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Downhill skiing demands a great deal of energy.

22

Aerobic Respiration and Energy Production

BIOCHEMISTRY

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1. Name the regions of the mitochondria and the function of each region.
2. Describe the reaction that results in the conversion of pyruvate to acetyl CoA, describing the location of the reaction and the components of the pyruvate dehydrogenase complex.
3. Summarize the reactions of aerobic respiration.
4. Looking at an equation representing any of the chemical reactions that occur in the citric acid cycle, describe the kind of reaction that is occurring and the significance of that reaction to the pathway.
5. Explain the mechanisms for the control of the citric acid cycle.
6. Describe the process of oxidative phosphorylation.
7. Describe the conversion of amino acids to molecules that can enter the citric acid cycle.
8. Explain the importance of the urea cycle and describe its essential steps.
9. Discuss the cause and effect of hyperammonemia.
10. Summarize the role of the citric acid cycle in catabolism and anabolism.

CHEMISTRY CONNECTION

Mitochondria from Mom

In this chapter we will be studying the amazing, intricate set of reactions that allow us to completely degrade fuel molecules such as sugars and amino acids. These oxygen-requiring reactions occur in cellular organelles called *mitochondria.*

We are used to thinking of the organelles as a collection of membrane-bound structures that are synthesized under the direction of the genetic information in the nucleus of the cell. Not so with the mitochondria. These organelles have their own genetic information and are able to make some of their own proteins. They grow and multiply in a way very similar to the simple bacteria. This, along with other information on the structure and activities of mitochondria, has led researchers to conclude that the mitochondria are actually the descendants of bacteria captured by eukaryotic cells millions of years ago.

Recent studies of the mitochondrial genetic information (DNA) have revealed fascinating new information. For instance, although each of us inherited half our genetic information from our mothers and half from our fathers, each of us inherited all of our mitochondria from our mothers. The reason for this is that when the sperm fertilizes the egg, only the sperm nucleus enters the cell.

The observation that all of our mitochondria are inherited from our mothers led Dr. A. Wilson to study the mitochondrial DNA of thousands of women around the world. He thought that by looking for similarities and differences in the mitochondrial DNA he would be able to identify a "Mitochondrial Eve"—the mother of all humanity. He didn't really think that he could identify a single woman who would have lived tens of thousands of years ago. But he hoped to determine the location of the first population of human women to help answer questions about the origin of humankind. Although the idea was a good one, the study had a number of experimental flaws. Currently, a hot debate is going on among hundreds of scientists about the Mitochondrial Eve. This controversy should encourage better experiments and analysis to help us identify our origins and to better understand the workings of the mitochondria.

Like the mitochondria themselves, some genetic diseases of energy metabolism are maternally inherited. One such disease, Leber's hereditary optic neuropathy (LHON), causes blindness and heart problems. People with LHON have a reduced ability to make ATP. As a result, sensitive tissues that demand a great deal of energy eventually die. LHON sufferers eventually lose their sight because the optic nerve dies from lack of energy.

Researchers have identified and cloned a mutant mitochondrial gene that is responsible for LHON. The defect is a mutant form of *NADH dehydrogenase.* NADH dehydrogenase is a huge, complex enzyme that accepts electrons from NADH and sends them on through an electron transport system. Passage of electrons through the electron transport system allows the synthesis of ATP. If NADH dehydrogenase is defective, passage of electrons through the electron transport system is less efficient, and less ATP is made. In LHON sufferers, the result is eventual blindness.

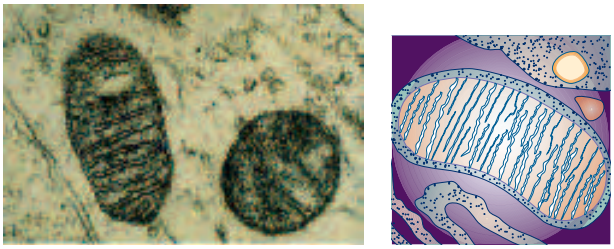
In this chapter and the next, we will study some of the important biochemical reactions that occur in the mitochondria. A better understanding of the function of healthy mitochondria will eventually allow us to help those suffering from LHON and other mitochondrial genetic diseases.

Introduction

As we have seen, the anaerobic glycolysis pathway begins the breakdown of glucose and produces a small amount of ATP and NADH. But it is aerobic catabolic pathways that complete the oxidation of glucose to CO_2 and H_2O and provide most of the ATP needed by the body. In fact, this process, called *aerobic respiration,* produces thirty-six ATP molecules using the energy harvested from each glucose molecule that enters glycolysis. These reactions occur in metabolic pathways located in *mitochondria,* the cellular "power plants." Mitochondria are a type of membrane-enclosed cell *organelle.*

An organelle is a compartment within the cytoplasm that has a specialized function.

Here, in the mitochondria, the final oxidations of carbohydrates, lipids, and proteins occur. Here, also, the electrons that are harvested in these oxidation reactions are used to make ATP. In these remarkably efficient reactions, nearly 40% of the potential energy of glucose is stored as ATP.



(a) (b)

Figure 22.1
Structure of the mitochondrion.
(a) Electron micrograph of mitochondria.
(b) Schematic drawing of the mitochondrion.

22.1 The Mitochondria

Learning Goal 1

Mitochondria are football-shaped organelles that are roughly the size of a bacterial cell. They are surrounded by an **outer mitochondrial membrane** and an **inner mitochondrial membrane** (Figure 22.1). The space between the two membranes is the **intermembrane space,** and the space inside of the inner membrane is the **matrix space.** The enzymes of the citric acid cycle, of the β-oxidation pathway for the breakdown of fatty acids, and for the degradation of amino acids are all found in the mitochondrial matrix space.

Structure and Function

The outer mitochondrial membrane has many small pores through which small molecules (less than 10,000 g/mol) can pass. Thus, the small molecules to be oxidized for the production of ATP can easily enter the mitochondrial intermembrane space.

The inner membrane is highly folded to create a large surface area. The folded membranes are known as **cristae.** The inner mitochondrial membrane is almost completely impermeable to most substances. For this reason it has many transport proteins to bring particular fuel molecules into the matrix space. Also embedded within the inner mitochondrial membrane are the protein electron carriers of the *electron transport system* and *ATP synthase.* ATP synthase is a large complex of many proteins that catalyzes the synthesis of ATP.

Origin of the Mitochondria

Not only are mitochondria roughly the size of bacteria, they have several other features that have led researchers to suspect that they may once have been free-living bacteria that were "captured" by eukaryotic cells. They have their own genetic information (DNA). They also make their own ribosomes that are very similar to those of bacteria. These ribosomes allow the mitochondria to synthesize some of their own proteins. Finally, mitochondria are actually self-replicating; they grow in size and divide to produce new mitochondria. All of these characteristics suggest that the mitochondria that produce the majority of the ATP for our cells evolved from bacteria "captured" perhaps as long as 1.5×10^9 years ago.

As we will see in Chapter 24, ribosomes are complexes of protein and RNA that serve as small platforms for protein synthesis.

What is the function of the mitochondria? **Question 22.1**

How do the mitochondria differ from the other components of eukaryotic cells? **Question 22.2**

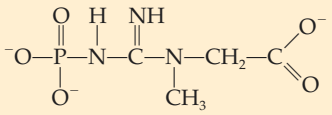
Draw a schematic diagram of a mitochondrion, and label the parts of this organelle. **Question 22.3**

A HUMAN PERSPECTIVE

Exercise and Energy Metabolism

The Olympic sprinters get set in the blocks. The gun goes off, and roughly ten seconds later the 100-m dash is over. Elsewhere, the marathoners line up. They will run 26 miles and 385 yards in a little over two hours. Both sports involve running, but they utilize very different sources of energy.

Let's look at the sprinter first. The immediate source of energy for the sprinter is stored ATP. But the quantity of stored ATP is very small, only about three ounces. This allows the sprinter to run as fast as he or she can for about three seconds. Obviously, another source of stored energy must be tapped, and that energy store is *creatine phosphate:*

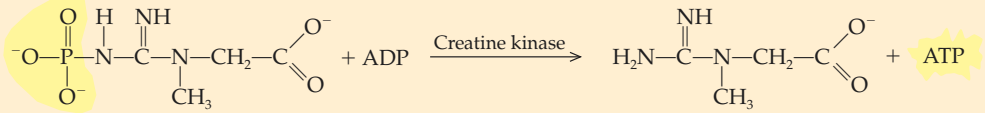


The structure of creatine phosphate.

Creatine phosphate, stored in the muscle, donates its high-energy phosphate to ADP to produce new supplies of ATP.

This will keep our runner in motion for another five or six seconds before the store of creatine phosphate is also depleted. This is almost enough energy to finish the 100-m dash, but in reality, all the runners are slowing down, owing to energy depletion, and the winner is the sprinter who is slowing down the least!

Consider a longer race, the 400-m or the 800-m. These runners run at maximum capacity for much longer. When they have depleted their ATP and creatine phosphate stores, they must synthesize more ATP. Of course, the cells have been making ATP all the time, but now the demand for energy is much greater. To supply this increased demand, the anaerobic energy-generating reactions (glycolysis and lactate fermentation, Chapter 21) and aerobic processes (citric acid cycle and oxidative phosphorylation) begin to function much more rapidly. Often, however, these athletes are running so strenuously that they cannot provide enough oxygen to the exercising muscle to allow oxidative phosphorylation to function efficiently. When this happens, the muscles must rely on glycolysis and lactate fermentation to provide *most* of the energy requirement. The chemical by-product of these anaerobic processes, lactate,



Phosphoryl group transfer from creatine phosphate to ADP is catalyzed by the enzyme creatine kinase.

Question 22.4

Describe the evidence that suggests that mitochondria evolved from free-living bacteria.

22.2 Conversion of Pyruvate to Acetyl CoA

Learning Goal 2

As we saw in Chapter 21, under *anaerobic* conditions, glucose is broken down into two pyruvate molecules that are then converted to a stable fermentation product. This limited degradation of glucose releases very little of the potential energy of glucose. Under *aerobic* conditions the cells can use oxygen and completely oxidize glucose to CO_2 in a metabolic pathway called the *citric acid cycle.*

This pathway is often referred to as the *Krebs cycle* in honor of Sir Hans Krebs who worked out the steps of this cyclic pathway from his own experimental data and that of other researchers. It is also called the *tricarboxylic acid (TCA) cycle* because several of the early intermediates in the pathway have three carboxyl groups.

builds up in the muscle and diffuses into the bloodstream. However, the concentration of lactate inevitably builds up in the working muscle and causes muscle fatigue and, eventually, muscle failure. Thus, exercise that depends primarily on anaerobic ATP production cannot continue for very long.

The marathoner presents us with a different scenario. This runner will deplete his or her stores of ATP and creatine phosphate as quickly as a short-distance runner. The anaerobic glycolytic pathway will begin to degrade glucose provided by the blood at a more rapid rate, as will the citric acid cycle and oxidative phosphorylation. The major difference in ATP production between the long-distance runner and the short- or middle-distance runner is that the muscles of the long-distance runner derive almost all the energy through aerobic pathways. These individuals continue to run long distances at a pace that allows them to supply virtually all the oxygen needed by the exercising muscle. In fact, only aerobic pathways can provide a constant supply of ATP for exercise that goes on for hours. Theoretically, under such conditions our runner could run indefinitely, utilizing first his or her stored glycogen and eventually stored lipids. Of course, in reality, other factors such as dehydration and fatigue place limits on the athlete's ability to continue.

We have seen, then, that long-distance runners must have a great capacity to produce ATP aerobically, in the mitochondria, whereas short- and middle-distance runners need a great capacity to produce energy anaerobically, in the cytoplasm of the muscle cells. It is interesting to note that the muscles of these runners reflect these diverse needs.

When one examines muscle tissue that has been surgically removed, one finds two predominant types of muscle fibers. *Fast twitch muscle fibers* are large, relatively plump, pale cells. They have only a few mitochondria but contain a large reserve of glycogen and high concentrations of the enzymes that are needed for glycolysis and lactate fermentation. These muscle fibers fatigue rather quickly because fermentation is inefficient, quickly depleting the cell's glycogen store and causing the accumulation of lactate.

Slow twitch muscle fiber cells are about half the diameter of fast twitch muscle cells and are red. The red color is a result of the high concentrations of myoglobin in these cells. Recall that myoglobin stores oxygen for the cell (Section 19.9) and facilitates rapid diffusion of oxygen throughout the cell. In addition, slow twitch muscle fiber cells are packed with mitochondria. With this abundance of oxygen and mitochondria these cells have the capacity for extended ATP production via aerobic pathways—ideal for endurance sports like marathon racing.

It is not surprising, then, that researchers have found that the muscles of sprinters have many more fast twitch muscle fibers and those of endurance athletes have many more slow twitch muscle fibers. One question that many researchers are trying to answer is whether the type of muscle fibers an individual has is a function of genetic makeup or training. Is a marathon runner born to be a long-distance runner, or are his or her abilities due to the type of training the runner undergoes? There is no doubt that the training regimen for an endurance runner does indeed increase the number of slow twitch muscle fibers and that of a sprinter increases the number of fast twitch muscle fibers. But there is intriguing new evidence to suggest that the muscles of endurance athletes have a greater proportion of slow twitch muscle fibers before they ever begin training. It appears that some of us truly were born to run.

Once pyruvate enters the mitochondria, it must be converted to a two-carbon acetyl group. This acetyl group must be "activated" to enter the reactions of the citric acid cycle. Activation occurs when the acetyl group is bonded to the thiol group of coenzyme A. **Coenzyme A** is a large thiol derived from ATP and the vitamin pantothenic acid (Figure 22.2). It is an acceptor of acetyl groups (in red in Figure 22.2), which are bonded to it through a high-energy thioester bond. The acetyl coenzyme A (**acetyl CoA**) formed is the "activated" form of the acetyl group.

Coenzyme A is described in Sections 13.9 and 15.4.
Thioester bonds are discussed in Section 15.4.

The structure and function of pantothenic acid are discussed in Appendix F, Water-Soluble Vitamins.

Figure 22.3 shows us the reaction that converts pyruvate to acetyl CoA. First, pyruvate is decarboxylated, which means that it loses a carboxyl group that is released as CO_2. Next it is oxidized, and the hydride anion that is removed is accepted by NAD^+. Finally, the remaining acetyl group, CH_3CO—, is linked to coenzyme A by a thioester bond. This very complex reaction is carried out by three enzymes and five coenzymes that are organized together in a single bundle called the **pyruvate dehydrogenase complex** (see Figure 22.3). This organization allows the substrate to be passed from one enzyme to the next as each chemical reaction occurs. A schematic representation of this "disassembly line" is shown in Figure 22.3b.

This single reaction requires four coenzymes made from four different vitamins, in addition to the coenzyme lipoamide. These are thiamine pyrophosphate, derived from thiamine (Vitamin B_1); FAD, derived from riboflavin (Vitamin B_2);

These vitamins are discussed in Appendix F, Water-Soluble Vitamins.

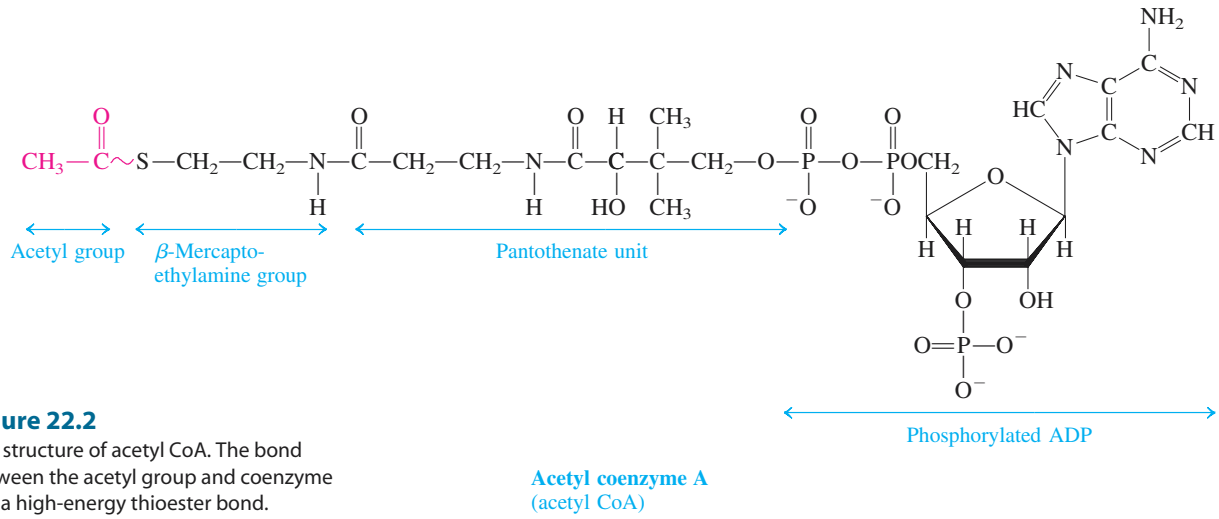
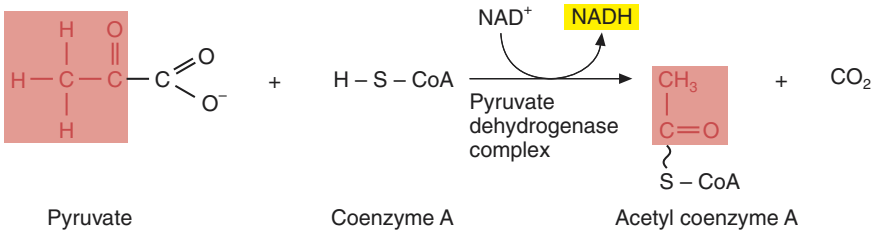
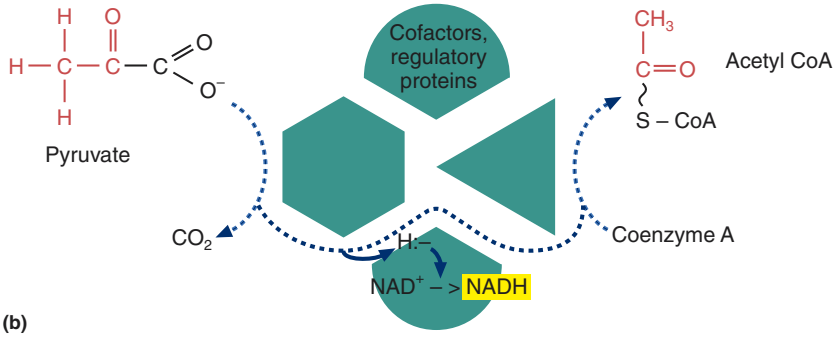


Figure 22.2
The structure of acetyl CoA. The bond between the acetyl group and coenzyme A is a high-energy thioester bond.



(a)



(b)

Figure 22.3
The decarboxylation and oxidation of pyruvate to produce acetyl CoA. (a) The overall reaction in which CO_2 and an $H:^-$ are removed from pyruvate and the remaining acetyl group is attached to coenzyme A. This requires the concerted action of three enzymes and five coenzymes. (b) The pyruvate dehydrogenase complex that carries out this reaction is actually a cluster of enzymes and coenzymes. The substrate is passed from one enzyme to the next as the reaction occurs.

NAD^+, derived from niacin; and coenzyme A, derived from pantothenic acid. Obviously, a deficiency in any of these vitamins would seriously reduce the amount of acetyl CoA that our cells could produce. This, in turn, would limit the amount of ATP that the body could make and would contribute to vitamin-deficiency diseases. Fortunately, a well-balanced diet provides an adequate supply of these and other vitamins.

In Figure 22.4 we see that acetyl CoA is a central character in cellular metabolism. It is produced by the degradation of glucose, fatty acids, and some amino

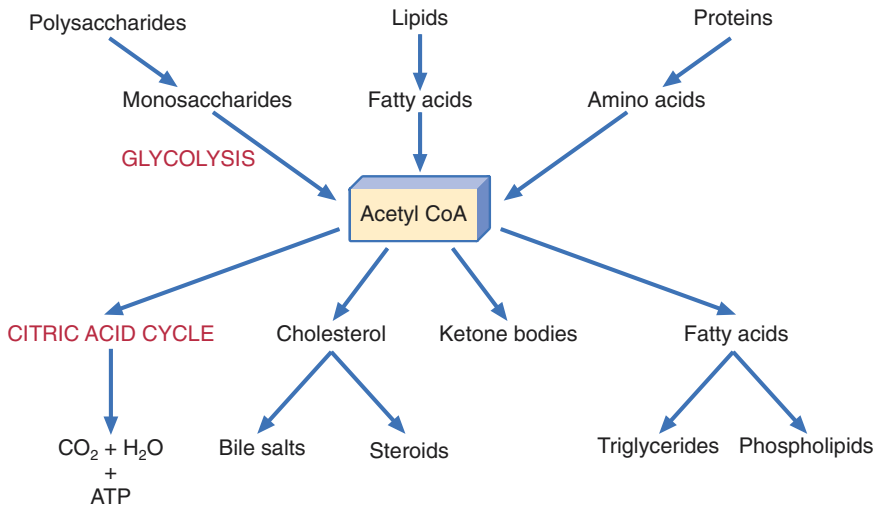


Figure 22.4
The central role of acetyl CoA in cellular metabolism.

acids. The major function of acetyl CoA in energy-harvesting pathways is to carry the acetyl group to the citric acid cycle, in which it will be used to produce large amounts of ATP. In addition to these catabolic duties, the acetyl group of acetyl CoA can also be used for *anabolic* or biosynthetic reactions to produce cholesterol and fatty acids. It is through this intermediate, acetyl CoA, that all the energy sources (fats, proteins, and carbohydrates) are interconvertible.

What vitamins are required for acetyl CoA production from pyruvate? **Question 22.5**

What is the major role of coenzyme A in catabolic reactions? **Question 22.6**

22.3 An Overview of Aerobic Respiration

Learning Goal 3

Aerobic respiration is the oxygen-requiring breakdown of food molecules and production of ATP. The different steps of aerobic respiration occur in different compartments of the mitochondria.

The enzymes for the citric acid cycle are found in the mitochondrial matrix space. The first enzyme catalyzes a reaction that joins the acetyl group of acetyl CoA (two carbons) to a four-carbon molecule (oxaloacetate) to produce citrate (six carbons). The remaining enzymes catalyze a series of rearrangements, decarboxylations (removal of CO_2), and oxidation–reduction reactions. The eventual products of this cyclic pathway are two CO_2 molecules and oxaloacetate—the molecule we began with.

At several steps in the citric acid cycle a substrate is oxidized. In three of these steps a pair of electrons is transferred from the substrate to NAD^+, producing NADH (three NADH molecules per turn of the cycle). At another step a pair of electrons is transferred from a substrate to FAD, producing $FADH_2$ (one $FADH_2$ molecule per turn of the cycle).

Remember (Section 20.7) that it is really the hydride anion with its pair of electrons ($H:^-$) that is transferred to NAD^+ to produce NADH. Similarly, a pair of hydrogen atoms (and thus two electrons) are transferred to FAD to produce $FADH_2$.

The electrons are passed from NADH or $FADH_2$, through an electron transport system located in the inner mitochondrial membrane, and finally to the terminal electron acceptor, molecular oxygen (O_2). The transfer of electrons through the electron transport system causes protons (H^+) to be pumped from the mitochondrial matrix into the intermembrane compartment. The result is a high-energy H^+ reservoir.

In the final step the energy of the H^+ reservoir is used to make ATP. This last step is carried out by the enzyme complex ATP synthase. As protons flow back into the mitochondrial matrix through a pore in the ATP synthase complex, the enzyme catalyzes the synthesis of ATP.

This long, involved process is called *oxidative phosphorylation*, because the energy of electrons from the *oxidation* of substrates in the citric acid cycle is used to *phosphorylate* ADP and produce ATP. The details of each of these steps will be examined in upcoming sections.

Question 22.7 What is meant by the term *oxidative phosphorylation?*

Question 22.8 What does the term *aerobic respiration* mean?

22.4 The Citric Acid Cycle (The Krebs Cycle)

Reactions of the Citric Acid Cycle

Learning Goal 4

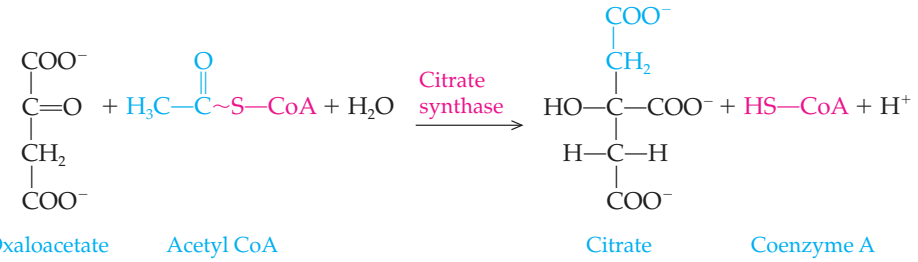
The **citric acid cycle** is sometimes called the *Krebs cycle*, in honor of its discoverer, Sir Hans Krebs. It is the final stage of the breakdown of carbohydrates, fats, and amino acids released from dietary proteins (Figure 22.5).

The formation of acetyl CoA was described in Section 22.2.

To understand this important cycle, let's follow the fate of the acetyl group of an acetyl CoA as it passes through the citric acid cycle. The numbered steps listed below correspond to the steps in the citric acid cycle that are summarized in Figure 22.5.

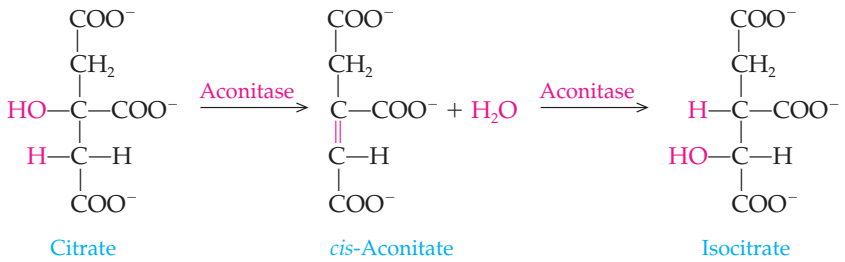
Aldol condensation reactions are reactions between aldehydes and ketones to form larger molecules (Section 14.4).

Reaction 1. This is a condensation reaction between the acetyl group of acetyl CoA and oxaloacetate. Actually, this is another biological example of an aldol condensation reaction. It is catalyzed by the enzyme *citrate synthase*. The product that is formed is citrate:



Notice that the conversion of citrate to cis-aconitate is a biological example of the dehydration of an alcohol to produce an alkene (Section 13.5). The conversion of cis-aconitate to isocitrate is a biochemical example of the hydration of an alkene to produce an alcohol (Sections 12.5 and 13.5).

Reaction 2. The enzyme *aconitase* catalyzes the dehydration of citrate, producing *cis*-aconitate. The same enzyme, aconitase, then catalyzes addition of a water molecule to the *cis*-aconitate, converting it to isocitrate. The net effect of these two steps is the isomerization of citrate to isocitrate:



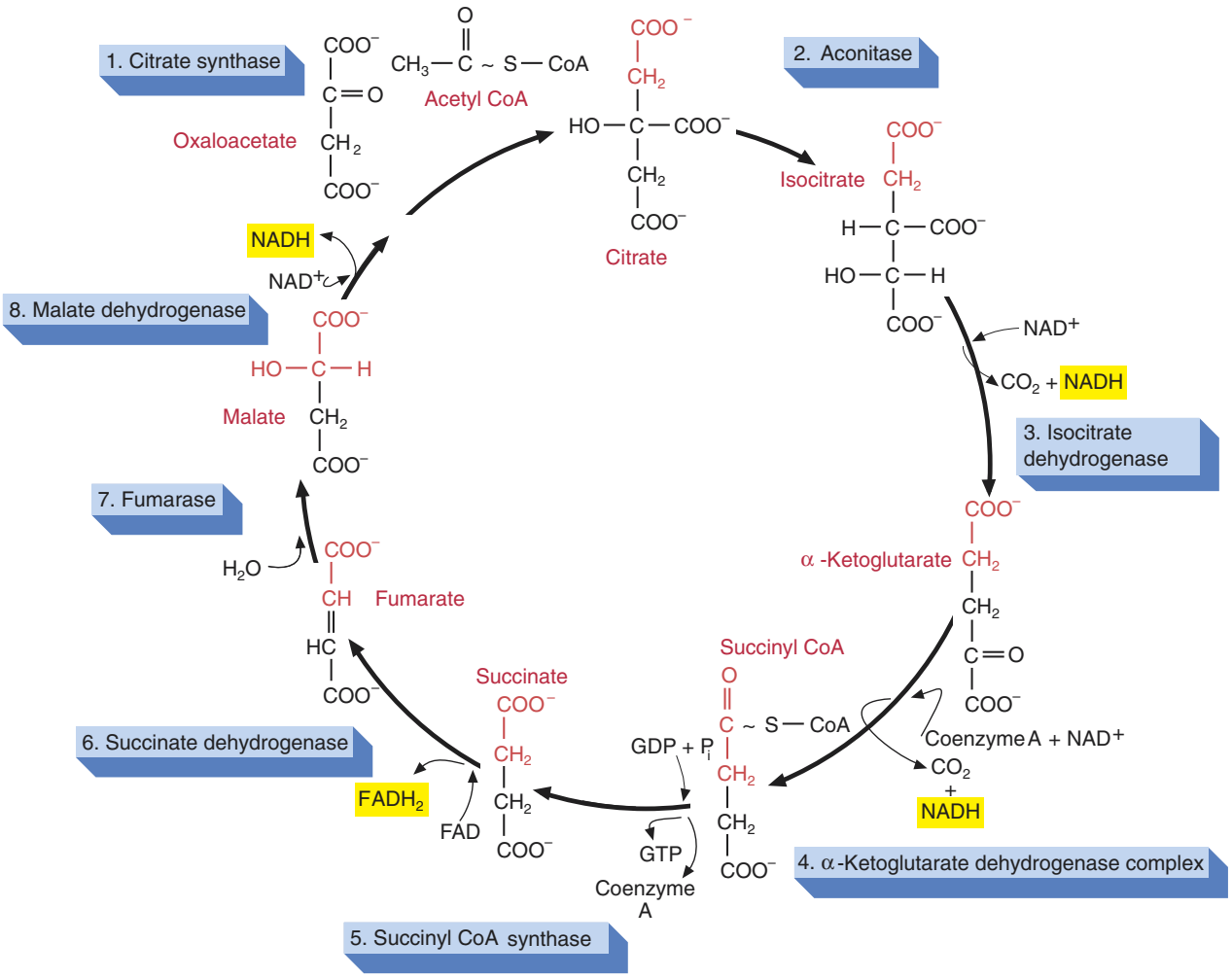
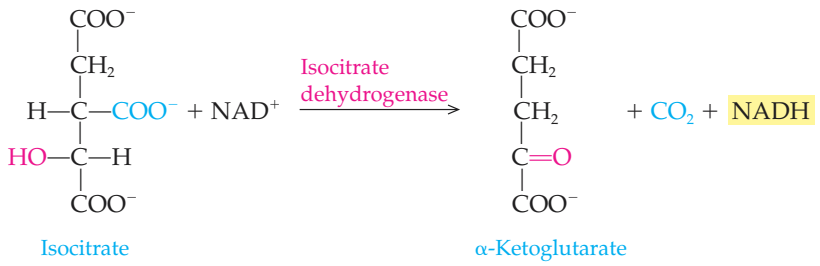


Figure 22.5
The reactions of the citric acid cycle.

Reaction 3. The first oxidative step of the citric acid cycle is catalyzed by *isocitrate dehydrogenase*. It is a complex reaction in which three things happen:

a. the hydroxyl group of isocitrate is oxidized to a ketone,
b. carbon dioxide is released, and
c. NAD^+ is reduced to NADH.

The product of this oxidative decarboxylation reaction is α-ketoglutarate:



The oxidation of a secondary alcohol produces a ketone (Sections 13.5 and 14.4).

The structure of NAD^+ and its reduction to NADH are shown in Figure 20.8.

Remember, in organic (and thus biochemical) reactions, oxidation can be recognized as a gain of oxygen or loss of hydrogen (Section 13.6).

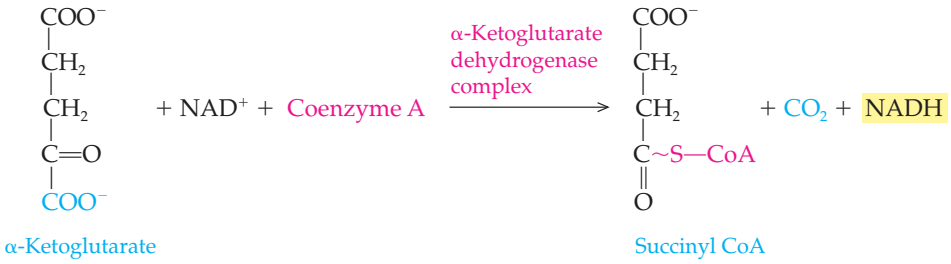
The pyruvate dehydrogenase complex was described in Section 22.2 and shown in Figure 22.3.

Reaction 4. Coenzyme A enters the picture again in this step of the citric acid cycle. The α-*ketoglutarate dehydrogenase* complex carries out this series of reactions. This complex is very similar to the pyruvate dehydrogenase complex and requires the same coenzymes. Once again, three chemical events occur:

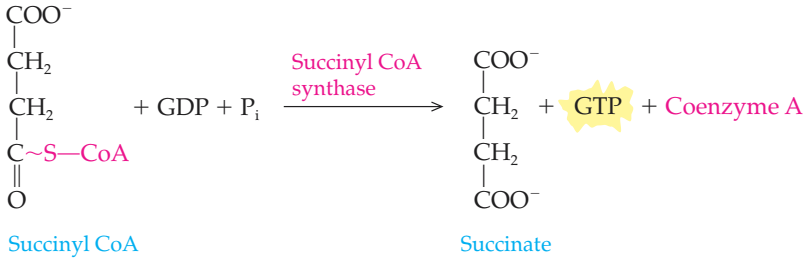
a. α-ketoglutarate loses a carboxylate group as CO_2,

b. it is oxidized and NAD^+ is reduced to NADH, and

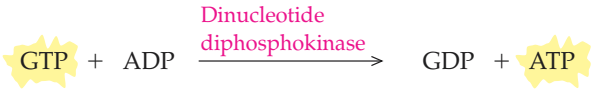
c. coenzyme A combines with the product, succinate, to form succinyl CoA. The bond thus formed between succinate and coenzyme A is a high-energy thioester linkage.



Reaction 5. Succinyl CoA is converted to succinate in this step, which once more is chemically very involved. The enzyme *succinyl CoA synthase* catalyzes a coupled reaction in which the high-energy thioester bond of succinyl CoA is hydrolyzed and an inorganic phosphate group is added to GDP to make GTP:



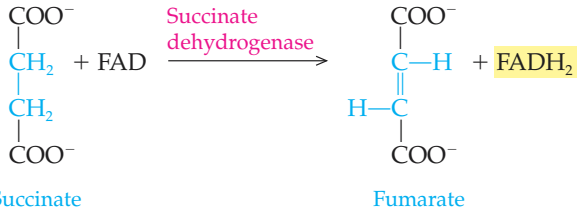
Another enzyme, *dinucleotide diphosphokinase*, then catalyzes the transfer of a phosphoryl group from GTP to ADP to make ATP:



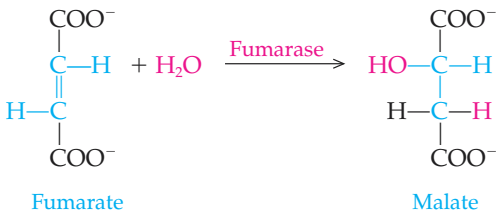
The structure of FAD was shown in Figure 20.8.

Reaction 6. *Succinate dehydrogenase* then catalyzes the oxidation of succinate to fumarate in the next step. The oxidizing agent, *flavin adenine dinucleotide (FAD)*, is reduced in this step:

We studied hydrogenation of alkenes to produce alkanes in Section 12.5. This is simply the reverse.

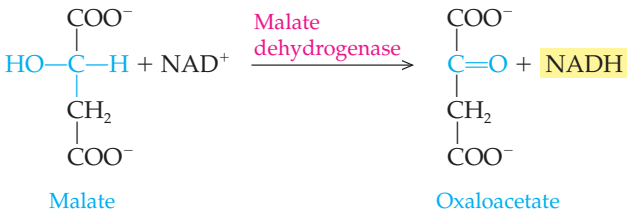


Reaction 7. Addition of H_2O to the double bond of fumarate gives malate. The enzyme *fumarase* catalyzes this reaction:



This reaction is a biological example of the hydration of an alkene to produce an alcohol (Sections 12.5 and 13.5).

Reaction 8. In the final step of the citric acid cycle, *malate dehydrogenase* catalyzes the reduction of NAD^+ to NADH and the oxidation of malate to oxaloacetate. Because the citric acid cycle "began" with the addition of an acetyl group to oxaloacetate, we have come full circle.



This reaction is a biochemical example of the oxidation of a secondary alcohol to a ketone, which we studied in Sections 13.5 and 15.4.

22.5 Control of the Citric Acid Cycle

Learning Goal 5

Just like glycolysis, the citric acid cycle is responsive to the energy needs of the cell. The pathway speeds up when there is a greater demand for ATP, and it slows down when ATP energy is in excess. In the last chapter we saw that several of the enzymes that catalyze the reactions of glycolysis are *allosteric enzymes.* Similarly, four enzymes or enzyme complexes involved in the complete oxidation of pyruvate via the citric acid cycle also are allosteric enzymes. They are able to bind to *effectors,* such as ATP or ADP, that alter the shape of the enzyme active site, either stimulating the rate of the reaction (positive allosterism) or inhibiting the reaction (negative allosterism).

Because the control of the pathway must be precise, there are several enzymatic steps that are regulated. These are summarized in Figure 22.6 and below:

1. *Conversion of pyruvate to acetyl CoA.* The pyruvate dehydrogenase complex is inhibited by high concentrations of ATP, acetyl CoA, and NADH. Of course, the presence of these compounds in abundance signals that the cell has an adequate supply of energy, and thus energy metabolism is slowed.
2. *Synthesis of citrate from oxaloacetate and acetyl CoA.* The enzyme citrate synthase is an allosteric enzyme. In this case the negative effector is ATP. Again, this is logical because an excess of ATP indicates that the cell has an abundance of energy.
3. *Oxidation and decarboxylation of isocitrate to α-ketoglutarate.* Isocitrate dehydrogenase is also an allosteric enzyme; however, the enzyme is controlled by the positive allosteric effector, ADP. ADP is a signal that the levels of ATP must be low, and therefore the rate of the citric acid cycle should be increased. Interestingly, isocitrate dehydrogenase is also *inhibited* by high levels of NADH and ATP.
4. *Conversion of α-ketoglutarate to succinyl CoA.* The α-ketoglutarate dehydrogenase complex is inhibited by high levels of the products of the reactions that it catalyzes, namely, NADH and succinyl CoA. It is further inhibited by high concentrations of ATP.

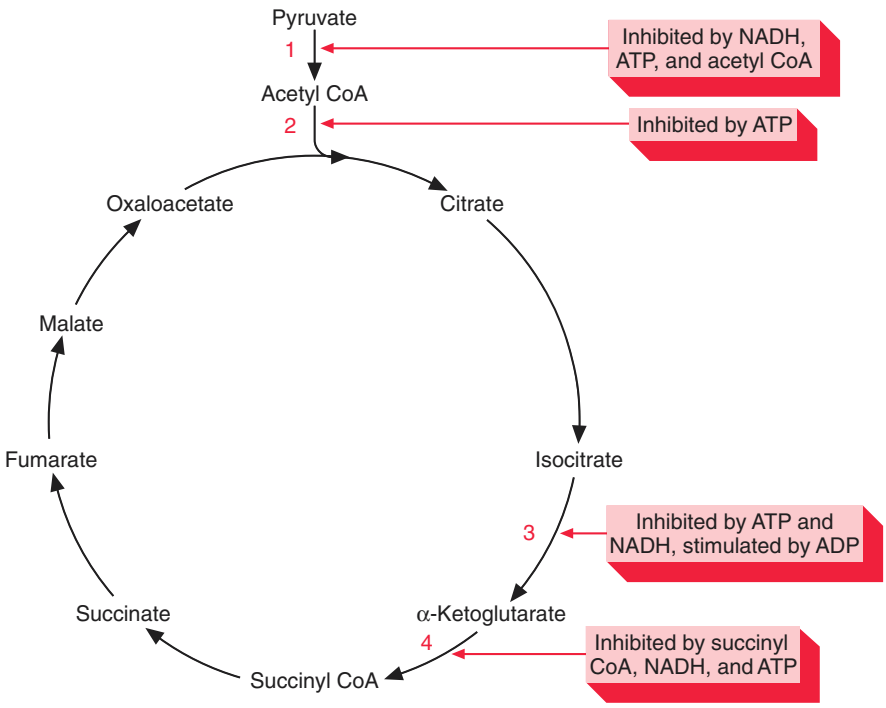


Figure 22.6
Regulation of the pyruvate dehydrogenase complex and the citric acid cycle.

22.6 Oxidative Phosphorylation

Learning Goal 6

In Section 22.3 we noted that the electrons carried by NADH can be used to produce three ATP molecules, and those carried by $FADH_2$ can be used to produce two ATP molecules. We turn now to the process by which the energy of electrons carried by these coenzymes is converted to ATP energy. It is a series of reactions called **oxidative phosphorylation,** which couples the oxidation of NADH and $FADH_2$ to the phosphorylation of ADP to generate ATP.

Electron Transport Systems and the Hydrogen Ion Gradient

Before we try to understand the mechanism of oxidative phosphorylation, let's first look at the molecules that carry out this complex process. Embedded within the mitochondrial inner membrane are **electron transport systems.** These are made up of a series of electron carriers, including coenzymes and cytochromes. All these molecules are located within the membrane in an arrangement that allows them to pass electrons from one to the next. This array of electron carriers is called the *respiratory electron transport system* (Figure 22.7). As you would expect in such sequential oxidation-reduction reactions, the electrons lose some energy with each transfer. Some of this energy is used to make ATP.

At three sites in the electron transport system, protons (H^+) can be pumped from the mitochondrial matrix to the intermembrane space. These H^+ contribute to a high-energy H^+ reservoir. At each of the three sites, enough H^+ are pumped into the H^+ reservoir to produce one ATP molecule. The first site is NADH dehydrogenase. Because electrons from NADH enter the electron transport system by being transferred to NADH dehydrogenase, all three sites actively pump H^+, and three ATP molecules are made (see Figure 22.7). $FADH_2$ is a less "powerful" electron donor. It transfers its electrons to an electron carrier that follows NADH dehydrogenase. As a result, when $FADH_2$ is oxidized, only the second and third sites pump H^+, and only two ATP molecules are made.

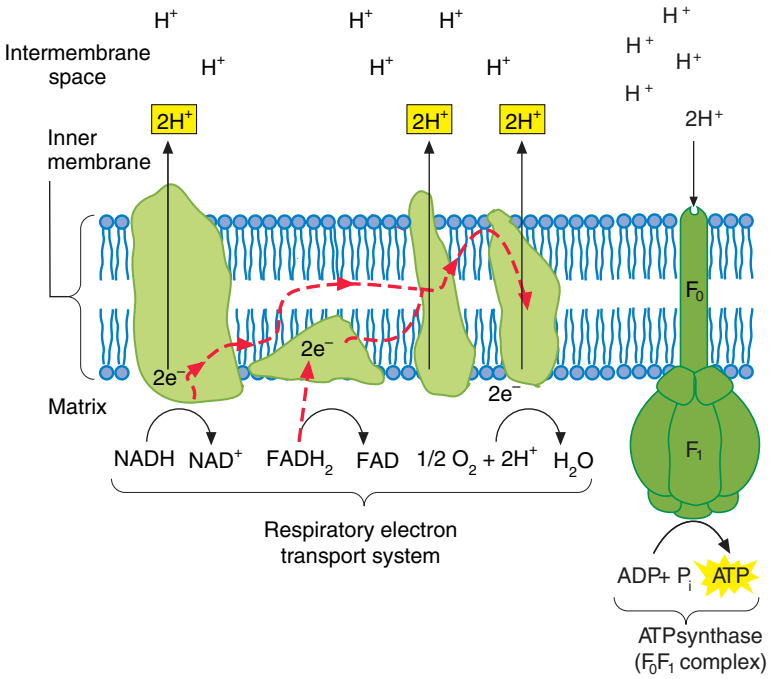


Figure 22.7
Electrons flow from NADH to molecular oxygen through a series of electron carriers embedded in the inner mitochondrial membrane. Protons are pumped from the mitochondrial matrix space into the intermembrane space. This results in a hydrogen ion reservoir in the intermembrane space. As protons pass through the channel in ATP synthase, their energy is used to phosphorylate ADP and produce ATP.

The last component needed for oxidative phosphorylation is a multiprotein complex called **ATP synthase,** also called the **F_0F_1 complex** (see Figure 22.7). The F_0 portion of the molecule is a channel through which H^+ pass. It spans the inner mitochondrial membrane, as shown in Figure 22.7. The F_1 part of the molecule is an enzyme that catalyzes the phosphorylation of ADP to produce ATP.

ATP Synthase and the Production of ATP

How does all this complicated machinery actually function? NADH carries electrons, originally from glucose, to the first carrier of the electron transport system, NADH dehydrogenase (see Figure 22.7). There, NADH is oxidized to NAD^+, which returns to the site of the citric acid cycle to be reduced again. As Figure 22.7 shows (dashed red line), the pair of electrons is passed to the next electron carrier, and H^+ are pumped to the intermembrane compartment. The electrons are passed sequentially through the electron transport system, and at two additional sites, H^+ from the matrix are pumped into the intermembrane compartment. With each transfer the electrons lose some of their potential energy. It is this energy that is used to transport H^+ across the inner mitochondrial membrane and into the H^+ reservoir. As mentioned above, $FADH_2$ donates its electrons to a carrier of lower energy and fewer H^+ are pumped into the reservoir.

The importance of keeping the electron transport system functioning becomes obvious when we consider what occurs in cyanide poisoning. Cyanide binds to the heme group iron of cytochrome oxidase, instantly stopping electron transfers and causing death within minutes!

Finally, the electrons arrive at the last carrier. They now have too little energy to accomplish any more work, but they *must* be donated to some final electron acceptor so that the electron transport system can continue to function. In aerobic organisms the **terminal electron acceptor** is molecular oxygen, O_2, and the product is water.

As the electron transport system continues to function, a high concentration of protons builds up in the intermembrane space. This creates an H^+ gradient across the inner mitochondrial membrane. Such a gradient is an enormous energy source, like water stored behind a dam. The mitochondria make use of the potential energy of the gradient to synthesize ATP energy.

A HUMAN PERSPECTIVE

Brown Fat: The Fat That Makes You Thin?

Humans have two types of fat, or adipose, tissue. *White fat* is distributed throughout the body and is composed of aggregations of cells having membranous vacuoles containing stored triglycerides. The size and number of these storage vacuoles determine whether a person is overweight or not. The other type of fat is *brown fat*. Brown fat is a specialized tissue for heat production, called *nonshivering thermogenesis*. As the name suggests, this is a means of generating heat in the absence of the shivering response. The cells of brown fat look nothing like those of white fat. They do contain small fat vacuoles; however, the distinguishing feature of brown fat is the huge number of mitochondria within the cytoplasm. In addition, brown fat tissue contains a great many blood vessels. These provide oxygen for the thermogenic metabolic reactions.

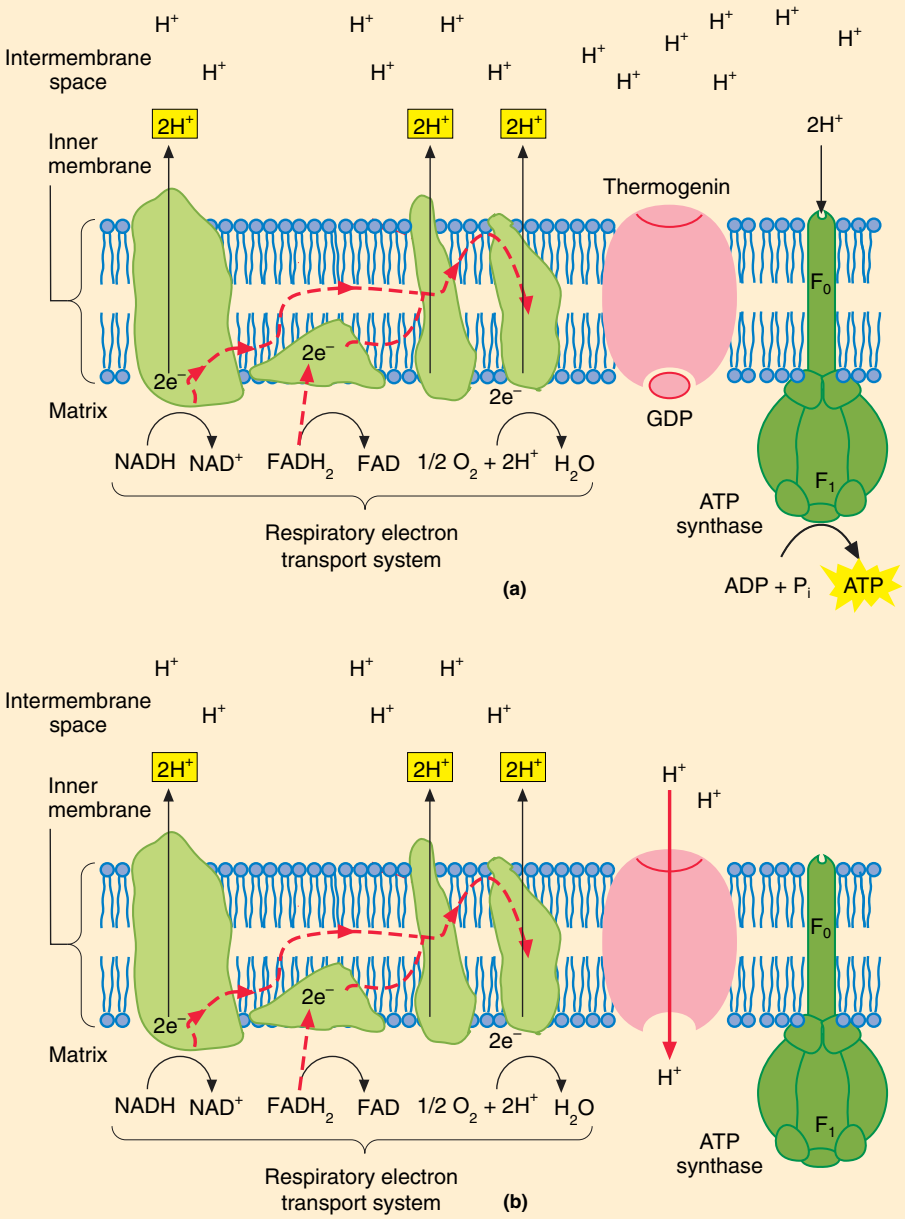
Brown fat is most pronounced in newborns, cold-adapted mammals, and hibernators. One major difficulty faced by a newborn is temperature regulation. The baby leaves an environment in which he or she was bathed in fluid of a constant 37°C, body temperature. Suddenly, the child is thrust into a world that is much colder and in which he or she must generate his or her own warmth internally. By having a good reserve of active brown fat to generate that heat, the newborn is protected against cold shock at the time of birth. However, this thermogenesis literally burns up most of the brown fat tissue, and adults typically have so little brown fat that it can be found only by using a special technique called *thermography*, which detects temperature differences throughout a body. However, in some individuals, brown fat is very highly developed. For instance, the Korean diving women who spend 6–7 hours every day diving for pearls in cold water have a massive amount of brown fat to warm them by nonshivering thermogenesis. Thus development of brown fat is a mechanism of cold adaptation.

When it was noticed that such cold-adapted individuals were seldom overweight, a correlation was made between the amount of brown fat in the body and the tendency to become overweight. Studies done with rats suggest that, to some degree, fatness is genetically determined. In other words, you are as lean as your genes allow you to be. In these studies, cold-adapted and non-cold-adapted rats were fed cafeteria food—as much as they wanted—and their weight gain was monitored. In every case the cold-adapted rats, with their greater quantity of brown fat, gained significantly less weight than their non-cold-adapted counterparts, despite the fact that they ate as much as the non-cold-adapted rats. This and other studies led researchers to conclude that brown fat burns excess fat in a highly caloric diet.

How does brown fat generate heat and burn excess calories? For the answer we must turn to the mitochondrion. In addition to the ATP synthase and the electron transport system proteins that are found in all mitochondria, there is a protein in the inner mitochondrial membrane of brown fat tissue called *thermogenin*. This protein has a channel in the center through which the protons (H^+) of the intermembrane space could pass back into the mitochondrial matrix. Under normal conditions this channel is plugged by a GDP molecule so that it remains closed and the proton gradient can continue to drive ATP synthesis by oxidative phosphorylation.

When brown fat is "turned on," by cold exposure or in response to certain hormones, there is an immediate increase in the rate of glycolysis and β-oxidation of the stored fat (Chapter 23). These reactions produce acetyl CoA, which then fuels the citric acid cycle. The citric acid cycle, of course, produces NADH and $FADH_2$, which carry electrons to the electron transport system. Finally, the electron transport system pumps protons into the intermembrane space. Under usual conditions the energy of the proton gradient would be used to synthesize ATP. However, when brown fat is stimulated, the GDP that had plugged the pore in thermogenin is lost. Now protons pass freely back into the matrix space, and the proton gradient is dissipated. The energy of the gradient, no longer useful for generating ATP, is released as *heat*, the heat that warms and protects newborns and cold-adapted individuals.

Brown fat is just one of the body's many systems for maintaining a constant internal environment regardless of the conditions in the external environment. Such mechanisms, called *homeostatic mechanisms*, are absolutely essential to allow the body to adapt to and survive in an ever-changing environment.



(a) The inner membrane of brown fat mitochondria contains thermogenin. In the normal state the pore in the center of thermogenin is plugged by a GDP molecule. (b) When brown fat is activated for thermogenesis, the GDP molecule is removed from the pore, and the protons from the H^+ reservoir are free to flow back into the matrix of the mitochondrion. As the gradient dissipates, heat energy is released.

ATP synthase harvests the energy of this gradient by making ATP. H^+ pass through the F_0 channel back into the matrix. This causes F_1 to become an active enzyme that catalyzes the phosphorylation of ADP to produce ATP. In this way the energy of the H^+ reservoir is harvested to make ATP.

Question 22.9

Write a balanced chemical equation for the reduction of NAD^+.

Question 22.10

Write a balanced chemical equation for the reduction of FAD.

Summary of the Energy Yield

One turn of the citric acid cycle results in the production of two CO_2 molecules, three NADH molecules, one $FADH_2$ molecule, and one ATP molecule. Oxidative phosphorylation yields three ATP molecules per NADH molecule and two ATP molecules per $FADH_2$ molecule. The only exception to these energy yields is the NADH produced in the cytoplasm during glycolysis. Oxidative phosphorylation yields only two ATP molecules per cytoplasmic NADH molecule. The reason for this is that energy must be expended to shuttle electrons from NADH in the cytoplasm to $FADH_2$ in the mitochondrion.

In some tissues of the body there is a more efficient shuttle system that results in the production of three ATP per cytoplasmic NADH. This system is described in Appendix G.

Knowing this information and keeping in mind that two turns of the citric acid cycle are required, we can sum up the total energy yield from the complete oxidation of one glucose molecule.

EXAMPLE 22.1 ***Determining the Yield of ATP from Aerobic Respiration***

Calculate the number of ATP produced by the complete oxidation of one molecule of glucose.

Solution

Glycolysis:	
Substrate-level phosphorylation	2 ATP
2 NADH × 2 ATP/cytoplasmic NADH	4 ATP
Conversion of 2 pyruvate molecules to 2 acetyl CoA molecules:	
2 NADH × 3 ATP/NADH	6 ATP
Citric acid cycle (two turns):	
2 GTP × 1 ATP/GTP	2 ATP
6 NADH × 3 ATP/NADH	18 ATP
2 $FADH_2$ × 2 ATP/$FADH_2$	4 ATP
	36 ATP

This represents an energy harvest of about 40% of the potential energy of glucose.

Aerobic metabolism is very much more efficient than anaerobic metabolism. The abundant energy harvested by aerobic metabolism has had enormous consequences for the biological world. Much of the energy released by the oxidation of fuels is not lost as heat but conserved in the form of ATP. Organisms that possess abundant energy have evolved into multicellular organisms and developed specialized functions. As a consequence of their energy requirements, all multicellular organisms are aerobic.

22.7 The Degradation of Amino Acids

Learning Goal 7

Carbohydrates are not our only source of energy. As we saw in Chapter 21, dietary protein is digested to amino acids that can also be used as an energy source, although this is not their major metabolic function. Most of the amino acids used for energy come from the diet. In fact, it is only under starvation conditions, when stored glycogen has been depleted, that the body begins to burn its own protein, for instance from muscle, as a fuel.

The fate of the mixture of amino acids provided by digestion of protein depends upon a balance between the need for amino acids for biosynthesis and the need for cellular energy. Only those amino acids that are not needed for protein synthesis are eventually converted into citric acid cycle intermediates and used as fuel.

The degradation of amino acids occurs primarily in the liver and takes place in two stages. The first stage is the removal of the α-amino group, and the second is the degradation of the carbon skeleton. In land mammals the amino group generally ends up in urea, which is excreted in the urine. The carbon skeletons can be converted into a variety of compounds, including citric acid cycle intermediates, pyruvate, acetyl CoA, or acetoacetyl CoA. The degradation of the carbon skeletons is summarized in Figure 22.8. Deamination reactions and the fate of the carbon skeletons of amino acids are the focus of this section.

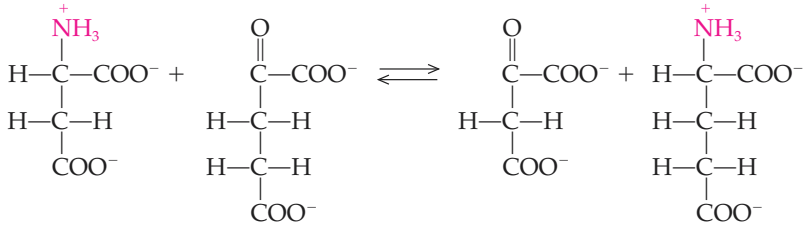
Removal of α-Amino Groups: Transamination

The first stage of amino acid degradation, the removal of the α-amino group, is usually accomplished by a **transamination** reaction. **Transaminases** catalyze the transfer of the α-amino group from an α-amino acid to an α-keto acid:

Donor amino acid | Acceptor keto acid | α-Keto acid of amino acid | New amino acid

The α-amino group of a great many amino acids is transferred to α-ketoglutarate to produce the amino acid glutamate and a new keto acid. This glutamate family of transaminases is especially important because the α-keto acid corresponding to glutamate is α-ketoglutarate, a citric acid cycle intermediate. The glutamate transaminases thus provide a direct link between amino acid degradation and the citric acid cycle.

Aspartate transaminase, catalyzes the transfer of the α-amino group of aspartate to α-ketoglutarate, producing oxaloacetate and glutamate:



Aspartate | α-Ketoglutarate | Oxaloacetate | Glutamate

Another important transaminase in mammalian tissues is *alanine transaminase*, which catalyzes the transfer of the α-amino group of alanine to α-ketoglutarate and produces pyruvate and glutamate:

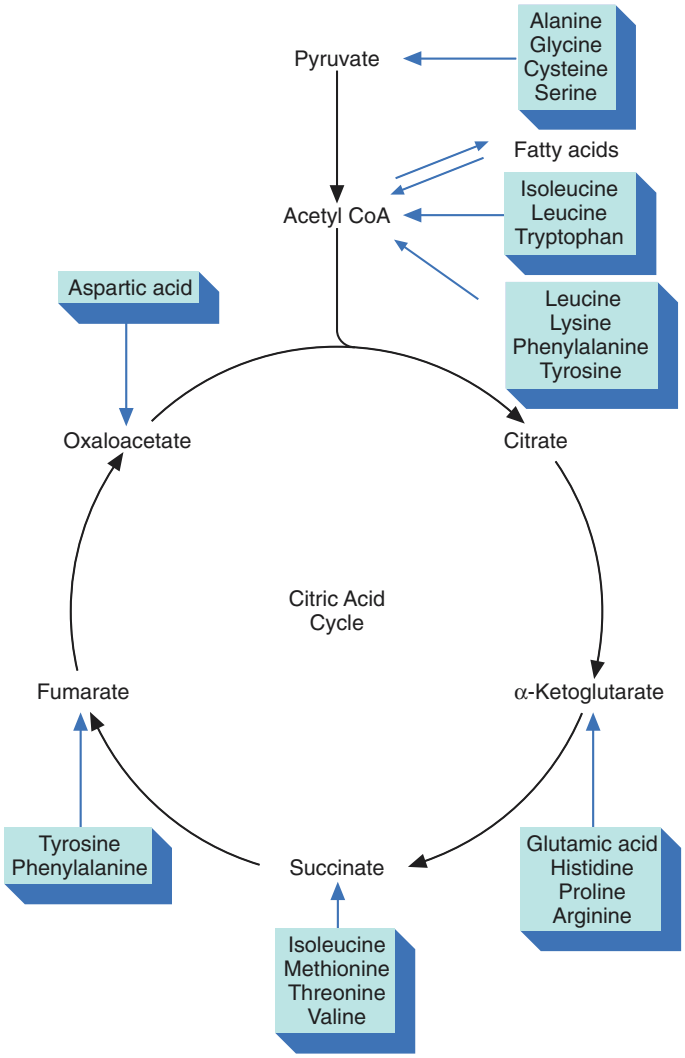
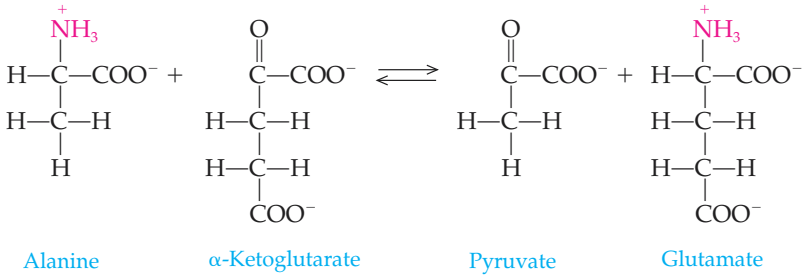


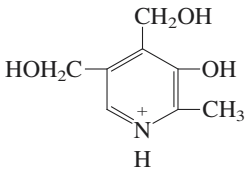
Figure 22.8
The carbon skeletons of amino acids can be converted to citric acid cycle intermediates and completely oxidized to produce ATP energy.



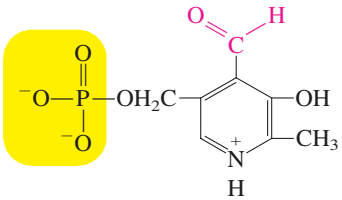
See Appendix F, Water-Soluble Vitamins, for more information on these vitamins and the coenzymes that are made from them.

All of the more than fifty transaminases that have been discovered require the coenzyme **pyridoxal phosphate.** This coenzyme is derived from vitamin B_6 (pyridoxine, Figure 22.9).

The transamination reactions shown above appear to be a simple transfer, but in reality, the reaction is much more complex. The transaminase binds the amino acid (aspartate in Figure 22.10a) in its active site. Then the α-amino group of aspartate is transferred to pyridoxal phosphate, producing pyridoxamine phosphate



Pyridoxine (vitamin B_6)



Pyridoxal phosphate

Figure 22.9
The structure of pyridoxal phosphate, the coenzyme required for all transamination reactions, and pyridoxine, vitamin B_6, the vitamin from which it is derived.

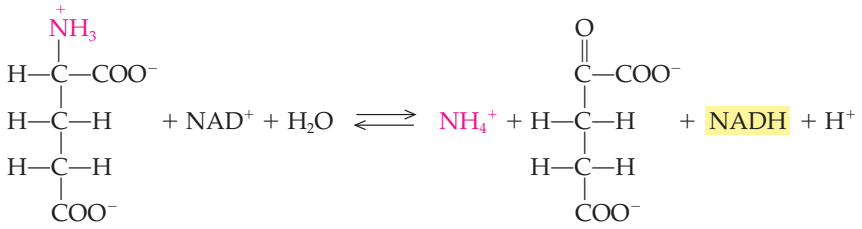
and oxaloacetate (Figure 22.10b). The amino group is then transferred to an α-keto acid, in this case, α-ketoglutarate (Figure 22.10c), to produce the amino acid glutamate (Figure 22.10d). Next we will examine the fate of the amino group that has been transferred to α-ketoglutarate to produce glutamate.

What is the role of pyridoxal phosphate in transamination reactions? **Question 22.11**

What is the function of a transaminase? **Question 22.12**

Removal of α-Amino Groups: Oxidative Deamination

In the next stage of amino acid degradation, ammonium ion is liberated from the glutamate formed by the transaminase. This breakdown of glutamate, catalyzed by the enzyme *glutamate dehydrogenase*, occurs as follows:



Glutamate α-Ketoglutarate

This is an example of an **oxidative deamination,** an oxidation-reduction process in which NAD^+ is reduced to NADH and the amino acid is deaminated (the amino group is removed). A summary of the deamination reactions described is shown in Figure 22.11.

The Fate of Amino Acid Carbon Skeletons

The carbon skeletons produced by these and other deamination reactions enter glycolysis or the citric acid cycle at many steps. For instance, we have seen that

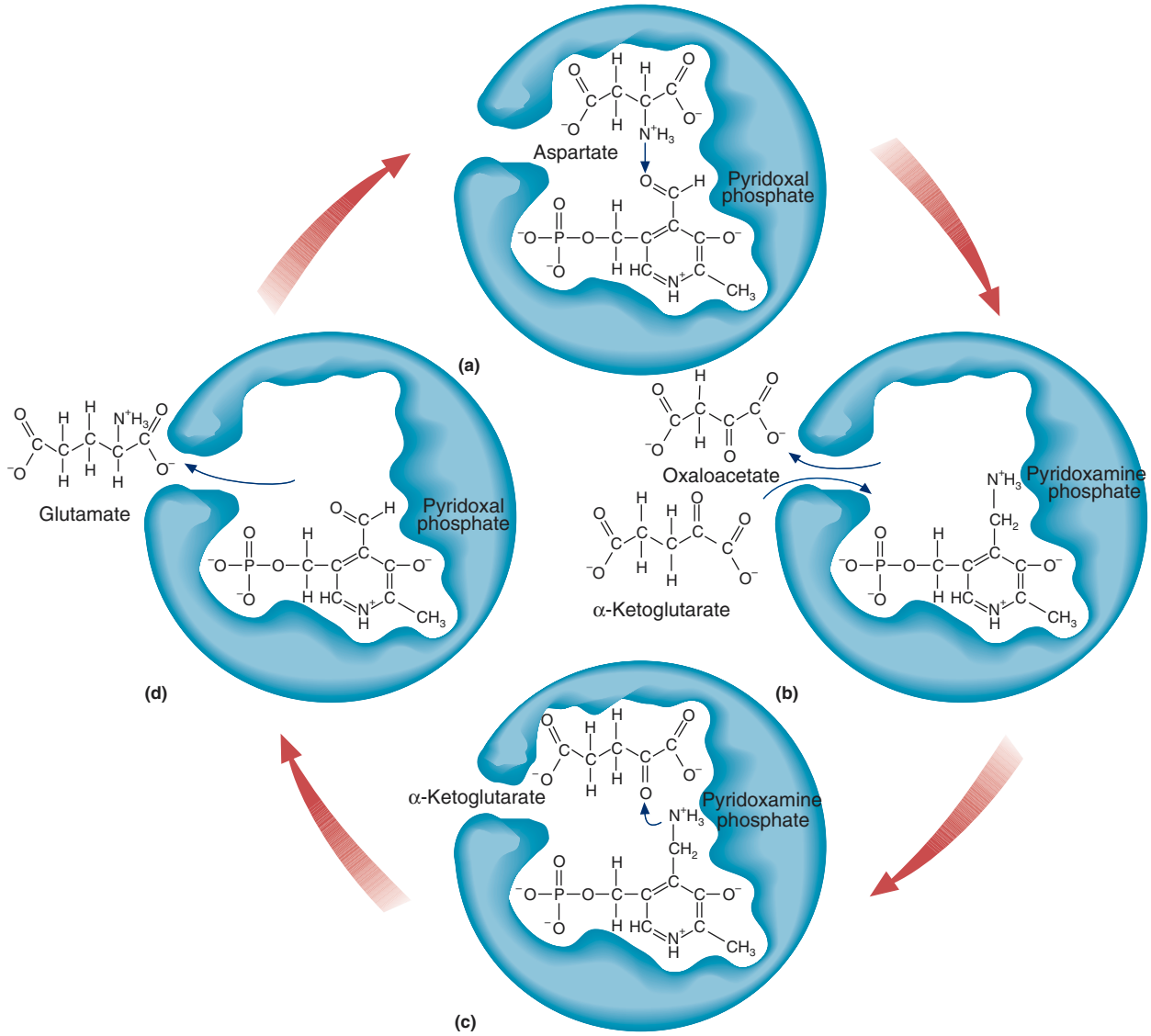


Figure 22.10
The mechanism of transamination.

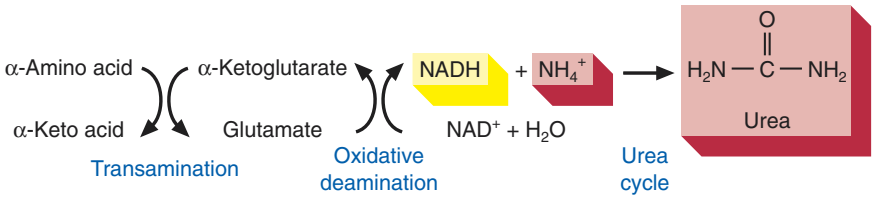


Figure 22.11
Summary of the deamination of an α-amino acid and the fate of the ammonium ion (NH_4^+).

transamination converts aspartate to oxaloacetate and alanine to pyruvate. The positions at which the carbon skeletons of various amino acids enter the energy-harvesting pathways are summarized in Figure 22.8.

22.8 The Urea Cycle

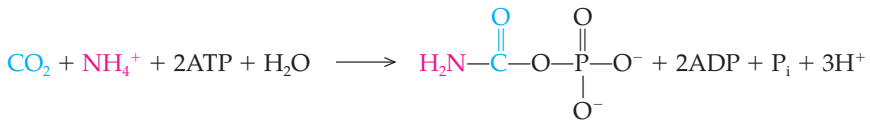
Learning Goal 8

Oxidative deamination produces large amounts of ammonium ion. Because ammonium ions are extremely toxic, they must be removed from the body, regardless of the energy expenditure required. In humans, they are detoxified in the liver by converting the ammonium ions into urea. This pathway, called the **urea cycle,** is the method by which toxic ammonium ions are kept out of the blood. The excess ammonium ions incorporated in urea are excreted in the urine (Figure 22.12).

Reactions of the Urea Cycle

The five reactions of the urea cycle are shown in Figure 22.12, and details of the reactions are summarized as follows.

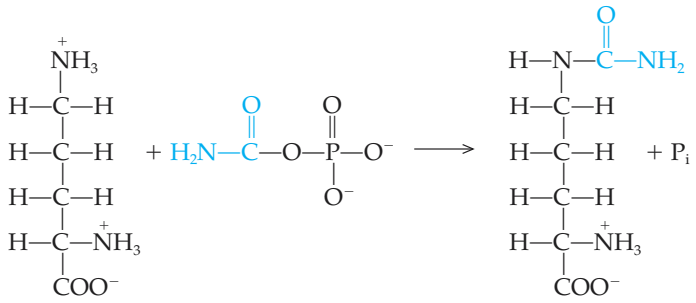
Step 1. The first step of the cycle is a reaction in which CO_2 and NH_4^+ form carbamoyl phosphate. This reaction, which also requires ATP and H_2O, occurs in the mitochondria and is catalyzed by the enzyme *carbamoyl phosphate synthetase*.



Carbamoyl phosphate

Step 2. The carbamoyl phosphate thus produced condenses with the amino acid ornithine to produce the amino acid citrulline. This reaction also occurs in the mitochondria and is catalyzed by the enzyme *ornithine transcarbamoylase*.

The urea cycle involves several unusual amino acids that are not found in polypeptides.



Ornithine Carbamoyl phosphate Citrulline

Step 3. Citrulline is transported into the cytoplasm and now condenses with aspartate to produce argininosuccinate. This reaction, which requires energy released by the hydrolysis of ATP, is catalyzed by the enzyme *argininosuccinate synthase*.

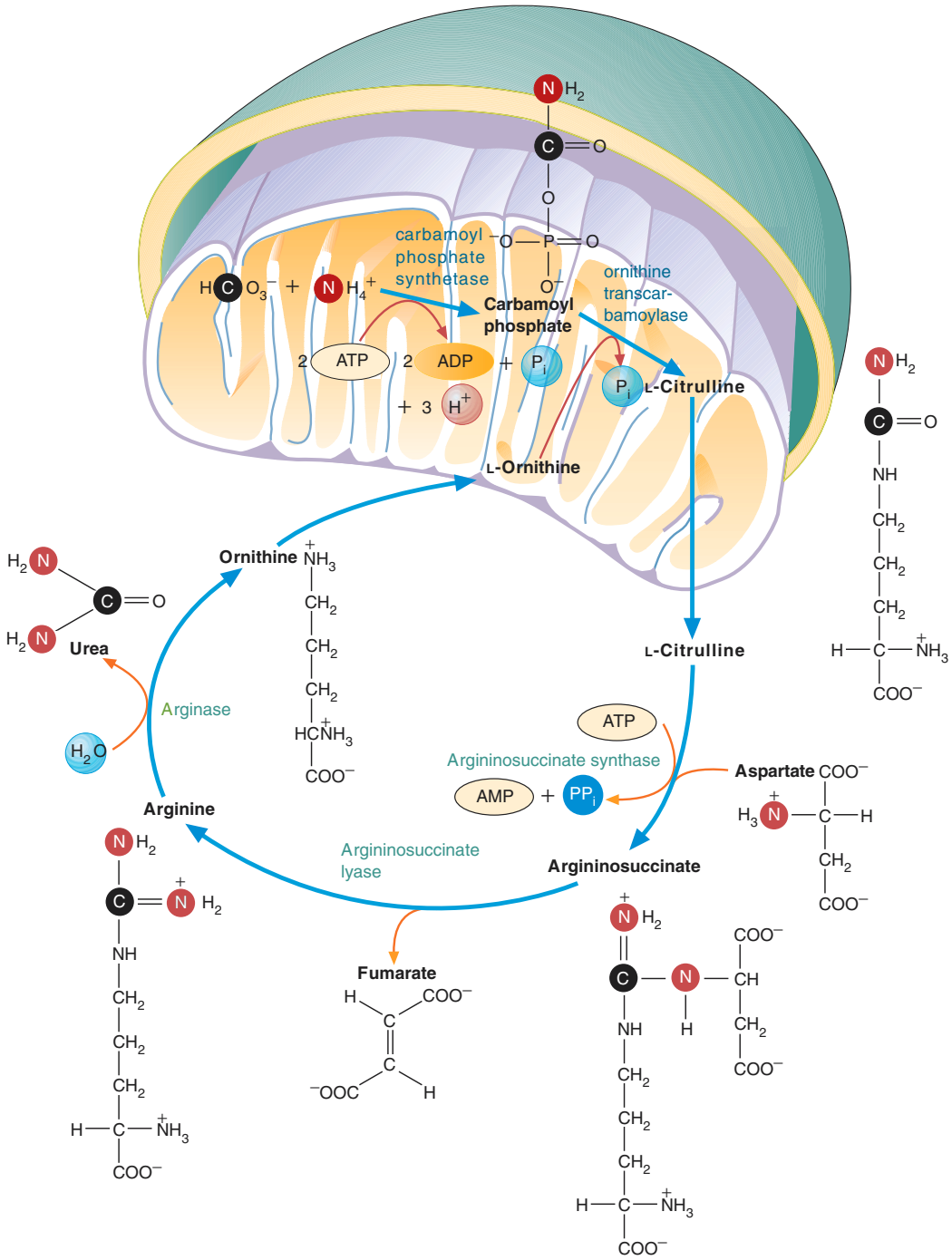
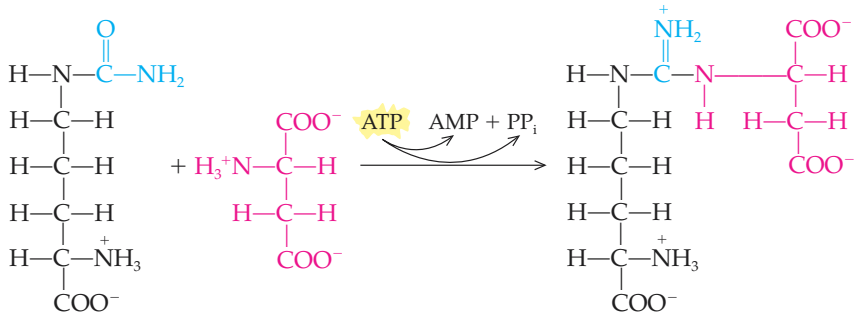


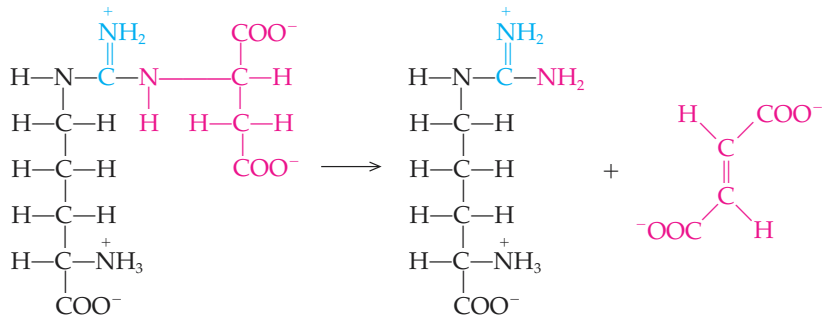
Figure 22.12

The urea cycle converts ammonium ions into urea, which is less toxic. The sources of the atoms are shown in color and the intracellular locations of the reactions are indicated. Citrulline, formed in the reaction between ornithine and carbamoyl phosphate, is transported out of the mitochondrion and into the cytoplasm. Ornithine, a substrate for the formation of citrulline, is transported from the cytoplasm into the mitochondrion.



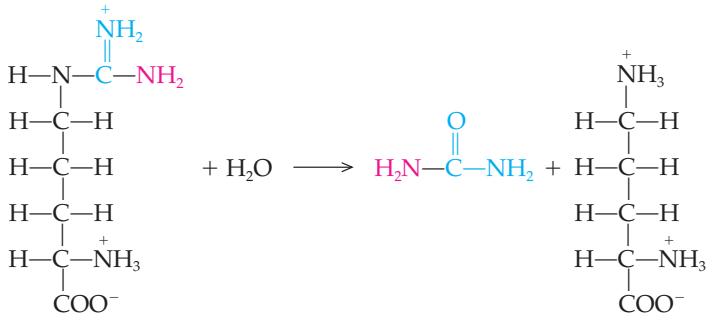
Citrulline Aspartate Argininosuccinate

Step 4. Now the argininosuccinate is cleaved to produce the amino acid arginine and the citric acid cycle intermediate fumarate. This reaction is catalyzed by the enzyme *argininosuccinate lyase*.



Argininosuccinate Arginine Fumarate

Step 5. Finally, arginine is hydrolyzed to generate urea, the product of the reaction to be excreted, and ornithine, the original reactant in the cycle. *Arginase* is the enzyme that catalyzes this reaction.

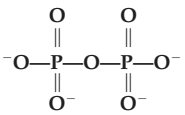


Arginine Water Urea Ornithine

Note that one of the amino groups in urea is derived from the ammonium ion and the second is derived from the amino acid aspartate.

There are genetically transmitted diseases that result from a deficiency of one of the enzymes of the urea cycle. The importance of the urea cycle is apparent when we consider the terrible symptoms suffered by afflicted individuals. A deficiency of urea cycle enzymes causes an elevation of the concentration of NH_4^+, a condition known as **hyperammonemia.** If there is a complete deficiency of one of

The abbreviation PP$_i$ represents the pyrophosphate group, which consists of two phosphate groups joined by a phosphoanhydride bond:



Learning Goal 9

A MEDICAL PERSPECTIVE

Pyruvate Carboxylase Deficiency

Pyruvate carboxylase is the enzyme that converts pyruvate to oxaloacetate.

$$\text{Pyruvate} + CO_2 + ATP + H_2O \longrightarrow \text{Oxaloacetate} + ADP + 2H^+$$

This reaction is important because it provides oxaloacetate for the citric acid cycle when the supplies have run low because of the demands of biosynthesis. It is also the enzyme that catalyzes the first step in gluconeogenesis, the pathway that provides the body with needed glucose in times of starvation or periods of exercise that deplete glycogen stores. But somehow these descriptions don't fill us with a sense of the importance of this enzyme and its jobs. It is not until we investigate a case study of a child born with pyruvate carboxylase deficiency that we see the full impact of this enzyme.

Pyruvate carboxylase deficiency is found in about 1 in 250,000 births; however, there is an increased incidence in native North American Indians who speak the Algonquin dialect and in the French. There are two types of genetic disorders that have been described. In the neonatal form of the disease, there is a complete absence of the enzyme. Symptoms are apparent at birth and the child is born with brain abnormalities. In the infantile form, the patient develops symptoms early in infancy. Again, it is neurological symptoms that draw attention to the condition. The infants do not develop mental or psychomotor skills. They may develop seizures and/or respiratory depression. In both cases, it is the brain that suffers the greatest damage. In fact, this is the case in most of the disorders that reduce energy metabolism because the brain has such high energy requirements.

Biochemically, patients exhibit quite a variety of symptoms. They show acidosis (low blood pH) due to accumulations of lactate and extremely high pyruvate concentrations in the blood. Blood levels of alanine are also high and large doses of alanine do not stimulate gluconeogenesis. Furthermore, a patient's cells accumulate lipid.

We can understand each of these symptoms by considering the pathways affected by the absence of this single enzyme. Lactic acidosis results from the fact that the body must rely on glycolysis and lactate fermentation for most of its energy needs. Alanine levels are high because it isn't being transaminated to pyruvate efficiently, because pyruvate levels are so high. In addition, alanine can't be converted to glucose by gluconeogenesis. Although the excess alanine is taken up by the liver and converted to pyruvate, the pyruvate can't be converted to glucose. Lipids accumulate because a great deal of pyruvate is converted to acetyl CoA. However, the acetyl CoA is not used to produce citrate as a result of the absence of oxaloacetate. So, the acetyl CoA is thus used to synthesize fatty acids, which are stored as triglycerides.

Dietary intervention has been tried. One such regimen is to supplement with aspartic acid and glutamic acid. The theory behind this treatment is as complex as the many symptoms of the disorder. Both amino acids can be aminated (amino groups added) in non-nervous tissue. This produces asparagine and glutamine, both of which are able to cross the blood-brain barrier. Glutamine is deaminated to glutamate, which is then transaminated to α-ketoglutarate, indirectly replenishing oxaloacetate. Asparagine can be deaminated to aspartate, which can be converted to oxaloacetate. This serves as a second supply of oxaloacetate. To date, these attempts at dietary intervention have not proved successful. Perhaps in time research will provide the tools for enzyme replacement therapy or gene therapy that could alleviate the symptoms.

the enzymes of the urea cycle, the result is death in early infancy. If there is a partial deficiency of one of the enzymes of the urea cycle, the result may be retardation, convulsions, and vomiting. In these milder forms of hyperammonemia, a low-protein diet leads to a lower concentration of NH_4^+ in blood and less severe clinical symptoms.

Question 22.13 What is the purpose of the urea cycle?

Question 22.14 Where do the reactions of the urea cycle occur?

22.9 Overview of Anabolism: The Citric Acid Cycle as a Source of Biosynthetic Intermediates

Learning Goal 10

So far, we have talked about the citric acid cycle only as an energy-harvesting mechanism. We have seen that dietary carbohydrates and amino acids enter the pathway at various stages and are oxidized to generate NADH and $FADH_2$, which, by means of oxidative phosphorylation, are used to make ATP.

However, the role of the citric acid cycle in cellular metabolism involves more than just **catabolism.** It plays a key role in **anabolism,** or biosynthesis, as well. Figure 22.13 shows the central role of glycolysis and the citric acid cycle as energy-harvesting reactions, as well as their role as a source of biosynthetic precursors.

As you may already suspect from the fact that amino acids can be converted into citric acid cycle intermediates, these same citric acid cycle intermediates can also be used as starting materials for the synthesis of amino acids. Oxaloacetate

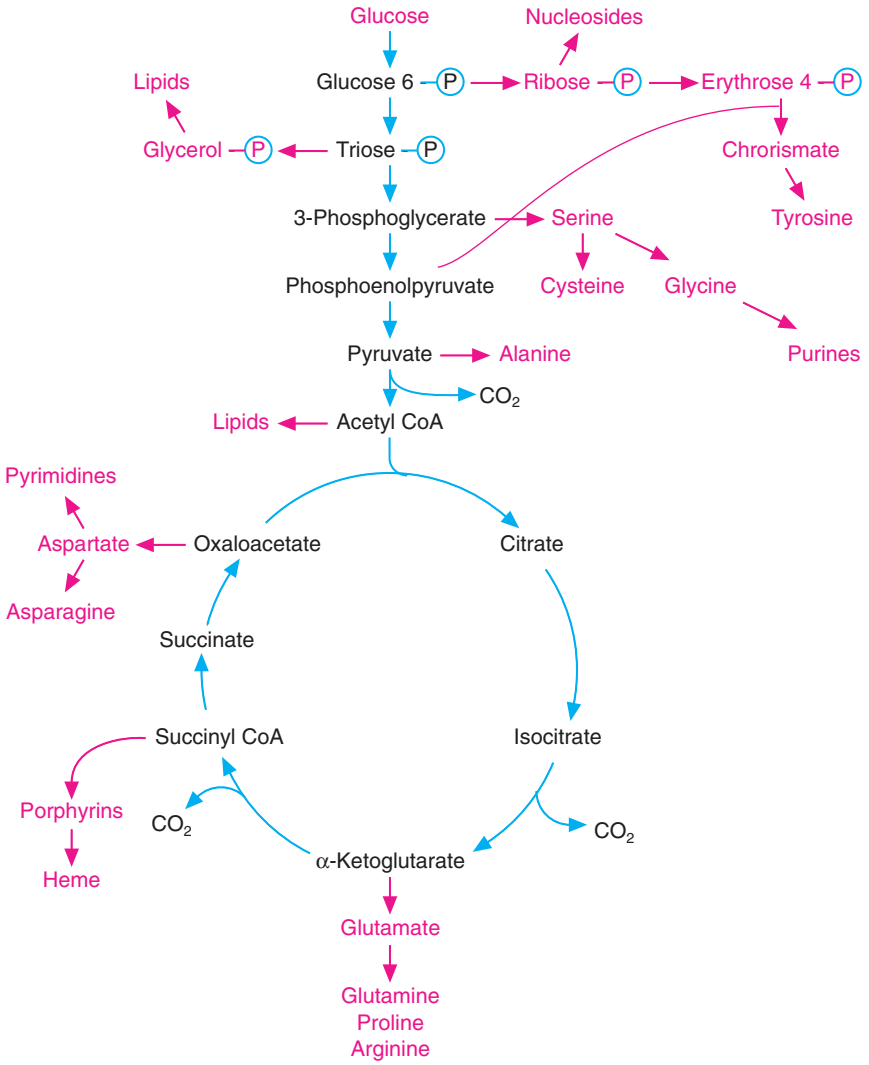


Figure 22.13
Glycolysis, the pentose phosphate pathway, and the citric acid cycle also provide a variety of precursors for the biosynthesis of amino acids, nitrogenous bases, and porphyrins.

provides the carbon skeleton for the one-step synthesis of the amino acid aspartate by the transamination reaction:

$$\text{oxaloacetate} + \text{glutamate} \rightleftharpoons \text{aspartate} + \alpha\text{-ketoglutarate}$$

Aside from providing aspartate for protein synthesis, this reaction provides aspartate for the urea cycle.

Asparagine is made from aspartate by the amination reaction

$$\text{aspartate} + NH_4^+ + \text{ATP} \longrightarrow \text{asparagine} + \text{AMP} + PP_1 + H^+$$

The nine amino acids not shown in Figure 22.13 (histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan, and valine) are called the essential amino acids (Section 19.11) because they cannot be synthesized by humans. Arginine is an essential amino acid for infants and adults under physical stress.

α-Ketoglutarate serves as the starting carbon chain for the family of amino acids including glutamate, glutamine, proline, and arginine. Glutamate is especially important because it serves as the donor of the α-amino group of almost all other amino acids. It is synthesized from NH_4^+ and α-ketoglutarate in a reaction mediated by glutamate dehydrogenase. This is the reverse of the reaction shown in Figure 22.11 and previously described. In this case the coenzyme that serves as the reducing agent is NADPH.

$$NH_4^+ + \alpha\text{-ketoglutarate} + \text{NADPH} \rightleftharpoons \text{L-glutamate} + NADP^+ + H_2O$$

Actually, in humans tyrosine is made from the essential amino acid phenylalanine.

Glutamine, proline, and arginine are synthesized from glutamate.

Examination of Figure 22.13 reveals that serine, glycine, and cysteine are synthesized from 3-phosphoglycerate; alanine is synthesized from pyruvate; and tyrosine is produced from phosphoenolpyruvate and the four-carbon sugar erythrose-4-phosphate, which, in turn, is synthesized from glucose-6-phosphate in the pentose phosphate pathway. In addition to the amino acid precursors, glycolysis and the citric acid cycle also provide precursors for lipids and the nitrogenous bases that are required to make DNA, the molecule that carries the genetic information. They also generate precursors for heme, the prosthetic group that is required for hemoglobin, myoglobin, and the cytochromes.

Clearly, the reactions of glycolysis and the citric acid cycle are central to both anabolic and catabolic cellular activities. Metabolic pathways that function in both anabolism and catabolism are called **amphibolic pathways.** Consider for a moment the difficulties that the dual nature of these pathways could present to the cell. When the cell is actively growing, there is a great demand for biosynthetic precursors to build new cell structures. A close look at Figure 22.13 shows us that periods of active cell growth and biosynthesis may deplete the supply of citric acid cycle intermediates. The problem is, the processes of growth and biosynthesis also require a great deal of ATP!

Carboxylation of pyruvate during gluconeogenesis is discussed in Section 21.6.

The solution to this problem is to have an alternative pathway for oxaloacetate synthesis that can produce enough oxaloacetate to supply the anabolic and catabolic requirements of the cell. Although bacteria and plants have several mechanisms, the only way that mammalian cells can produce more oxaloacetate is by the carboxylation of pyruvate, a reaction that is also important in gluconeogenesis. This reaction is

$$\text{pyruvate} + CO_2 + \text{ATP} \longrightarrow \text{oxaloacetate} + \text{ADP} + P_1$$

The enzyme that catalyzes this reaction is *pyruvate carboxylase.* It is a conjugated protein having as its covalently linked prosthetic group the vitamin *biotin.* This enzyme is "turned on" by high levels of acetyl CoA. A high concentration of acetyl CoA is a signal that the cell requires high levels of the citric acid cycle intermediates, particularly oxaloacetate, the beginning substrate.

The reaction catalyzed by pyruvate carboxylase is called an **anaplerotic reaction.** The term *anaplerotic* means to fill up. Indeed, this critical enzyme must constantly replenish the oxaloacetate and thus indirectly all the citric acid cycle intermediates that are withdrawn as biosynthetic precursors for the reactions summarized in Figure 22.13.

Explain how the citric acid cycle serves as an amphibolic pathway.

Question 22.15

What is the function of an anaplerotic reaction?

Question 22.16

Summary

22.1 The Mitochondria

The *mitochondria* are aerobic cell organelles that are responsible for most of the ATP production in eukaryotic cells. They are enclosed by a double membrane. The outer membrane permits low-molecular-weight molecules to pass through. The *inner mitochondrial membrane*, by contrast, is almost completely impermeable to most molecules. The inner mitochondrial membrane is the site where *oxidative phosphorylation* occurs. The enzymes of the *citric acid cycle*, of amino acid catabolism, and of fatty acid oxidation are located in the *matrix space* of the mitochondrion.

22.2 Conversion of Pyruvate to Acetyl CoA

Under aerobic conditions, pyruvate is oxidized by the pyruvate dehydrogenase complex. Acetyl CoA, formed in this reaction, is a central molecule in both catabolism and anabolism.

22.3 An Overview of Aerobic Respiration

Aerobic respiration is the oxygen-requiring degradation of food molecules and production of ATP. *Oxidative phosphorylation* is the process that uses the high-energy electrons harvested by oxidation of substrates of the citric acid cycle to produce ATP.

22.4 The Citric Acid Cycle (The Krebs Cycle)

The *citric acid cycle* is the final pathway for the degradation of carbohydrates, amino acids, and fatty acids. The citric acid cycle occurs in the matrix of the mitochondria. It is a cyclic series of biochemical reactions that accomplishes the complete oxidation of the carbon skeletons of food molecules.

22.5 Control of the Citric Acid Cycle

Because the rate of ATP production by the cell must vary with the amount of available oxygen and the energy requirements of the body at any particular time, the citric acid cycle is regulated at several steps. This allows the cell to generate more energy when needed, as for exercise, and less energy when the body is at rest.

22.6 Oxidative Phosphorylation

Oxidative phosphorylation is the process by which NADH and $FADH_2$ are oxidized and ATP is produced. Two molecules of ATP are produced when $FADH_2$ is oxidized, and three molecules of ATP are produced when NADH is oxidized. The complete oxidation of one glucose molecule by glycolysis, the citric acid cycle, and oxidative phosphorylation yields thirty-six molecules of ATP versus two molecules of ATP for anaerobic degradation of glucose by glycolysis and fermentation.

22.7 The Degradation of Amino Acids

Amino acids are oxidized in the mitochondria. The first step of amino acid catabolism is deamination, the removal of the amino group. The carbon skeletons of amino acids are converted into molecules that can enter the citric acid cycle.

22.8 The Urea Cycle

In the *urea cycle* the toxic ammonium ions released by deamination of amino acids are incorporated in urea, which is excreted in the urine.

22.9 Overview of Anabolism: The Citric Acid Cycle as a Source of Biosynthetic Intermediates

In addition to its role in *catabolism*, the citric acid cycle also plays an important role in cellular *anabolism*, or biosynthetic reactions. Many of the citric acid cycle intermediates are precursors for the synthesis of amino acids and macromolecules required by the cell. A pathway that functions in both catabolic and anabolic reactions is called an *amphibolic pathway*.

Key Terms

acetyl CoA (22.2)
aerobic respiration (22.3)
amphibolic pathway (22.9)
anabolism (22.9)
anaplerotic reaction (22.9)
ATP synthase (22.6)
catabolism (22.9)
citric acid cycle (22.4)
coenzyme A (22.2)
cristae (22.1)

electron transport system (22.6)
F_0F_1 complex (22.6)
hyperammonemia (22.8)
inner mitochondrial membrane (22.1)
intermembrane space (22.1)
matrix space (22.1)
mitochondria (22.1)
outer mitochondrial membrane (22.1)
oxidative deamination (22.7)
oxidative phosphorylation (22.6)
pyridoxal phosphate (22.7)
pyruvate dehydrogenase complex (22.2)
terminal electron acceptor (22.6)
transaminase (22.7)
transamination (22.7)
urea cycle (22.8)

Questions and Problems

The Mitochondria

22.17 What is the function of the intermembrane compartment of the mitochondria?
22.18 What biochemical processes occur in the matrix space of the mitochondria?
22.19 In what important way do the inner and outer mitochondrial membranes differ?
22.20 What kinds of proteins are found in the inner mitochondrial membrane?

Conversion of Pyruvate to Acetyl CoA

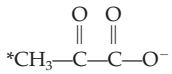
22.21 Under what metabolic conditions is pyruvate converted to acetyl CoA?
22.22 Write a chemical equation for the production of acetyl CoA from pyruvate. Under what conditions does this reaction occur?
22.23 How could a deficiency of riboflavin, thiamine, niacin, or pantothenic acid reduce the amount of ATP the body can produce?
22.24 In what form are the vitamins riboflavin, thiamine, niacin, and pantothenic acid needed by the pyruvate dehydrogenase complex?

The Citric Acid Cycle

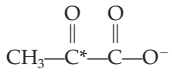
22.25 Label each of the following statements as true or false:
- **a.** Both glycolysis and the citric acid cycle are aerobic processes.
- **b.** Both glycolysis and the citric acid cycle are anaerobic processes.
- **c.** Glycolysis occurs in the cytoplasm, and the citric acid cycle occurs in the mitochondria.
- **d.** The inner membrane of the mitochondria is virtually impermeable to most substances.

22.26 Fill in the blanks:
- **a.** The proteins of the electron transport system are found in the _____, the enzymes of the citric acid cycle are found in the _____, and the hydrogen ion reservoir is found in the _____ of the mitochondria.
- **b.** The infoldings of the inner mitochondrial membrane are called _____.
- **c.** Energy released by oxidation in the citric acid cycle is conserved in the form of phosphoanhydride bonds in _____.
- **d.** The purpose of the citric acid cycle is the _____ of the acetyl group.

22.27 **a.** To what metabolic intermediate is the acetyl group of acetyl CoA transferred in the citric acid cycle?
b. What is the product of this reaction?
22.28 To what final products is the acetyl group of acetyl CoA converted during oxidation in the citric acid cycle?
22.29 How many ions of NAD^+ are reduced to molecules of NADH during one turn of the citric acid cycle?
22.30 How many molecules of FAD are converted to $FADH_2$ during one turn of the citric acid cycle?
22.31 What is the net yield of ATP for anaerobic glycolysis?
22.32 How many molecules of ATP are produced by the complete degradation of glucose via glycolysis, the citric acid cycle, and oxidative phosphorylation?
22.33 What is the function of acetyl CoA in the citric acid cycle?
22.34 What is the function of oxaloacetate in the citric acid cycle?
22.35 GTP is formed in one step of the citric acid cycle. How is this GTP converted into ATP?
22.36 What is the chemical meaning of the term *decarboxylation?*
22.37 The first reaction in the citric acid cycle is an aldol condensation. Write the equation for this reaction and explain its significance.
22.38 A bacterial culture is given ^{14}C-labeled pyruvate as its sole source of carbon and energy. The following is the structure of the radiolabeled pyruvate.



Follow the fate of the radioactive carbon through the reactions of the citric acid cycle.
22.39 The enzyme aconitase catalyzes the isomerization of citrate into isocitrate. Discuss the two reactions catalyzed by aconitase in terms of the chemistry of alcohols and alkenes.
22.40 A bacterial culture is given ^{14}C-labeled pyruvate as its sole source of carbon and energy. The following is the structure of the radiolabeled pyruvate.



Follow the fate of the radioactive carbon through the reactions of the citric acid cycle.
22.41 In the oxidation of malate to oxaloacetate, what is the structural evidence that an oxidation reaction has occurred? What functional groups are involved?
22.42 In the oxidation of succinate to fumarate, what is the structural evidence that an oxidation reaction has occurred? What functional groups are involved?
22.43 To what class of enzymes does dinucleotide diphosphokinase belong? Explain your answer.
22.44 To what class of enzymes does succinate dehydrogenase belong? Explain your answer.

Control of the Citric Acid Cycle

22.45 What is the importance of the regulation of the citric acid cycle?
22.46 Explain the role of allosteric enzymes in control of the citric acid cycle.
22.47 What molecule serves as a signal to increase the rate of the reactions of the citric acid cycle?
22.48 What molecules serve as signals to decrease the rate of the reactions of the citric acid cycle?

Oxidative Phosphorylation

22.49 How many molecules of ATP are produced when one molecule of NADH is oxidized by oxidative phosphorylation?

22.50 How many molecules of ATP are produced when one molecule of $FADH_2$ is oxidized by oxidative phosphorylation?
22.51 What is the source of energy for the synthesis of ATP in mitochondria?
22.52 What is the name of the enzyme that catalyzes ATP synthesis in mitochondria?
22.53 What is the function of the electron transport systems of the mitochondria?
22.54 What is the cellular location of the electron transport systems?
22.55 **a.** Compare the number of molecules of ATP produced by glycolysis to the number of ATP molecules produced by oxidation of glucose by aerobic respiration.
b. Which pathway produces more ATP? Explain.
22.56 At which steps in the citric acid cycle do oxidation–reduction reactions occur?

The Degradation of Amino Acids

22.57 What chemical transformation is carried out by transaminases?
22.58 Write a chemical equation for the transfer of an amino group from alanine to α-ketoglutarate, catalyzed by a transaminase.
22.59 Why is the glutamate family of transaminases so important?
22.60 What biochemical reaction is catalyzed by glutamate dehydrogenase?
22.61 Into which citric acid cycle intermediate is each of the following amino acids converted?
a. Alanine
b. Glutamate
c. Aspartate
d. Phenylalanine
e. Threonine
f. Arginine
22.62 What is the net ATP yield for degradation of each of the amino acids listed in problem 22.62?
22.63 Write a balanced equation for the synthesis of glutamate that is mediated by the enzyme glutamate dehydrogenase.
22.64 Write a balanced equation for the transamination of aspartate.

The Urea Cycle

22.65 What metabolic condition is produced if the urea cycle does not function properly?
22.66 What is hyperammonemia? How are mild forms of this disease treated?
22.67 The structure of urea is

$$NH_2—\overset{\overset{\displaystyle O}{\|}}{C}—NH_2$$

a. What substances are the sources of each of the amino groups in the urea molecule?
b. What substance is the source of the carbonyl group?
22.68 What is the energy source used for the urea cycle?

Overview of Anabolism: The Citric Acid Cycle as a Source of Biosynthetic Intermediates

22.69 From which citric acid cycle intermediate is the amino acid glutamate synthesized?
22.70 What amino acids are synthesized from α-ketoglutarate?
22.71 What is the role of the citric acid cycle in biosynthesis?
22.72 How are citric acid cycle intermediates replenished when they are in demand for biosynthesis?
22.73 What is meant by the term *essential amino acid?*
22.74 What are the nine essential amino acids?
22.75 Write a balanced equation for the reaction catalyzed by pyruvate carboxylase.
22.76 How does the reaction described in Problem 22.75 allow the citric acid cycle to fulfill its roles in both catabolism and anabolism?

Critical Thinking Problems

1. A one-month-old baby boy was brought to the hospital showing severely delayed development and cerebral atrophy. Blood tests showed high levels of lactate and pyruvate. By three months of age, very high levels of succinate and fumarate were found in the urine. Fumarase activity was absent in the liver and muscle tissue. The baby died at five months of age. This was the first reported case of fumarase deficiency and the defect was recognized too late for effective therapy to be administered. What reaction is catalyzed by fumarase? How would a deficiency of this mitochondrial enzyme account for the baby's symptoms and test results?
2. A certain bacterium can grow with ethanol as its only source of energy and carbon. Propose a pathway to describe how ethanol can enter a pathway that would allow ATP production and synthesis of precursors for biosynthesis.
3. Fluoroacetate has been used as a rat poison and can be fatal when eaten by humans. Patients with fluoroacetate poisoning accumulate citrate and fluorocitrate within the cells. What enzyme is inhibited by fluoroacetate? Explain your reasoning.
4. The pyruvate dehydrogenase complex is activated by removal of a phosphoryl group from pyruvate dehydrogenase. This reaction is catalyzed by the enzyme pyruvate dehydrogenase phosphate phosphatase. A baby is born with a defect in this enzyme. What effects would this defect have on the rate of each of the following pathways: aerobic respiration, glycolysis, lactate fermentation? Explain your reasoning.
5. Pyruvate dehydrogenase phosphate phosphatase is stimulated by Ca^{2+}. In muscles, the Ca^{2+} concentration increases dramatically during muscle contraction. How would the elevated Ca^{2+} concentration affect the rate of glycolysis and the citric acid cycle?
6. Liver contains high levels of nucleic acids. When excess nucleic acids are degraded, ribose-5-phosphate is one of the degradation products that accumulate in the cell. Can this substance be used as a source of energy? What pathway would be used?
7. In birds, arginine is an essential amino acid. Can birds produce urea as a means of removing ammonium ions from the blood? Explain your reasoning.