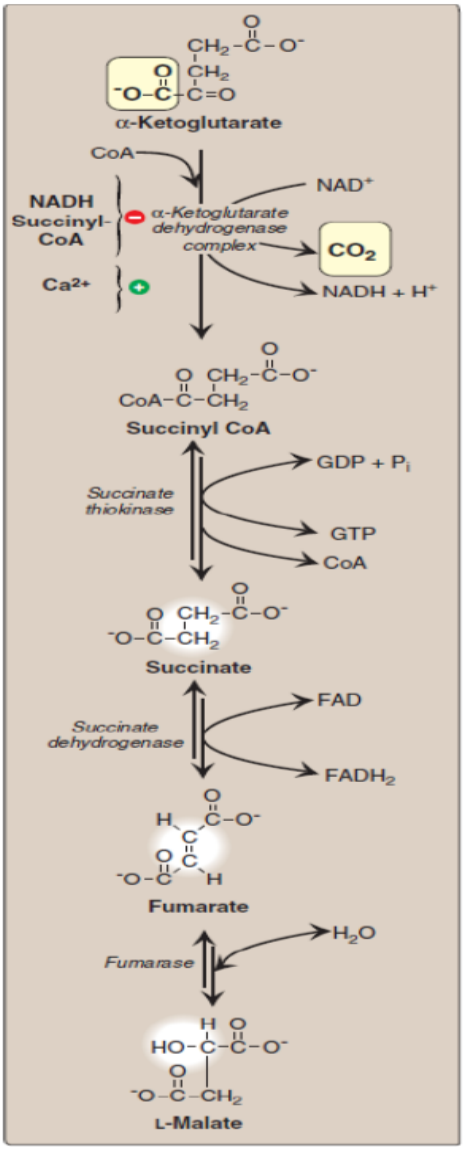


Figure 9.5
Formation of malate from α-ketoglutarate.

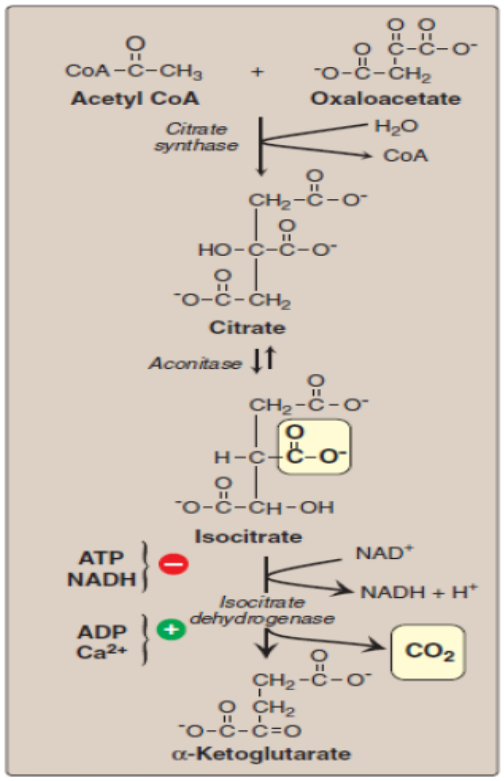


Figure 9.4
Formation of α-ketoglutarate from acetyl CoA and oxaloacetate.

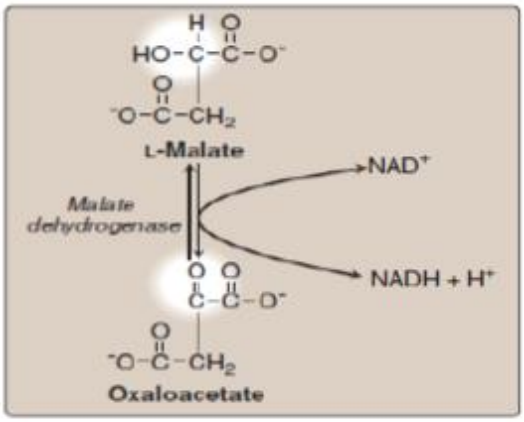


Figure 9.6
Formation of oxaloacetate from malate.

I

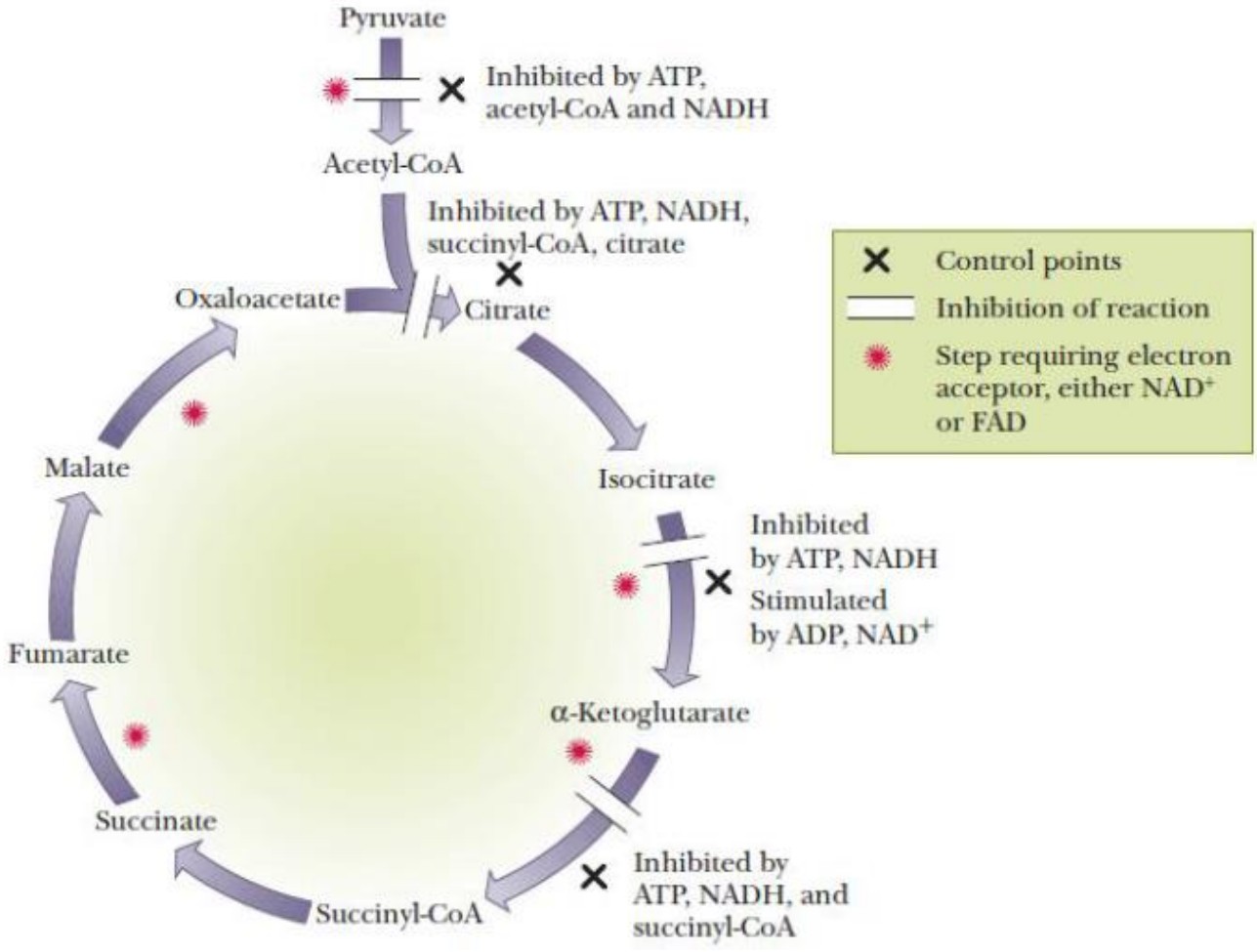
III. ENERGY PRODUCED BY THE TCA CYCLE:

Two carbon atoms enter the cycle as acetyl CoA and leave as CO2. The cycle does not involve net consumption or production of oxaloacetate or of any other intermediate. Four pairs of electrons are transferred during one turn of the cycle: three pairs of electrons reducing three NAD+ to NADH and one pair reducing FAD to FADH2. Oxidation of one NADH by the electron transport chain leads to formation of approximately three ATP, whereas oxidation of FADH2 yields approximately two ATP.

Energy producing reaction	Number of ATP produced
3 NADH ⟶ 3 NAD^+	9
$FADH_2$ ⟶ FAD	2
GDP + P_i ⟶ GTP	1
	12 ATP/acetyl CoA oxidized

Figure 9.7
Number of ATP molecules produced from the oxidation of one molecule of acetyl CoA (using both substrate-level and oxidative phosphorylation).

IV. REGULATION OF THE TCA CYCLE:



TCA CYCLE INTERMEDIATES AND ANAPLEROTIC REACTIONS

A. TCA Cycle Intermediates are Precursors for Biosynthetic Pathways

The intermediates of the TCA cycle serve as precursors for a variety of different pathways present in different cell types. This is particularly important in the central metabolic role of the liver. The TCA cycle in the liver is often called an “open cycle” because there is such a high efflux of intermediates.

After a high carbohydrate meal, citrate efflux and cleavage to acetyl CoA provides acetyl units for fatty acid synthesis. During fasting, gluconeogenic precursors are converted to malate, which leaves the mitochondria for cytosolic gluconeogenesis. The liver also uses TCA cycle intermediates to synthesize carbon skeletons of amino acids. Succinyl CoA may be removed from the TCA cycle to form heme in cells of the liver and bone marrow.

In the brain, α-ketoglutarate is converted to glutamate and then to α-aminobutyric acid (GABA), a neurotransmitter. In skeletal muscle, α-ketoglutarate is converted to glutamine, which is transported through the blood to other tissues.

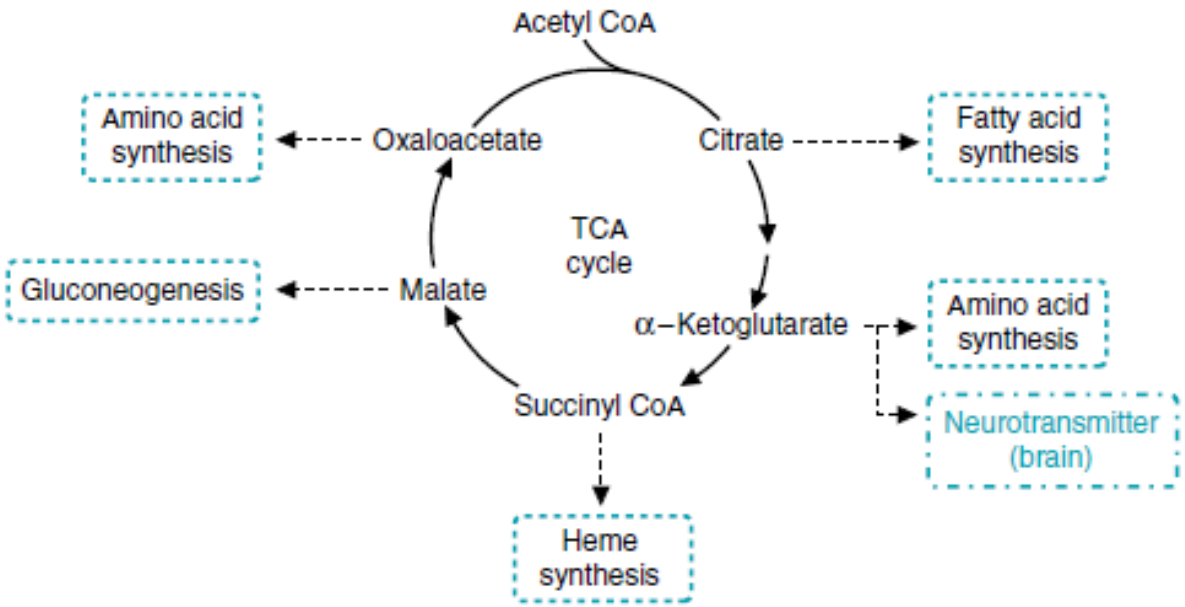


Fig. 20.17. Efflux of intermediates from the TCA cycle. In the liver, TCA cycle intermediates are continuously withdrawn into the pathways of fatty acid synthesis, amino acid synthesis, gluconeogenesis, and heme synthesis. In brain, α-ketoglutarate is converted to glutamate and GABA, both neurotransmitters.

Vitamins and minerals required for TCA

- Niacin (NAD+)
- Riboflavin (FAD)
- Pentothenate
- Thiamine
- Biotin
- Magnesium
- Calcium
- Fe+2
- Phosphate.

Gluconeogenesis

Gluconeogenesis, the process by which glucose is synthesized from noncarbohydrate precursors, occurs mainly in the liver under fasting conditions.

I. OVERVIEW

Some tissues, such as the brain, red blood cells, kidney medulla, lens and cornea of the eye, testes, and exercising muscle, require a continuous supply of glucose as a metabolic fuel. Liver glycogen, an essential postprandial source of glucose, can meet these needs for only 10–18 hours in the absence of dietary intake of carbohydrate. During a prolonged fast, however, hepatic glycogen stores are depleted, and glucose is formed from precursors such as lactate, pyruvate, glycerol (derived from the backbone of triacylglycerols), and α-ketoacids (derived from the catabolism of glucogenic amino acids).

The formation of glucose does not occur by a simple reversal of glycolysis, because the overall equilibrium of glycolysis strongly favors pyruvate formation. Instead, glucose is synthesized by a special pathway, gluconeogenesis that requires both mitochondrial and cytosolic enzymes. During an overnight fast, approximately 90% of gluconeogenesis occurs in the liver, with the kidneys providing 10% of the newly synthesized glucose molecules. However, during prolonged fasting, the kidneys become major glucose-producing organs, contributing an estimated 40% of the total glucose production.

II. SUBSTRATES FOR GLUCONEOGENESIS:

Gluconeogenic precursors are molecules that can be used to produce a net synthesis of glucose. They include:

- intermediates of glycolysis and the tricarboxylic acid (TCA) cycle.
- Glycerol, lactate, and the amino acids are the most important gluconeogenic precursors.

A. Glycerol

Glycerol is released during the hydrolysis of triacylglycerols in adipose tissue, and is delivered by the blood to the liver.

1- Glycerol is phosphorylated by **glycerol kinase** to glycerol phosphate.
2- Glycerol phosphate oxidized by **glycerol phosphate dehydrogenase** to dihydroxy acetone phosphate—an intermediate of glycolysis.

B. Lactate

Lactate is released into the blood by exercising skeletal muscle, and by cells that lack mitochondria, such as red blood cells.

In the **cori cycle**: Lactate produces by exercising muscles diffuses into the blood. This lactate is taken up by the liver and reconverted to glucose, which is released back into the circulation.

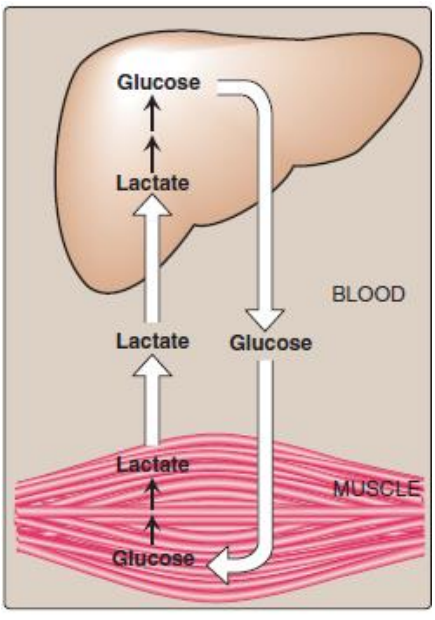


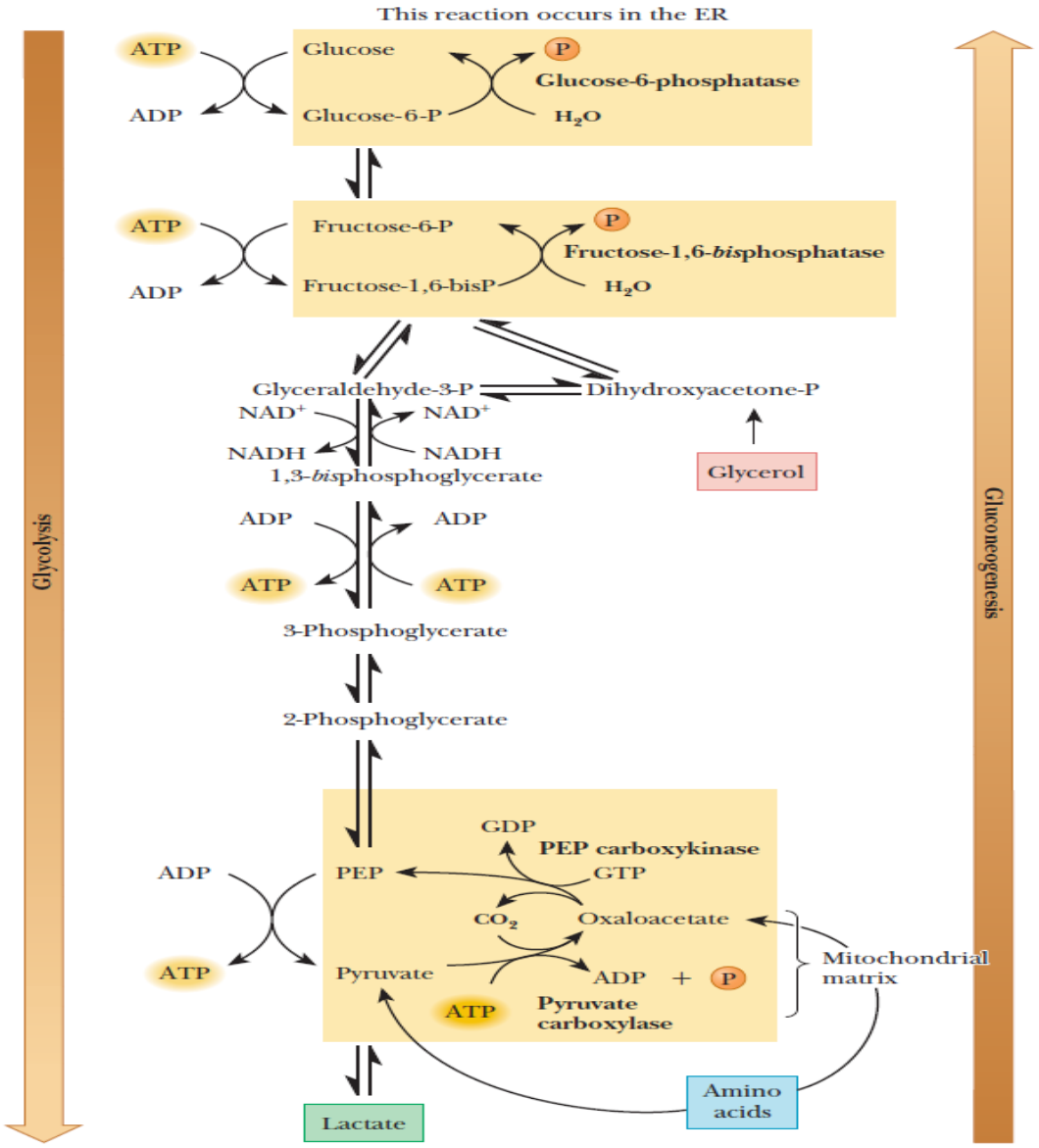
Figure 10.2

C. Amino acids

Amino acids derived from hydrolysis of tissue proteins are the major sources of glucose during a fast. α-Ketoacids, such as α-ketoglutarate, are derived from the metabolism of **glucogenic amino acids.** These α-ketoacids can enter the TCA cycle and form oxaloacetate (OAA)—a direct precursor of phosphoenol pyruvate (PEP).

III. REACTIONS UNIQUE TO GLUCONEOGENESIS:

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



A. Carboxylation of pyruvate: Conversion of pyruvate to OAA

In gluconeogenesis, pyruvate is first carboxylated by **pyruvate carboxylase** to oxaloacetate OAA.(this step in mitochondria)

1-Biotin is a coenzyme of pyruvate carboxylase derived from vitamin B6.

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

B. Transport of oxaloacetate to the cytosol

- PEP generated in mitochondria can be transported to cytosol by a specific transporter.
- OAA that is generated by pyruvate, can't cross the mitochondrial membrane readily it must be first converted to malate which can transport into the cytosol, where it is reoxidized to OAA.

C. Decarboxylation of cytosolic oxaloacetate

- Oxaloacetate is decarboxylated and phosphorylated to PEP in the cytosol by **PEP-carboxykinase**.
- **PEP-carboxykinase** found in cytosol and mitochondria.
- The reaction is driven by hydrolysis of guanosine triphosphate (GTP, see Figure 10.3).
- The combined actions of **pyruvate carboxylase** and **PEP carboxykinase** provide an energetically favorable pathway from pyruvate to PEP.

- Then, PEP is acted on by the reactions of glycolysis running in the reverse direction until it becomes fructose 1,6-bisphosphate.

D. Dephosphorylation of fructose 1,6-bisphosphate

- Hydrolysis of fructose 1,6-bisphosphate by **fructose 1,6-bisphosphatase** bypasses the irreversible phosphofructokinase-1 reaction.
- This reaction is an important regulatory site of gluconeogenesis.

Regulation by energy levels within the cell:

- Fructose 1,6-bisphosphatase is inhibited by elevated levels of adenosine monophosphate (AMP), which signal an "energy-poor" state in the cell.
- Conversely, high levels of ATP and low concentrations of AMP stimulate gluconeogenesis, an energy-requiring pathway.

E. Dephosphorylation of glucose 6-phosphate

Hydrolysis of glucose 6-phosphate by **glucose 6-phosphatase** bypasses the irreversible hexokinase reaction, and provides an energetically favorable pathway for the formation of free glucose (Figure 10.6). Liver and kidney are the only organs that release free glucose from glucose 6-phosphate.
This process actually requires two proteins:

- **glucose 6-phosphate translocase**, which transports glucose 6-phosphate across the endoplasmic reticulum (ER) membrane.
- **The ER enzyme**, **glucose 6-phosphatase** (found only in gluconeogenic cells), which removes the phosphate, producing free glucose (see Figure 10.6).

Specific transporters are responsible for releasing free glucose and phosphate back into the cytosol and, for glucose, into blood. [Note: Muscle lacks glucose 6-phosphatase, and therefore muscle glycogen cannot be used to maintain blood glucose levels.]

Advantages of Gluconeogenesis

1) Gluconeogenesis meets the requirements of glucose in the body when carbohydrates are not available in sufficient amounts.
2) Regulate Blood glucose level
3) Source of energy for Nervous tissue and Erythrocytes
4) Maintains level of intermediates of TCA cycle
5) Clear the products of metabolism of other tissues (Muscle)

IV. REGULATION OF GLUCONEOGENESIS

The moment-to-moment regulation of gluconeogenesis is determined primarily by:

1- The circulating level of glucagon.
2- The availability of gluconeogenic substrates.

A. Glucagon

This hormone from the α-cells of pancreatic islets stimulates gluconeogenesis.

B. Substrate availability

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

C. Allosteric activation by acetyl CoA

- Allosteric activation of hepatic **pyruvate carboxylase** by acetyl CoA occurs during fasting.
- As a result of increased lipolysis in adipose tissue, the liver is flooded with fatty acids. The rate of formation of acetyl CoA by β-oxidation of these fatty acids exceeds the capacity of the liver to oxidize it to CO_2 and H_2O. As a result, acetyl CoA accumulates and leads to activation of pyruvate carboxylase.

D. Allosteric inhibition by AMP

Fructose 1,6-bisphosphatase is inhibited by AMP—a compound that activates phosphofructokinase-1. This results in a reciprocal regulation of glycolysis and gluconeogenesis seen previously with fructose 2,6-bisphosphate.

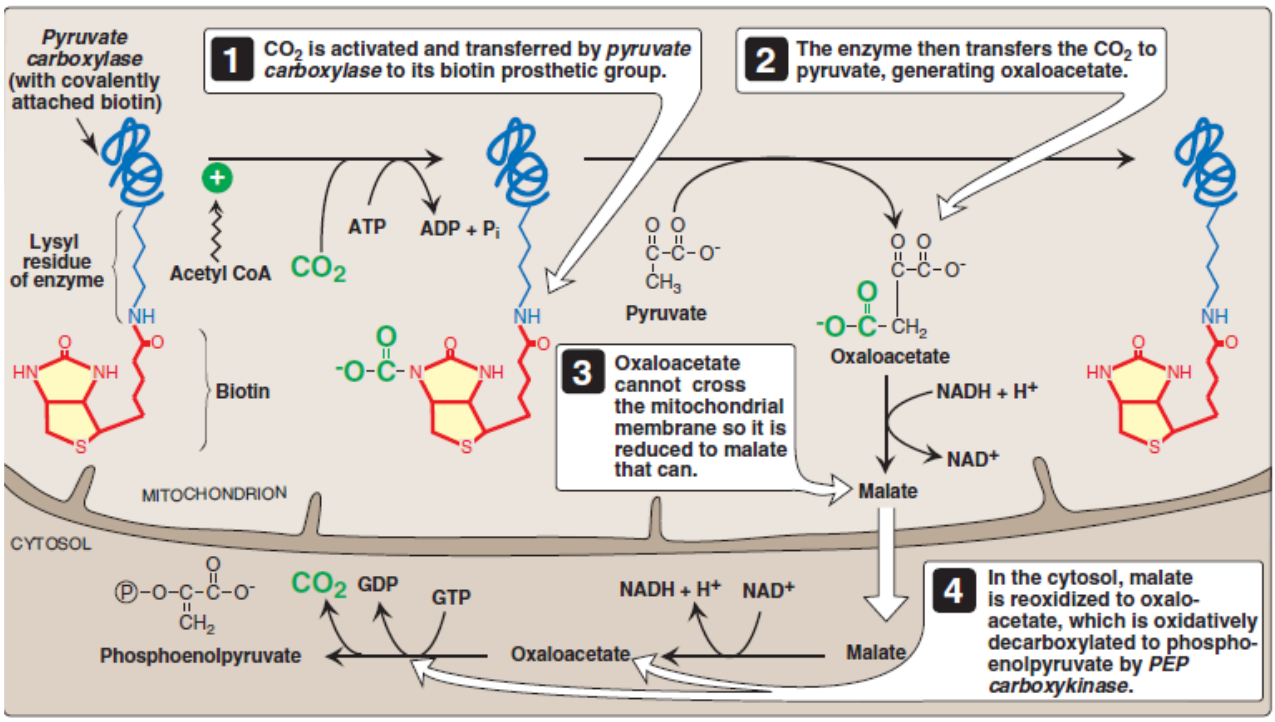


Figure 10.3
Activation and transfer of CO_2 to pyruvate, followed by transport of oxaloacetate to the cytosol and subsequent decarboxylation. Alternatively, OAA can be converted to PEP that is transported out of the mitochondria.

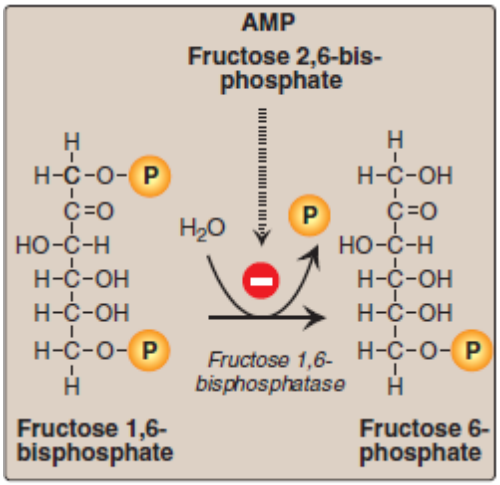


Figure 10.4
Dephosphorylation of fructose 1,6-bisphosphate.

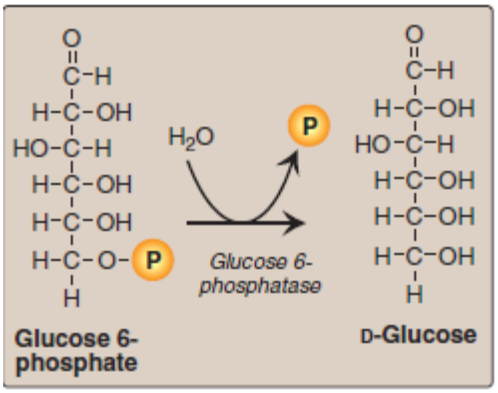
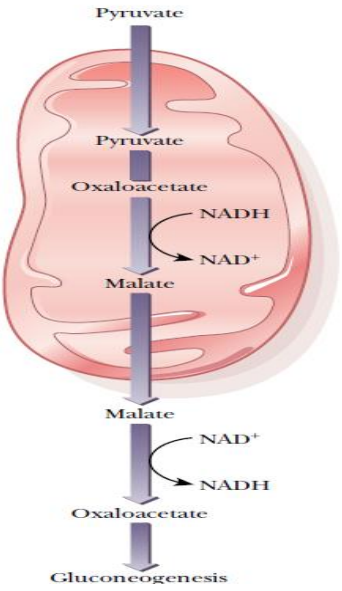


Figure 10.6
Dephosphorylation of glucose 6-phosphate allows release of free glucose from liver and kidney into blood.



Energy is required for the synthesis of glucose---------
During the gluconeogenic reactions 6 moles of high energy phosphate bonds are cleaved. Two moles of pyruvate are required for the synthesis of 1 mole of glucose.

Under fasting conditions, the energy required for gluconeogenesis is obtained from fatty acids metabolism.

GLYCOGEN METABOLISM.

Formation and degradation of glycogen

I. OVERVIEW

Glycogen----- is the storage form of glucose found in most types of cells. The liver and skeletal muscles contain the largest glycogen stores.

A constant source of blood glucose is an absolute requirement for human life. **Glucose** is the **greatly preferred energy source** for the brain, and the **required energy source** for cells with few or no mitochondria, such as mature erythrocytes. Glucose is also **essential as energy source** for exercising muscle, where it is the substrate for anaerobic glycolysis.

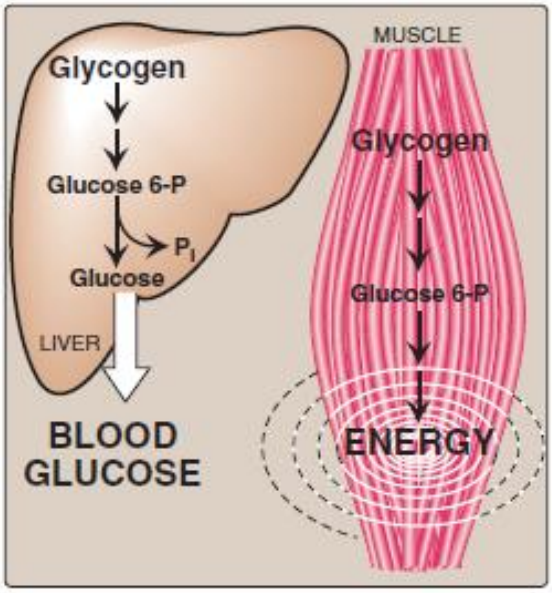


Figure 11.2
Functions of muscle and liver glycogen.

Blood glucose can be obtained from **three primary sources:**

1- The diet.
2- Degradation of glycogen.
3- Gluconeogenesis.

Dietary intake of glucose is not always a reliable source of blood glucose. In contrast, gluconeogenesis can provide sustained synthesis of glucose, but it is somewhat slow in responding to a falling blood glucose level. Therefore, the body has developed mechanisms for storing a supply of glucose in a rapidly mobilizable form, namely, **glycogen**.

In the absence of a dietary source of glucose, this sugar is rapidly released from liver and kidney glycogen. Similarly, muscle glycogen is extensively degraded in exercising muscle to provide that tissue with an important energy source. When glycogen stores are depleted, specific tissues synthesize glucose de novo, using amino acids from the body's proteins as a primary source of carbons for the gluconeogenic pathway.

II. STRUCTURE AND FUNCTION OF GLYCOGEN

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

A. Structure of glycogen

Glycogen is a branched-chain polysaccharide made exclusively from α-D-glucose. The primary glycosidic bond is an α(1-4) linkage. After an average of eight to ten glucosyl residues, there is a branch containing an α(1-6) linkage (Figure 11.3).

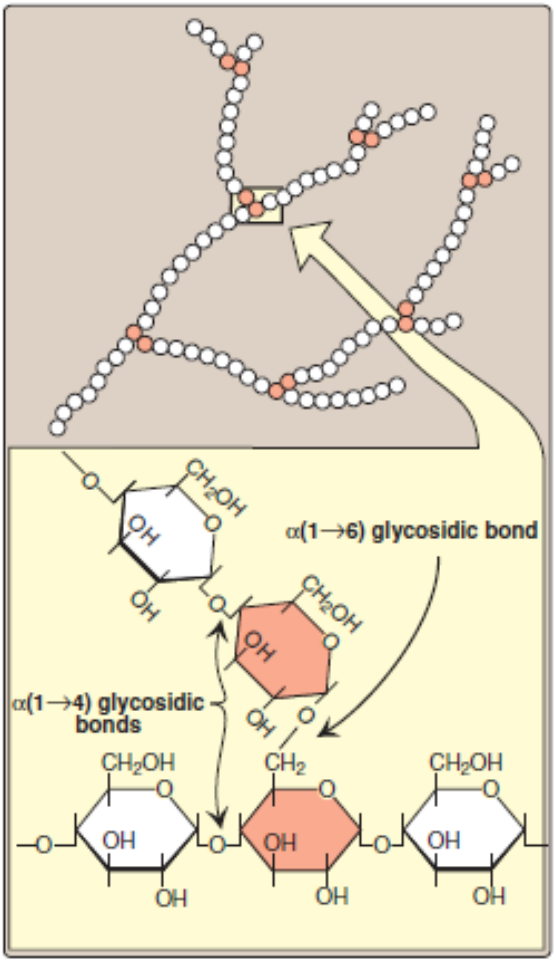


Figure 11.3
Branched structure of glycogen, showing α(1→4) and α(1→6) glycosidic bonds.

B. Fluctuation of glycogen stores
Liver glycogen stores increase during the well-fed state, and are depleted during a fast. Muscle glycogen is not affected by short periods of fasting (a few days) and is only moderately decreased in prolonged fasting (weeks). Muscle glycogen is synthesized to replenish muscle stores after they have been depleted following strenuous exercise.

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

A. Synthesis of UDP-glucose
- α-D-Glucose attached to uridine diphosphate (UDP) is the source of all the glucosyl residues that are added to the growing glycogen molecule. **UDP-glucose** (Figure 11.4)

- **UDP- glucose** is synthesized from glucose1**-phosphate and UTP by UDP-glucose pyrophosphorylase** (Figure11.5). The high-energy bond in pyrophosphate (PPi), the second product of the reaction, is hydrolyzed to two inorganic phosphates (Pi) by pyrophosphatase, which ensures that the UDP-glucose pyrophosphorylase reaction proceeds in the direction of UDP-glucose production

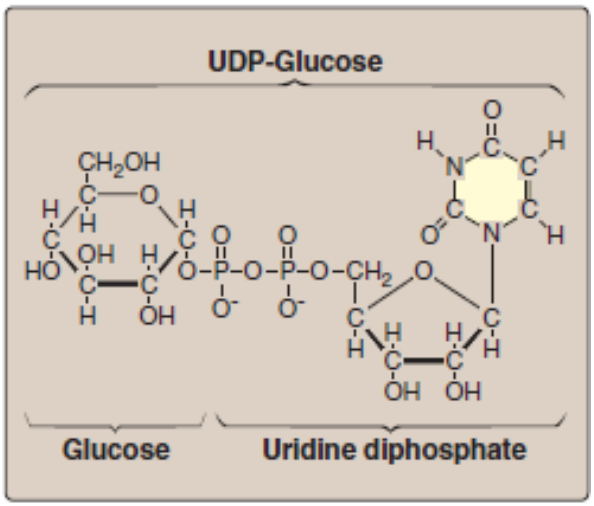


Figure 11.4
The structure of UDP-glucose, a nucleotide sugar.

B. Synthesis of a primer to initiate glycogen synthesis

Glycogen synthase is responsible for making the α(1-4) linkages in glycogen. This enzyme cannot initiate chain synthesis using free glucose as an acceptor of a molecule of glucose from UDP-glucose**. Instead, it can only elongate already existing chains of glucose.**

Therefore**, a fragment of glycogen can serve as a primer in cells** whose glycogen stores are not totally depleted. In the **absence of** a **glycogen** fragment, a protein,

called **glycogenin**, can serve as an acceptor of glucose residues from UDP-glucose (see Figure 11.5). The side chain hydroxyl group of a specific tyrosine serves as the site at which the initial glucosyl unit is attached.

The reaction is catalyzed by glycogenin itself via autoglucosylation; thus, glycogenin **is an enzyme. Glycogenin then catalyzes the transfer of the next few molecules of glucose from UDP-glucose, producing a short, α(1-4) linked glucosyl chain.** This short chain serves as a primer that is able to be elongated by glycogen synthase as described below [Note: Glycogenin stays associated with and forms the core of a glycogen granule.]

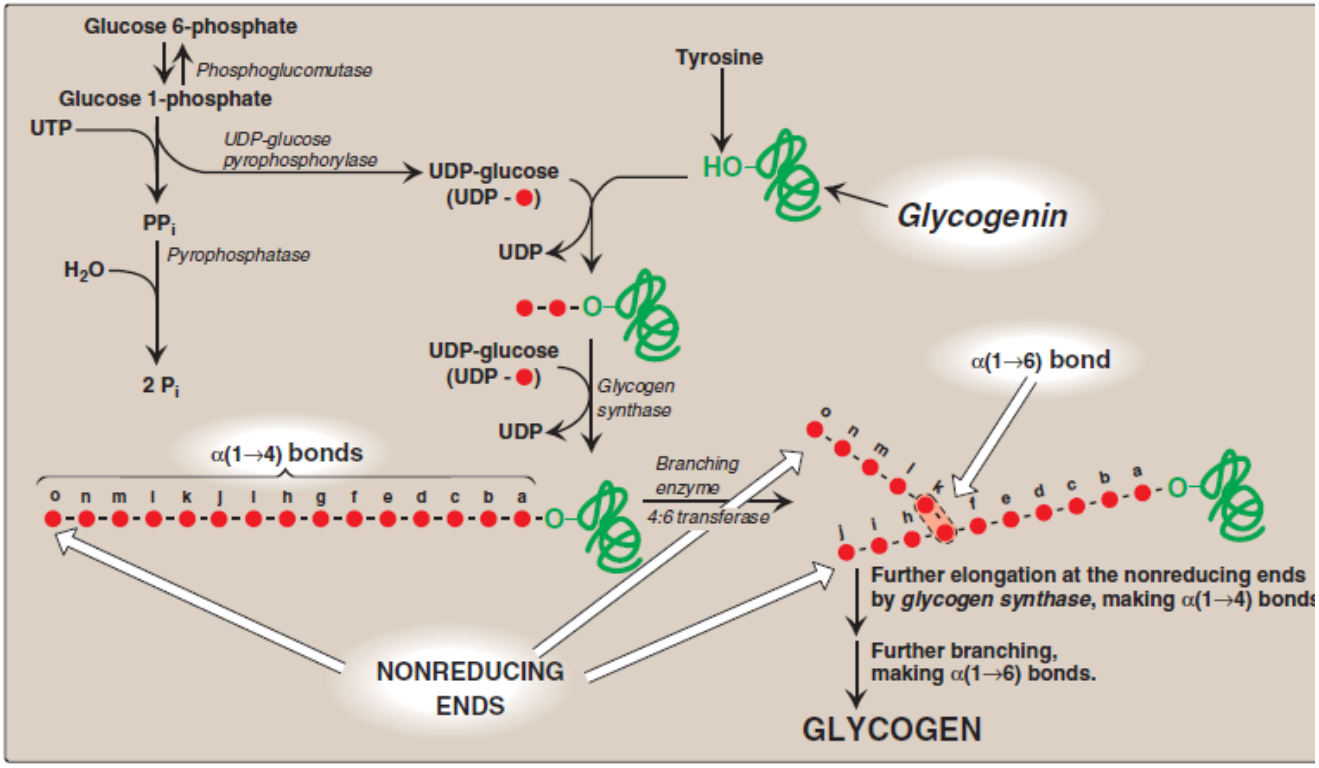


Figure 11.5
Glycogen synthesis.

C. Elongation of glycogen chains by glycogen synthase

Elongation of a glycogen chain involves the transfer of glucose from UDP-glucose to the nonreducing end of the growing chain, forming a new glycosidic bond between the anomeric hydroxyl of carbon 1 of the activated glucose and carbon 4 of the accepting glucosyl residue (see Figure 11.5). The enzyme responsible for making the α(1-4) linkages in glycogen is glycogen synthase.

D. Formation of branches in glycogen

Branching form of glycogen:

- More soluble than unbranching form.
- Branching also increases the number of nonreducing ends to which new glucosyl residues can be added or removed. Thereby greatly accelerating the rate at which glycogen synthesis can occur, and dramatically increasing the size of the molecule.

1. Synthesis of branches: Branches are made by the action of the branching enzyme, amylo-**α(1-4)- α(1-6)-transglucosidase**.

This enzyme removes a chain of **six to eight** glucosyl residues from the nonreducing end of the glycogen chain, breaking an α(1-4) bond to another residue on the chain, and attaches it to a non-terminal glucosyl residue by an α(1-6) linkage.

The resulting new, nonreducing end (see "j" in Figure 11.5), as well as the old nonreducing end from which the six to eight residues were removed (see "o" in Figure 11.5), can now be further elongated by glycogen synthase.

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

IV. DEGRADATION OF GLYCOGEN (GLYCOGENOLYSIS)

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

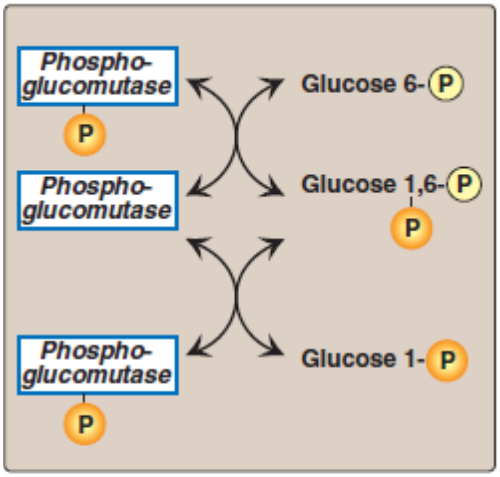


Figure 11.6
Interconversion of glucose 6-phosphate and glucose 1-phosphate by *phosphoglucomutase*.

A. Shortening of chains

Glycogen phosphorylase sequentially cleaves the α(1-4) glycosidic bonds between the glucosyl residues at the nonreducing ends of the glycogen chains by simple phosphorolysis (producing glucose 1-phosphate) **until four glucosyl units remain on each chain** before a branch point (Figure 11.7). The resulting structure is called a **limit dextrin**, and phosphorylase cannot degrade it any further (Figure 11.8).

B. Removal of branches

Branches are removed by the two enzymic activities of a **single bifunctional protein**, the **debranching enzyme** (see Figure 11.8).

First, oligo-α(1-4)□α(1-4)-glucan transferase activity **removes the outer three of the four glucosyl residues attached at a branch.** It next **transfers** them to the nonreducing end of another chain, lengthening it accordingly. Thus, an α(1-4) bond is broken and an α(1-4) bond is made, and the enzyme functions as a 4:4 transferase.

Next, the remaining single glucose residue attached in an α(1-6) linkage is removed hydrolytically by amylo-α(1-6)-glucosidase activity, releasing free glucose. The glucosyl chain is now available again for degradation by glycogen phosphorylase until four glucosyl units from the next branch are reached.

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

Glucose 1-phosphate, produced by **glycogen phosphorylase**, is converted in the cytosol to glucose 6-phosphate by phosphoglucomutase—a reaction that produces glucose 1,6-bisphosphate as a temporary but essential intermediate (see Figure 11.6).

In the liver, glucose 6-phosphate is transported into the endoplasmic reticulum (ER) by glucose 6-phosphate translocase. There it is converted to glucose by glucose 6-phosphatase—the same enzyme used in the last step of gluconeogenesis.

The glucose then moves from the ER to the cytosol. Hepatocytes release glycogen-derived glucose into the blood to help maintain blood glucose levels until the gluconeogenic pathway is actively producing glucose.

[Note: In the muscle, glucose 6-phosphate cannot be dephosphorylated because of a lack of glucose 6-phosphatase. Instead, it enters glycolysis, providing energy needed for muscle contraction.]

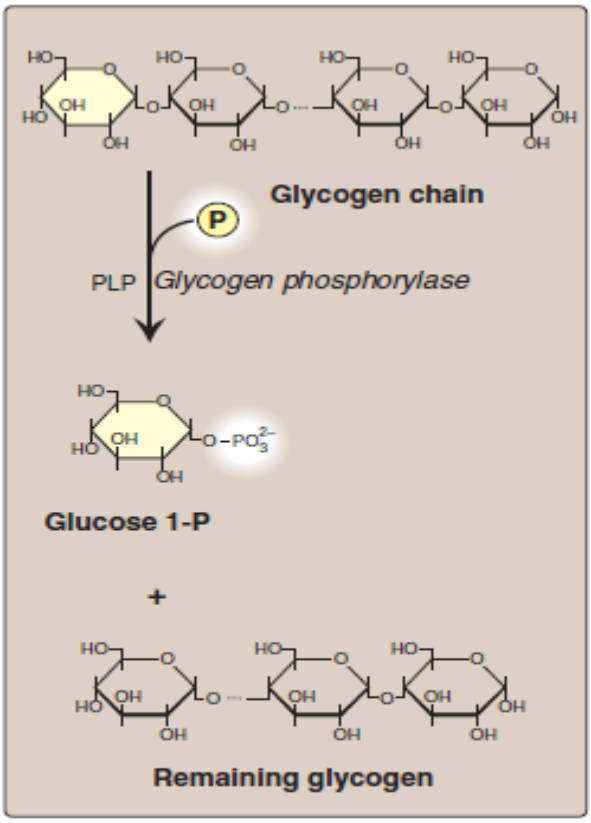


Figure 11.7
Cleavage of an $\alpha(1\rightarrow 4)$-glycosidic bond. PLP= pyridoxal phosphate.

V. REGULATION OF GLYCOGEN SYNTHESIS AND DEGRADATION

Because of the importance of maintaining blood glucose levels, the synthesis and degradation of its glycogen storage form are tightly regulated. In the liver, glycogenesis accelerates during periods when the body has been well fed, whereas glycogenolysis accelerates during periods of fasting. In skeletal muscle, glycogenolysis occurs during active exercise, and glycogenesis begins as soon as the muscle is again at rest.

Regulation of glycogen synthesis and degradation is accomplished on two levels. First, glycogen synthase and glycogen phosphorylase are hormonally regulated to meet the needs of the body as a whole. Second, the pathways of glycogen synthesis and degradation are allosterically controlled to meet the needs of a particular tissue.

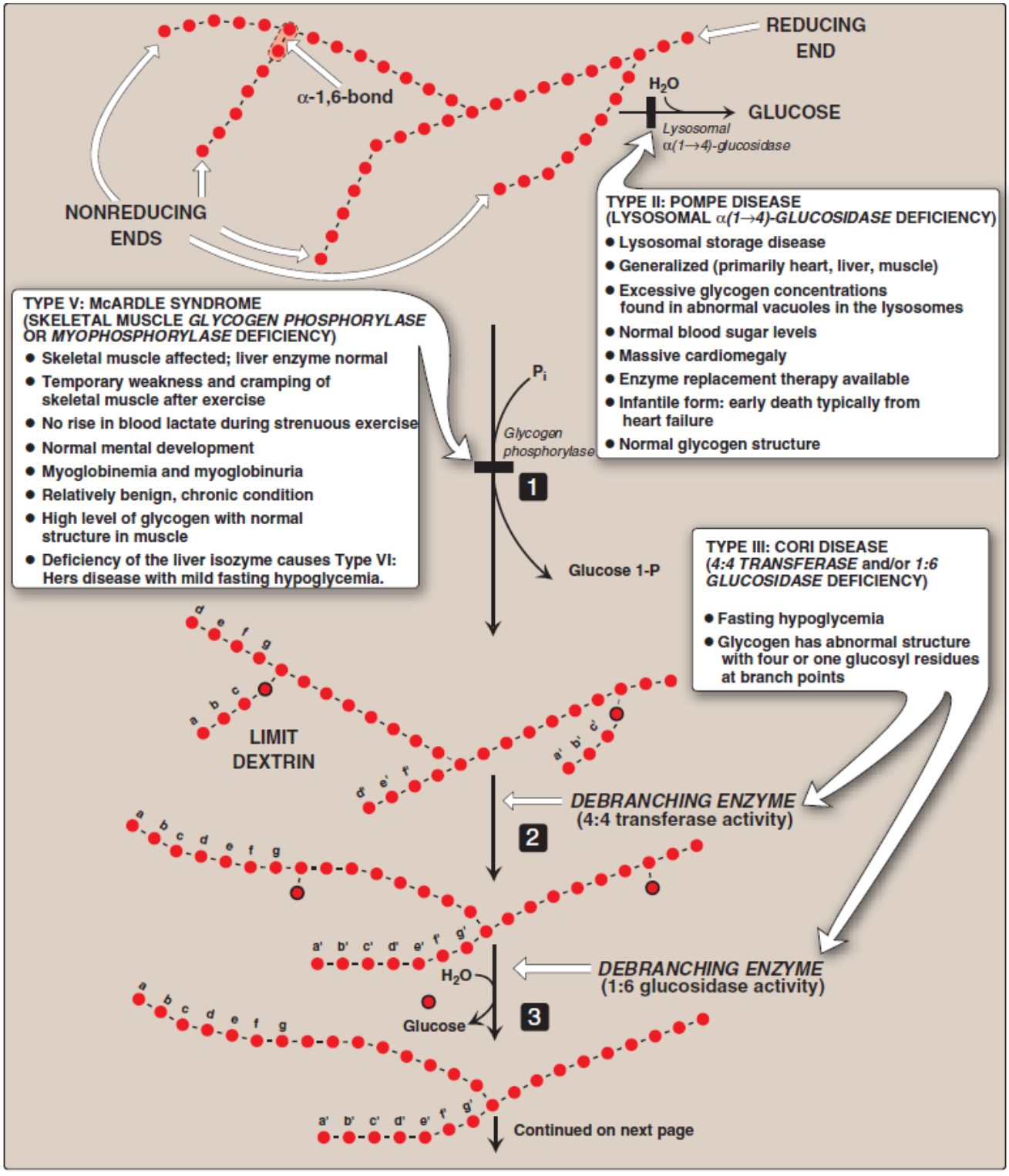
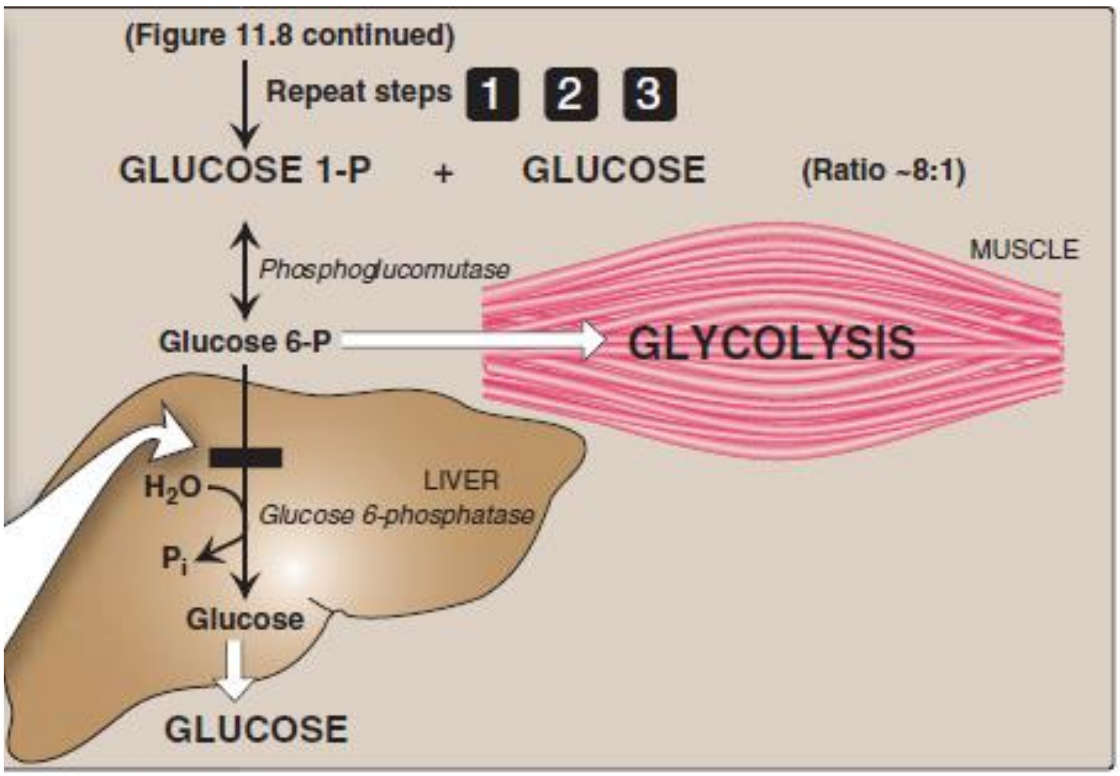
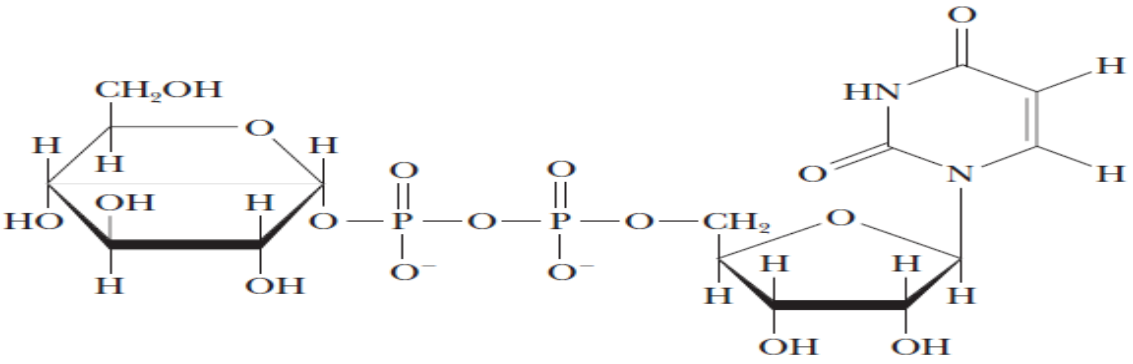


Figure 11.8
Glycogen degradation, showing some of the glycogen storage diseases (GSD). [Note: A GSD can also be caused by defects in *branching enzyme*, an enzyme of synthesis, resulting in Type IV: Andersen disease and causing death in early childhood.](*Continued on next page.*)



Synthesis of glycogen:



Uridine disphosphate glucose (UDPG)

$$\text{Glucose-1-phosphate} + \text{UTP} \rightleftharpoons \text{UDPG} + PP_i$$
$$H_2O + PP_i \rightarrow 2\ P_i$$
$$\text{Overall:}\quad \text{Glucose-1-phosphate} + \text{UTP} \rightarrow \text{UDPG} + 2\ P_i$$

Pentose Phosphate Pathway and NADPH

The pentose phosphate pathway also called:

- The hexose monophosphate pathway.
- 6-phosphogluconate pathway.

It Occurs in the cytosol of the cell. It includes two, irreversible oxidative reactions, followed by a series of reversible sugar–phosphate interconversions (Figure 13.1). No ATP is directly consumed or produced in the cycle.

Carbon 1 of glucose 6-phosphate is released as CO_2, and two NADPH are produced for each glucose 6-phosphate molecule entering the oxidative part of the pathway. The rate and direction of the reversible reactions of the pentose phosphate pathway are determined by the supply of and demand for intermediates of the cycle.

The importance of this pentose phosphate pathway PPP:

1- The pathway provides a major portion of the body's NADPH, which functions as a biochemical reductant.
2- It also produces ribose 5-phosphate, required for the biosynthesis of nucleotides.
3- provides a mechanism for the metabolic use of five-carbon sugars obtained from the diet or the degradation of structural carbohydrates in the body.

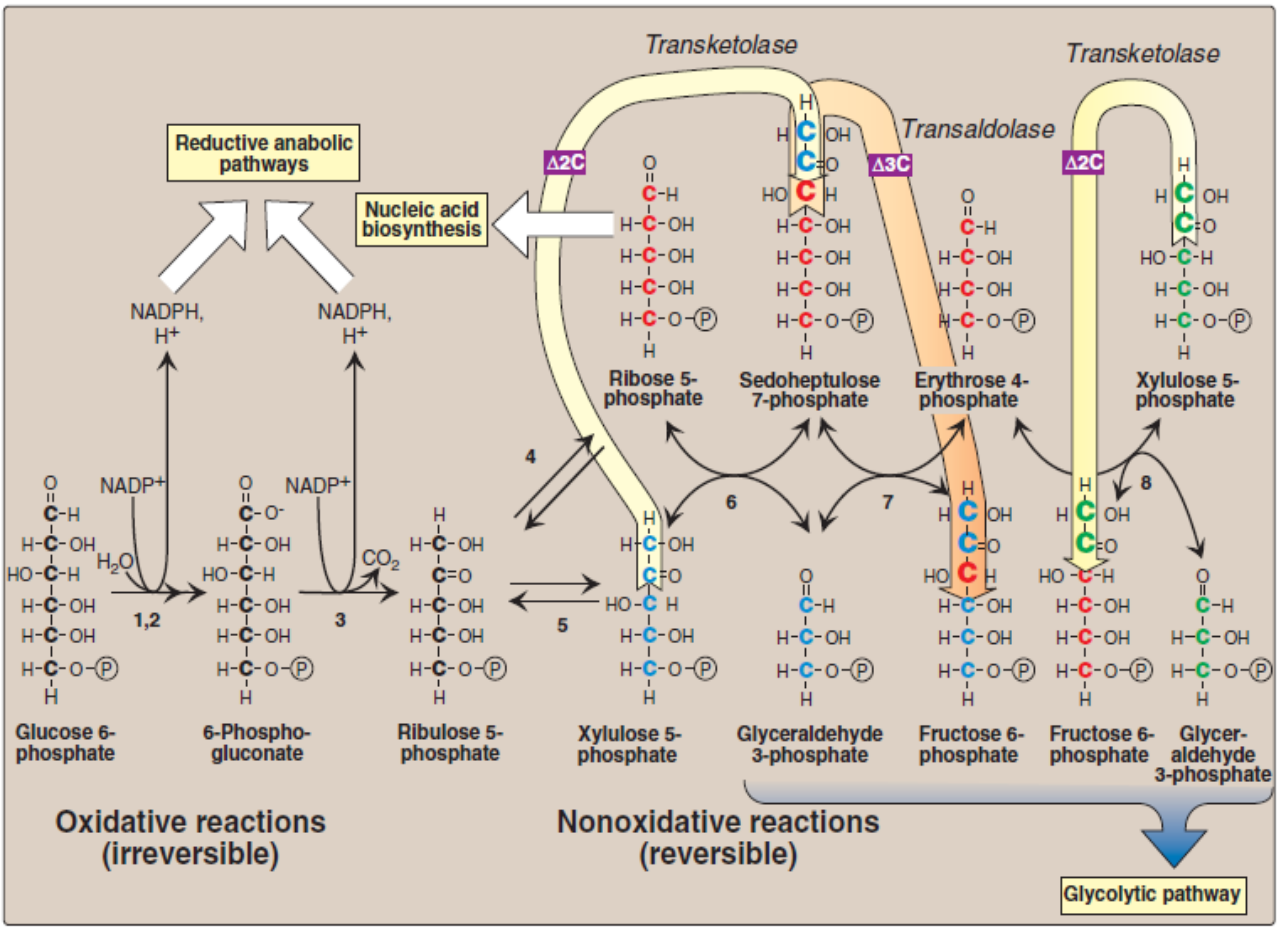


Figure 13.2
Reactions of the hexose monophosphate pathway. Enzymes numbered above are: 1,2) *glucose 6-phosphate dehydrogenase* and *6-phosphogluconolactone hydrolase*, 3) *6-phosphogluconate dehydrogenase*, 4) *ribose 5-phosphate isomerase*, 5) *phosphopentose epimerase*, 6) and 8) *transketolase* (coenzyme: thiamine pyrophosphate), and 7) *transaldolase*. Δ2C = two carbons are transferred in *transketolase* reactions; Δ3C = three carbons are transferred in the *transaldolase* reaction.

I. IRREVERSIBLE OXIDATIVE REACTIONS: The oxidative portion of the pentose phosphate pathway consists of three reactions that lead to the formation of ribulose 5-phosphate, CO_2, and two molecules of NADPH for each molecule of glucose 6-phosphate oxidized (Figure 13.2).

This portion of the pathway is particularly important in:

1- The liver, lactating mammary glands, and adipose, which are active in the NADPH-dependent biosynthesis of fatty acids.
2- The testes, ovaries, placenta and adrenal cortex, which are active in the NADPH-dependent biosynthesis of steroid hormones.
3- Erythrocytes, which require NADPH to keep glutathione reduced.

A. Dehydrogenation of glucose 6-phosphate

Glucose 6-phosphate dehydrogenase (G6PD) catalyzes an irreversible oxidation of glucose 6-phosphate to 6-phosphogluconolactone in a reaction that is specific for $NADP^+$ as its coenzyme. The pentose phosphate pathway is regulated primarily at the G6PD reaction. NADPH is a potent competitive inhibitor of the enzyme.

under most metabolic conditions, the ratio of NADPH/$NADP^+$ is sufficiently high to substantially inhibit enzyme activity. With increased demand for NADPH, the ratio of NADPH/$NADP^+$ decreases and enhanced activity of G6PD. Insulin upregulates expression of the gene for G6PD, and flux through the pathway increases in the wellfed state.

B. Formation of ribulose 5-phosphate

6-Phosphogluconolactone is hydrolyzed by 6-phosphogluconol actone hydrolase. The reaction is irreversible and not rate-limiting.

The oxidative decarboxylation of the product, 6-phosphogluconate is catalyzed by 6-phosphogluconate dehydrogenase. **This irreversible reaction produces a pentose sugar–phosphate (ribulose 5-phosphate), CO_2 (from carbon 1 of glucose), and a second molecule of NADPH (see Figure 13.2).**

III. REVERSIBLE NONOXIDATIVE REACTIONS

The nonoxidative reactions of the pentose phosphate pathway occur in all cell types synthesizing nucleotides and nucleic acids. These reactions catalyze the interconversion of sugars containing three to seven carbons (see Figure 13.2). These reversible reactions permit ribulose 5-phosphate (produced by the oxidative portion of the pathway) to be converted either to ribose 5-phosphate (needed for nucleotide synthesis) or to intermediates of glycolysis—fructose 6-phosphate and glyceraldehyde 3-phosphate.

For example, many cells that carry out reductive biosynthetic reactions have a greater need for NADPH than for ribose 5-phosphate. In this case, ribulose-5-phosphate produced as an endproduct of the oxidative reactions
Converted to glyceraldehyde 3-phosphate and fructose 6-phosphate, which are intermediates of glycolysis.

In contrast, under conditions in which the demand for ribose for incorporation into nucleotides and nucleic acids is greater than the need for NADPH, the nonoxidative reactions can provide the biosynthesis of ribose 5-phosphate from glyceraldehydes 3-phosphate and fructose 6-phosphate in the absence of the oxidative steps (Figure 13.3).

Cells have a greater need for NADPH than for ribose-5 phosphate, ribulose-5-phosphate convertd to glyceraldehydes-3-phosphate and fructose-6-phosphate. And in conditions in which the demand for ribose-5-phosphate is greater than NADPH, reactions will produce ribose-5-phosphate from glyceraldehydes-3-phosphate and fructose-6-phosphate.

Uses of NADPH:

The coenzyme $NADP^+$ differs from NAD^+ only by the presence of a phosphate group on one of the ribose units (Figure 13.4). This seemingly small change in structure allows $NADP^+$ to interact with $NADP^+$-specific enzymes that have unique roles in the cell.

This section summarizes some important $NADP^+$ or NADPH-specific functions.

A. NADPH is used for biosynthesis of several important compounds

- In the liver used for fatty acids synthesis, cholesterol synthesis, and bile acid synthesis.
- In the adrenal cortex, used for hormone synthesis.

B. Reduction of hydrogen peroxide

Hydrogen peroxide is one of a family of reactive oxygen species (ROS) that are formed from the partial reduction of molecular oxygen (Figure 13.5A).

These compounds are formed continuously as by-products of aerobic metabolism, through reactions with drugs and environmental toxins, or when the level of antioxidants is diminished, all creating the condition of oxidative stress. The highly reactive oxygen intermediates can cause serious chemical damage to DNA, proteins, and unsaturated lipids, and can lead to cell death.

These ROS have been implicated in a number of pathologic processes, including reperfusion injury, cancer, inflammatory disease, and aging. **The cell has several protective mechanisms that minimize the toxic potential of these compounds.**

1. Enzymes that catalyze antioxidant reactions:

Reduced glutathione, present in most cells, can chemically detoxify hydrogen peroxide (Figure 13.5B). This reaction, forms oxidized glutathione, which no longer has protective properties.

The cell regenerates reduced glutathione in a reaction catalyzed by glutathione reductase, using NADPH as a source of reducing equivalents.
Thus, NADPH indirectly provides electrons for the reduction of hydrogen peroxide (Figure 13.6). [Note: Erythrocytes are totally dependent on the pentose phosphate pathway for their supply of NADPH because, unlike other cell types, erythrocytes do not have an alternate source for this essential coenzyme.].

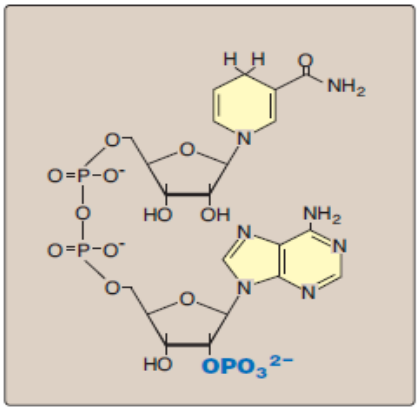


Figure 13.4
Structure of NADPH.

2. Antioxidant chemicals: A number of intracellular reducing agents, such as ascorbate, vitamin E, and beta-carotene, are able to reduce and, thus, detoxify oxygen intermediates in the laboratory. Consumption of foods rich in these antioxidant compounds has been correlated with a reduced risk for certain types of cancers, as well as decreased frequency of certain other chronic health problems.

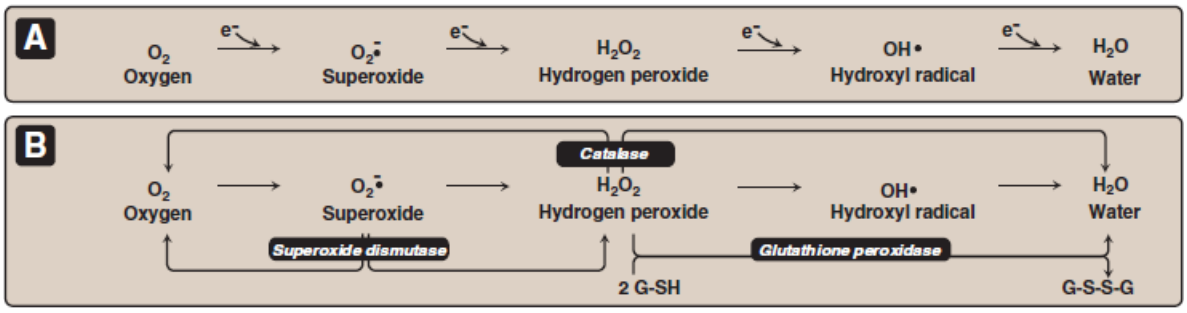


Figure 13.5
A. Formation of reactive intermediates from molecular oxygen. B. Actions of antioxidant enzymes. G-SH = reduced glutathione; G-S-S-G = oxidized glutathione.

C. Phagocytosis by white blood cells

Phagocytosis is the ingestion by receptor-mediated endocytosis of microorganisms, foreign particles, and cellular debris by cells such as neutrophils and macrophages (monocytes). It is an important body defense mechanism, particularly in bacterial infections.

D. Synthesis of nitric oxide

Nitric oxide (NO) is recognized as a mediator in a broad array of biologic systems. NO is the endothelium-derived relaxing factor, which causes vasodilation by relaxing vascular smooth muscle. NO also acts as a neurotransmitter, prevents platelet aggregation, and plays an essential role in macrophage function. NO has a very short half-life in tissues (3–10 seconds) because it reacts with oxygen and superoxide, and then is converted into nitrates and nitrites including peroxynitrite ($O=NOO^-$), a reactive nitrogen species (RNS).

V. GLUCOSE 6-P DEHYDROGENASE DEFICIENCY

Glucose 6-phosphate dehydrogenase (G6PD) deficiency is an inherited disease characterized by hemolytic anemia caused by the inability to detoxify oxidizing agents.

G6PD deficiency is the most common disease-producing enzyme abnormality in humans, affecting more than 400 million individuals worldwide. G6PD deficiency is X-linked, and is, in fact, a family of deficiencies caused by more than 400 different mutations in the gene coding for G6PD. Only some of these mutations cause clinical symptoms. [Note: In addition to hemolytic anemia, a clinical manifestation of G6PD deficiency is neonatal jaundice appearing 1–4 days after birth. The jaundice, which may be severe, typically results from increased production of unconjugated bilirubin.]

A. Role of G6PD in red blood cells

Diminished G6PD activity impairs the ability of the cell to form the NADPH that is essential for the maintenance of the reduced glutathione pool. This result in a decrease in the cellular detoxification of free radicals and peroxides formed within the cell (Figure 13.10).

Glutathione also helps maintain the reduced states of sulfhydryl groups in proteins, including hemoglobin. Oxidation of those sulfhydryl groups leads to the formation of denatured proteins that form insoluble masses (called Heinz bodies) that attach to the red cell membranes.
Additional oxidation of membrane proteins causes the red cells to be rigid (less deformable), and they are removed from the circulation by macrophages in the spleen and liver. Although G6PD deficiency occurs in all cells of the affected individual, it is most severe in erythrocytes, where the pentose phosphate pathway provides the only means of generating NADPH

B. Precipitating factors in G6PD deficiency

Most individuals who have inherited one of the many G6PD mutations do not show clinical manifestations, that is, they are asymptomatic. However, some patients with G6PD deficiency develop hemolytic anemia if they are treated with an oxidant drug, ingest fava beans, or contract a severe infection.

1. **Oxidant drugs:** Commonly used drugs that produce hemolytic anemia in patients with G6PD deficiency are best remembered from the mnemonic AAA—Antibiotics (for example, sulfamethoxazole and chloramphenicol), Antimalarials (for example, primaquine but not quinine), and Antipyretics (for example, acetanilide but not aceta minophen).

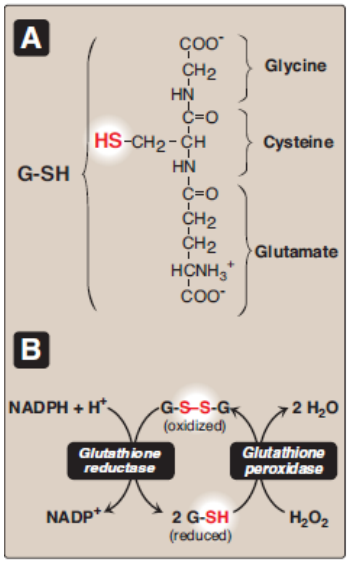


Figure 13.6
A. Structure of glutathione (G-SH). [Note: Glutamate is linked to cysteine through a γ-carboxyl, rather than an α-carboxyl.] B. Glutathione-mediated reduction of hydrogen peroxide by NADPH.

2. **Favism:** Some forms of G6PD deficiency, for example the Mediterranean variant, are particularly susceptible to the hemolytic effect of the fava (broad) bean, a dietary staple in the hemolytic effect of the fava (broad) bean, a dietary staple in the Mediterranean region. Favism, the hemolytic effect of ingesting fava beans, is not observed in all individuals with G6PD deficiency, but all patients with favism have G6PD deficiency.

3. **Infection:** Infection is the most common precipitating factor of hemolysis in G6PD deficiency. The inflammatory response to infection results in the generation of free radicals in macro phages, which can diffuse into the red blood cells and cause oxidative damage.

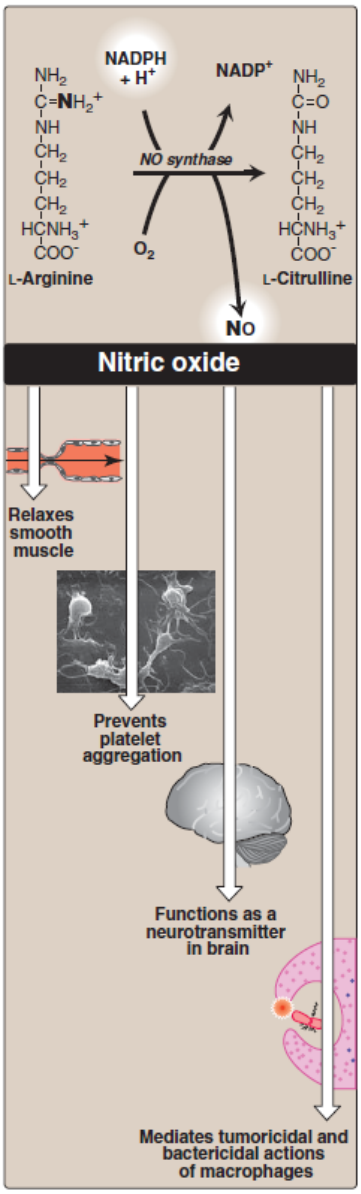


Figure 13.9
Synthesis and some of the actions of nitric oxide. [Note: FMN, FAD, heme, and tetrahydrobiopterin are additional coenzymes required by *NOS*.]

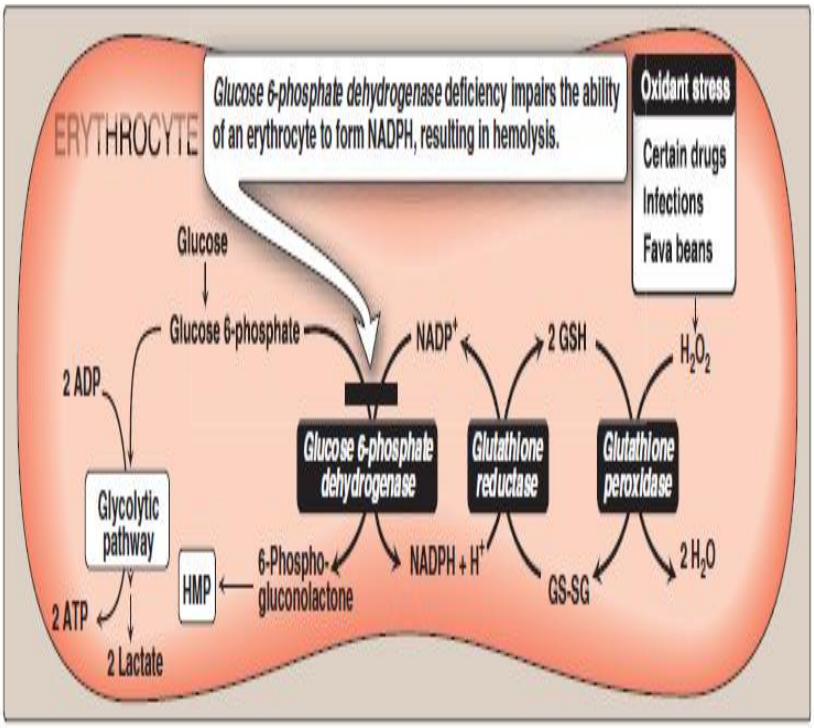


Figure 13.10
Pathways of glucose 6-phosphate metabolism in the erythrocyte. HMP = hexose monophosphate pathway.