These U.S. runners have just won the gold medal in the 4 × 400 m relay race at the 1996 Olympic games. The buildup of lactic acid can cause severe muscle pain. Inset: A model of S-lactic acid. (MCT via Getty Images)

**KEY QUESTIONS**

21.1 What Are the Key Participants in Glycolysis, the \( \beta \)-Oxidation of Fatty Acids, and the Citric Acid Cycle?

21.2 What Is Glycolysis?

21.3 What Are the Ten Reactions of Glycolysis?

21.4 What Are the Fates of Pyruvate?

21.5 What Are the Reactions of the \( \beta \)-Oxidation of Fatty Acids?

21.6 What Are the Reactions of the Citric Acid Cycle?

**WE HAVE NOW** studied the structures and typical reactions of the major types of organic functional groups. Further, we have examined the structures of carbohydrates, amino acids and proteins, nucleic acids, and lipids. Now let us apply this background to the study of the organic chemistry of metabolism. In this chapter, we study three key metabolic pathways: glycolysis, the citric acid cycle, and the \( \beta \)-oxidation of fatty acids. The first is a pathway by which glucose is converted to pyruvate and then to acetyl coenzyme A. The second is a pathway by which the hydrocarbon chains of fatty acids are degraded, two carbons at a time, to acetyl coenzyme A. The third is the pathway by which the carbon skeletons of carbohydrates, fatty acids, and proteins are oxidized to carbon dioxide.

Those of you who go on to courses in biochemistry will undoubtedly study these metabolic pathways in considerable detail, including their role in energy production and conservation, their regulation, and the diseases associated with errors in particular metabolic steps. Our concern in this chapter is more limited. Our goal is to show that the reactions of these pathways are biochemical equivalents of organic functional group reactions we have already...
studied in detail. For instance, we find examples of keto–enol tautomerism; oxidation of an aldehyde to a carboxylic acid; oxidation of a secondary alcohol to a ketone; an aldol reaction and a reverse aldol reaction; a reverse Claisen condensation; and the formation and hydrolysis of esters, imines, thioesters, and anhydrides. In addition, we will use the mechanisms we have studied earlier to give us insights into the mechanisms of these reactions, all of which are enzyme catalyzed.

### 21.1 What Are the Key Participants in Glycolysis, the β-Oxidation of Fatty Acids, and the Citric Acid Cycle?

To understand the reactions of functional groups that play a role in the β-oxidation of fatty acids, the tricarboxylic acid cycle, and glycolysis, we first need to introduce several of the principal compounds participating in these and a great many other metabolic pathways. Three of these compounds (ATP, ADP, and AMP) are central to the storage and transfer of phosphate groups. Four (NAD+/NADH and FAD/FADH$_2$) are coenzymes involved in the oxidation–reduction of metabolic intermediates. The final compound (coenzyme A) is an agent for the storage and transfer of acetyl groups.

#### A. ATP, ADP, and AMP: Agents for the Storage and Transfer of Phosphate Groups

Following is a structural formula of adenosine triphosphate (Section 20.1), a compound involved in the storage and transfer of phosphate groups:

![Adenosine triphosphate (ATP) structure](image)

A building block for ATP as well as for five other key participants is adenosine, which consists of a unit of adenine bonded to a unit of β-ribofuranose by a β-N-glycosidic bond. Three phosphate groups are bonded to the terminal CH$_2$OH of ribose: one by a phosphoric ester bond, the remaining two by phosphoric anhydride bonds. Hydrolysis of the terminal phosphate group of ATP gives ADP. In the following abbreviated structural formulas, adenosine and its single phosphoric ester group are represented by the symbol AMP (adenosine monophosphate):
The reaction shown is the hydrolysis of a phosphoric anhydride; the phosphate acceptor is water. In the first two reactions of glycolysis, the phosphate acceptors are —OH groups of glucose and fructose, respectively, and are used to form phosphoric esters of these monosaccharides. In two reactions of glycolysis, ADP is the phosphate acceptor and is converted to ATP.

**B. NAD$^+$//NADH: Agents for Electron Transfer in Biological Oxidation–Reductions**

Nicotinamide adenine dinucleotide (NAD$^+$) is one of the central agents for the transfer of electrons in metabolic oxidations and reductions. NAD$^+$ is constructed of a unit of ADP, joined by a phosphoric ester bond to the terminal $-\text{CH}_2\text{OH}$ of $\beta$-d-ribofuranose, which is in turn joined to the pyridine ring of nicotinamide by a $\beta$-N-glycosidic bond:

![Nicotinamide adenine dinucleotide (NAD$^+$)](image)

When NAD$^+$ acts as an oxidizing agent, it is reduced to NADH, which, in turn, is a reducing agent and is oxidized to NAD$^+$. In these abbreviated structural formulas, the adenine dinucleotide part of each molecule is represented by the symbol Ad:

![Nicotinamide adenine dinucleotide](image)

NAD$^+$ is involved in a variety of enzyme-catalyzed oxidation–reduction reactions. The three types of oxidations we deal with in this chapter are the following:
What Are the Key Participants in Glycolysis, β-Oxidation of Fatty Acids, and the Citric Acid Cycle?

- Oxidation of a secondary alcohol to a ketone:
  \[
  \begin{array}{c}
  \text{A 2° alcohol} \\
  \text{CH—OH} \\
  \end{array} 
  \begin{array}{c}
  \text{A ketone} \\
  \text{C—O} \\
  \text{NAD}^+ \\
  \text{H}^+ \\
  \text{NADH} \\
  \end{array}
  \]

- Oxidation of an aldehyde to a carboxylic acid:
  \[
  \begin{array}{c}
  \text{An aldehyde} \\
  \text{C—H} \\
  \text{NAD}^+ + \text{H}_2\text{O} \\
  \text{C—OH} \\
  \text{NADH} + \text{H}^+ \\
  \end{array}
  \]

- Oxidation of an α-ketoacid to a carboxylic acid and carbon dioxide:
  \[
  \begin{array}{c}
  \text{An α-ketoacid} \\
  \text{C—COOH} \\
  \text{NAD}^+ + \text{H}_2\text{O} \\
  \text{C—OH} \\
  \text{CO}_2 \\
  \text{NADH} + \text{H}^+ \\
  \end{array}
  \]

As the following mechanism shows, the oxidation of each functional group involves the transfer of a hydride ion to NAD⁺.

**Mechanism**

**Oxidation of an Alcohol by NAD⁺**

Reactions in biological systems are often catalyzed by enzymes. This allows their mechanisms to proceed more efficiently and in ways that may not occur in typical solution-phase chemistry. Because of this, we do not indicate the common mechanistic pattern for each of the mechanistic steps in this chapter. When studying these mechanisms, try to focus on the flow of electrons that achieves the chemical transformations.

**STEP 1:** A basic group, B⁻, on the surface of the enzyme removes H⁺ from the —OH group.

**STEP 2:** Electrons of the H—O sigma bond become the pi electrons of the C=O bond.

**STEP 3:** A hydride ion is transferred from carbon to NAD⁺ to create a new C—H bond.

**STEP 4:** Electrons within the ring flow to the positively charged nitrogen.

The hydride ion, H⁺, which is transferred from the secondary alcohol to NAD⁺, contains two electrons; thus, NAD⁺ and NADH function exclusively in two-electron oxidations and two-electron reductions.
C. **FAD/FADH₂: Agents for Electron Transfer in Biological Oxidation–Reductions**

Flavin adenine dinucleotide (FAD) is also a central component in the transfer of electrons in metabolic oxidations and reductions. In FAD, flavin is bonded to the five-carbon monosaccharide ribitol, which is, in turn, bonded to the terminal phosphate group of ADP.

![Flavin Adenine Dinucleotide (FAD) Structure]

Flavin adenine dinucleotide (FAD) participates in several types of enzyme-catalyzed oxidation–reduction reactions. Our concern in this chapter is its role in the oxidation of a carbon–carbon single bond in the hydrocarbon chain of a fatty acid to a carbon–carbon double bond, in the process of which FAD is reduced to FADH₂:

\[
\begin{align*}
-\text{CH}_2-\text{CH}_2- & + \text{FAD} \rightarrow -\text{CH}=\text{CH}- + \text{FADH}_2 \\
& \text{a portion of the hydrocarbon chain of a fatty acid}
\end{align*}
\]

The mechanism by which FAD oxidizes \(-\text{CH}_2-\text{CH}_2-\) to \(-\text{CH}=\text{CH}-\) involves the transfer of a hydride ion from the hydrocarbon chain of the fatty acid to FAD.

**Mechanism**

**Oxidation of a Fatty Acid \(-\text{CH}_2-\text{CH}_2-\) to \(-\text{CH}=\text{CH}-\) by FAD**

The individual curved arrows in this mechanism are numbered 1–6 to help you follow the flow of electrons in the transformation.

**STEP 1:** A basic group, B⁺, on the surface of the enzyme removes a hydrogen atom from the carbon adjacent to the carboxyl group.

**STEP 2:** Electrons from this C–H sigma bond become the pi electrons of the new C–C double bond.
**STEP 3:** A hydride ion transfers from the carbon beta to the carboxyl group, to a nitrogen atom of flavin.

**STEP 4:** The pi electrons within flavin become redistributed.

**STEP 5:** Electrons of the C–N bond remove a hydrogen atom from the enzyme.

**STEP 6:** A new basic group forms on the surface of the enzyme.

![Diagram showing the transfer of a hydride ion from the carbon beta to the carboxyl group, and the redistribution of pi electrons within flavin.](image)

Note that, of the two hydrogen atoms added to FAD to produce FADH₂, one comes from the hydrocarbon chain undergoing oxidation and the other comes from an acidic group on the surface of the enzyme catalyzing this oxidation. Note also that one group on the enzyme functions as a proton acceptor and another functions as a proton donor.

### D. Coenzyme A: The Carrier of Acetyl Groups

Coenzyme A is derived from four subunits. On the left is a two-carbon unit derived from 2-mercaptoethanamine. This unit is in turn joined by an amide bond to the carboxyl group of 3-aminopropanoic acid (β-alanine). The amino group of β-alanine is joined by an amide bond to the carboxyl group of pantothenic acid, a vitamin of the B complex. Finally, the –OH group of pantothenic acid is joined by a phosphoric ester bond to the terminal phosphate group of ADP:

![Diagram showing the structure of coenzyme A and its subunits](image)

A key feature of the structure of coenzyme A is the terminal sulfhydryl (–SH) group. During the degradation of foodstuffs for the production of energy, the carbon skeletons of glucose, fructose, and galactose, along with those of fatty acids, glycerol, and several amino
acids, are converted to acetate in the form of a thioester named acetyl coenzyme A, or, more commonly, acetyl-CoA:

\[
\text{monosaccharides} \quad \text{fatty acids} \quad \text{glycerol} \quad \text{amino acids} \quad \text{CH}_3 - \text{C} - \text{S} - \text{CoA} \quad \text{Acetyl coenzyme A (Acetyl-CoA)}
\]

In Section 21.6, we will see how the two-carbon acetyl group is oxidized to carbon dioxide and water by the reactions of the citric acid cycle.

## 21.2 What Is Glycolysis?

Nearly every living cell carries out glycolysis. Living things first appeared in an environment lacking O\(_2\), and glycolysis was an early and important pathway for extracting energy from nutrient molecules because it takes place in the absence of oxygen. Glycolysis played a central role in anaerobic metabolic processes for the first billion or so years of biological evolution on earth. Modern organisms still employ it to provide precursor molecules for aerobic pathways, such as the citric acid cycle, and as a short-term energy source when the supply of oxygen is limited.

Glycolysis is a series of ten enzyme-catalyzed reactions that brings about the oxidation of glucose to two molecules of pyruvate. The oxidizing agent is NAD\(^+\). Furthermore, two molecules of ATP are produced for each molecule of glucose oxidized to pyruvate. Following is the net reaction of glycolysis:

\[
\text{C}_6\text{H}_{12}\text{O}_6 + 2\text{NAD}^+ + 2\text{HPO}_4^{2-} + 2\text{ADP} \xrightarrow{10 \text{ enzyme-catalyzed steps}} 2\text{CH}_3\text{C} \text{COO}^- + 2\text{H}^+ + 2\text{NADH} + 2\text{ATP}
\]

**EXAMPLE 21.1**

Show that the conversion of glucose to two molecules of pyruvate is an oxidation. (Hint: That it is an oxidation is easiest to see if you take the product to be pyruvic acid; recognize, of course, that, under the pH conditions at which this reaction takes place in cells, pyruvic acid is ionized to pyruvate.)

**STRATEGY**

Write a balanced equation for the conversion of glucose to pyruvate, and examine it to see if the starting material gains oxygens or loses hydrogens (oxidation is the gain of oxygens and/or the loss of hydrogens).

**SOLUTION**

Glucose is \(\text{C}_6\text{H}_{12}\text{O}_6\). Two molecules of pyruvic acid are \(2(\text{C}_3\text{H}_4\text{O}_3) = \text{C}_6\text{H}_8\text{O}_6\). The number of O atoms remains the same in this conversion but four H are lost. Therefore, the conversion of glucose to pyruvate is an oxidation.

**PROBLEM 21.1**

Under anaerobic (without oxygen) conditions, glucose is converted to lactate by a metabolic pathway called anaerobic glycolysis or, alternatively, lactate fermentation:

\[
\text{C}_6\text{H}_{12}\text{O}_6 \xrightarrow{\text{anaerobic glycolysis}} \text{2CH}_3\text{CHCOO}^- + 2\text{H}^+
\]

Is anaerobic glycolysis a net oxidation, a net reduction, or neither?
21.3 What Are the Ten Reactions of Glycolysis?

Although writing the net reaction of glycolysis is simple, it took several decades of patient, intensive work by scores of scientists to discover the separate reactions by which glucose is converted to pyruvate. Glycolysis is frequently called the Embden–Meyerhof pathway, in honor of the two German biochemists, Gustav Embden and Otto Meyerhof, who contributed so greatly to our present knowledge of it. Figure 21.1 shows the ten reactions of glycolysis.

**Reaction 1: Phosphorylation of α-D-Glucose**
The transfer of a phosphate group from ATP to glucose gives α-D-glucose 6-phosphate. This conversion is an example of the reaction of an anhydride with an alcohol to form an ester (Section 14.4B); in this case, a phosphoric anhydride reacts with the primary alcohol group of glucose to form a phosphoric ester. In Section 14.6, we saw that a more reactive carboxyl functional group can be transformed into a less reactive carboxyl functional group. The

![Diagram of glycolysis reactions](image_url)
same principle applies to functional derivatives of phosphoric acid. In Reaction 1 of glycolysis, a phosphoric anhydride, a more reactive functional group, is converted to a phosphoric ester, a less reactive functional group:

\[
\begin{align*}
\alpha-\text{D-Glucose} & \quad \text{hexokinase} \quad \text{Mg}^{2+} \quad \text{ATP} \\
\alpha-\text{D-Glucose 6-phosphate} & \quad \text{ADP}
\end{align*}
\]

Hexokinase, the enzyme catalyzing this reaction, requires divalent magnesium ion, \(\text{Mg}^{2+}\), whose function is to coordinate with two negatively charged oxygens of the terminal phosphate group of ATP and to facilitate attack by the \(\text{—OH}\) group of glucose on the phosphorus atom of the \(\text{P==O}\) group.

The phosphorylation of glucose gives the molecule an overall \(-2\) charge and prevents it from passing through the plasma membrane. This prevents glucose from leaving the cell.

**Reaction 2: Isomerization of Glucose 6-Phosphate to Fructose 6-Phosphate**

In this reaction, \(\alpha-\text{D-glucose 6-phosphate}\), an aldohexose, is converted to \(\alpha-\text{D-fructose 6-phosphate}\), a 2-ketohexose:

\[
\begin{align*}
\text{phosphogluco-isomerase} & \\
\alpha-\text{D-Glucose 6-phosphate} & \quad \text{a} \quad \text{D-Fructose 6-phosphate}
\end{align*}
\]

The chemistry involved in this isomerization is easiest to see by considering the open-chain (Fisher projection) forms of these two monosaccharides:

\[
\begin{align*}
\text{Glucose 6-phosphate (an aldohexose phosphate)} & \\
\text{An enediol} & \\
\text{Fructose 6-phosphate (a 2-ketohexose phosphate)}
\end{align*}
\]

One keto–enol tautomerism forms an enediol; a second then forms the ketone carbonyl group in fructose 6-phosphate. (See Section 12.8A and Problems 12.34 and 12.35.) The conversion of the aldose to a ketose is necessary to facilitate the chemistry in Reaction 4.

**Reaction 3: Phosphorylation of Fructose 6-Phosphate**

In the third reaction, a second mole of ATP converts \(\text{d-fructose 6-phosphate}\) to \(\text{d-fructose 1,6-bisphosphate}\), an unstable product. Its instability helps to facilitate Reaction 4:

\[
\begin{align*}
\text{phosphofructokinase} & \\
\text{Mg}^{2+} & \\
\text{d-Fructose 6-phosphate} & \quad \text{d-Fructose 1,6-bisphosphate}
\end{align*}
\]
Reaction 4: Cleavage of Fructose 1,6-Bisphosphate to Two Triose Phosphates
In the fourth reaction, \( \Delta \)-fructose 1,6-bisphosphate is cleaved to dihydroxyacetone phosphate and \( \Delta \)-glyceraldehyde 3-phosphate by a reaction that is the reverse of an aldol reaction. Recall from Section 15.2 that an aldol reaction takes place between the \( \alpha \)-carbon of one carbonyl-containing compound and the carbonyl carbon of another and that the functional group of the product of an aldol reaction is a \( \beta \)-hydroxyaldehyde or ketone:

The characteristic structural features of the product of an aldol reaction are:

(a) a carbonyl group and
(b) a \( \beta \)-hydroxyl group

\( \Delta \)-Fructose 1,6-bisphosphate

\( \Delta \)-Dihydroxyacetone phosphate

\( \Delta \)-Glyceraldehyde 3-phosphate

Reaction 5: Isomerization of Dihydroxyacetone Phosphate to \( \Delta \)-Glyceraldehyde 3-Phosphate
This interconversion of triose phosphates occurs by the same type of keto–enol tautomerism (Section 12.8A) and enediol intermediate we have already seen in the isomerization of \( \Delta \)-glucose 6-phosphate to \( \Delta \)-fructose 6-phosphate. Note that \( \Delta \)-glyceraldehyde 3-phosphate is chiral and that two enantiomers are possible for it. The enzyme catalyzing this reaction has a high stereospecificity, and only the D enantiomer is formed.

This isomerization makes it so that only one molecule, \( \Delta \)-glyceraldehyde 3-phosphate, need be metabolized during the remaining steps.

Reaction 6: Oxidation of the Aldehyde Group of \( \Delta \)-Glyceraldehyde 3-Phosphate
To simplify structural formulas in Reaction 6, \( \Delta \)-glyceraldehyde 3-phosphate is abbreviated G—CHO. Two changes occur in this reaction. First, the aldehyde group is oxidized to a carboxyl group, which is, in turn, converted to a mixed anhydride, and second, the oxidizing agent is NAD\(^+\), which is reduced to NADH:

The reaction is considerably more complicated than might appear from the balanced equation. As the following mechanism indicates, it involves (1) the formation of a thiohemiacetal, (2) hydride ion transfer to form a thioester, and (3) the conversion of a thioester to a mixed anhydride.
Mechanism

Oxidation of D-Glyceraldehyde 3-Phosphate to 1,3-Bisphosphoglycerate

**STEP 1:** Reaction between D-glyceraldehyde 3-phosphate and a sulfhydryl group of the enzyme gives a thiohemiacetal (Section 12.6):

\[
\begin{align*}
\text{D-Glyceraldehyde} & \quad \text{3-phosphate} \\
\text{Enz} & \quad \text{Enz} \\
\end{align*}
\]

**STEP 2:** Oxidation occurs by the transfer of a hydride ion from the thiohemiacetal to NAD:\n
\[
\begin{align*}
\text{NAD} & \quad \text{NADH} \\
\text{Enz} & \quad \text{Enz} \\
\end{align*}
\]

**STEP 3:** Reaction of the thioester with phosphate ion gives a tetrahedral carbonyl addition intermediate, which then collapses to regenerate the enzyme and give a mixed anhydride of phosphoric acid and glyceric acid:

\[
\begin{align*}
\text{G} & \quad \text{C} \quad \text{C} \quad \text{H} \quad \text{O} \quad \text{S} \quad \text{Enz} \\
\text{Enz} & \quad \text{Enz} \\
\end{align*}
\]

The higher energy anhydride bond is necessary for the next step to create a molecule of ATP.

**Reaction 7: Transfer of a Phosphate Group from 1,3-Bisphosphoglycerate to ADP**

The transfer of a phosphate group in this reaction involves the exchange of one anhydride group for another, namely, the mixed anhydride of 1,3-bisphosphoglycerate for the new phosphoric anhydride in ATP:

\[
\begin{align*}
\text{1,3-Bisphosphoglycerate} & \quad \text{ADP} \\
\text{3-Phosphoglycerate} & \quad \text{ATP} \\
\end{align*}
\]
21.4 What Are the Fates of Pyruvate?

Pyruvate does not accumulate in cells, but rather undergoes one of three enzyme-catalyzed reactions, depending on the state of oxygenation and the type of cell in which it is produced. A key to understanding the biochemical logic responsible for two of these possible fates of pyruvate is to recognize that this compound is produced by the oxidation of glucose through the reactions of glycolysis. NAD$^+$ is the oxidizing agent and is reduced to NADH. For glycolysis to continue, there must be a continuing supply of NAD$^+$; therefore, under anaerobic conditions (in which there is no oxygen present for the reoxidation of NADH), two of the metabolic pathways we describe use pyruvate in ways that regenerate NAD$^+$. 

Reaction 8: Isomerization of 3-Phosphoglycerate to 2-Phosphoglycerate

A phosphate group is transferred from the primary $-\text{OH}$ group on carbon 3 to the secondary $-\text{OH}$ group on carbon 2:

\[
\text{3-Phosphoglycerate} \xrightarrow{\text{mutase}} \text{2-Phosphoglycerate}
\]

Reaction 9: Dehydration of 2-Phosphoglycerate

Dehydration of the primary alcohol (Section 8.2E) of 2-phosphoglycerate gives phosphoenolpyruvate, which is the ester of phosphoric acid and the enol form of pyruvic acid:

\[
\text{2-Phosphoglycerate} \xrightarrow{\text{enolase}} \text{Phosphoenolpyruvate}
\]

Reaction 10: Transfer of a Phosphate Group from Phosphoenolpyruvate to ADP

Reaction 10 is divided into two steps: the transfer of a phosphate group to ADP to produce ATP and the conversion of the enol form of pyruvate to its keto form by keto–enol tautomerism (Section 12.8A). It is common in writing biochemical reactions to show some reactants and products by a curved arrow set either over or under the main reaction arrow. We use this convention here to show that in this reaction ADP is converted to ATP.

\[
\text{Phosphoenolpyruvate} \xrightarrow{\text{pyruvate kinase}} \text{Enol of pyruvate} \xrightarrow{\text{ATP}} \text{Pyruvate}
\]

Summing these ten reactions gives a balanced equation for the net reaction of glycolysis:

\[
\text{C}_6\text{H}_{12}\text{O}_6 + 2\text{NAD}^+ + 2\text{HPO}_4^{2-} + 2\text{ADP} \xrightarrow{\text{10 enzyme-catalyzed steps}} 2\text{CH}_3\text{C}O\text{O}^- + 2\text{H}^+ + 2\text{NADH} + 2\text{ATP}
\]

Glucose Pyruvate
A. Reduction to Lactate—Lactate Fermentation

In vertebrates, the most important pathway for the regeneration of NAD\(^+\) under anaerobic conditions is the reduction of pyruvate to lactate, catalyzed by the enzyme lactate dehydrogenase:

\[
\text{Pyruvate} \stackrel{\text{lactate dehydrogenase}}{\longrightarrow} \text{Lactate}
\]

\[
\text{CH}_3\text{COO}^- + \text{NADH} + \text{H}_3\text{O}^+ \rightarrow \text{CH}_3\text{CHOO}^- + \text{NAD}^+ + \text{H}_2\text{O}
\]

Even though lactate fermentation allows glycolysis to continue in the absence of oxygen, it also brings about an increase in the concentration of lactate, and, perhaps more importantly, it increases the concentration of hydronium ion, H\(_3\)O\(^+\), in muscle tissue and in the bloodstream. This buildup of lactate and H\(_3\)O\(^+\) is associated with muscle fatigue. When blood lactate reaches a concentration of about 0.4 mg/100 mL, muscle tissue becomes almost completely exhausted.

**EXAMPLE 21.2**

Show that glycolysis, followed by the reduction of pyruvate to lactate (lactate fermentation), leads to an increase in the hydrogen ion concentration in the bloodstream.

**SOLUTION**

Lactate fermentation produces lactic acid, which is completely ionized at pH 7.4, the normal pH of blood plasma. Therefore, the hydronium ion concentration increases:

\[
\text{C}_6\text{H}_{12}\text{O}_6 + 2\text{H}_2\text{O} \rightarrow 2\text{CH}_3\text{CHOO}^- + 2\text{H}_3\text{O}^+
\]

**PROBLEM 21.2**

Does lactate fermentation result in an increase or a decrease in blood pH?

B. Reduction to Ethanol—Alcoholic Fermentation

Yeast and several other organisms have an alternative pathway to regenerate NAD\(^+\) under anaerobic conditions. In the first step of this pathway, pyruvate undergoes enzyme-catalyzed decarboxylation to give acetaldehyde:

\[
\text{Pyruvate} \stackrel{\text{pyruvate decarboxylase}}{\longrightarrow} \text{Acetaldehyde}
\]

\[
\text{CH}_3\text{COO}^- + \text{H}_3\text{O}^+ \rightarrow \text{CH}_3\text{CH} + \text{CO}_2 + \text{H}_2\text{O}
\]

The carbon dioxide produced in this reaction is responsible for the foam on beer and the carbonation of naturally fermented wines and champagnes. In the second step, acetaldehyde is reduced by NADH to ethanol:

\[
\text{Acetaldehyde} \stackrel{\text{alcohol dehydrogenase}}{\longrightarrow} \text{Ethanol}
\]

\[
\text{CH}_3\text{CH} + \text{NADH} + \text{H}_3\text{O}^+ \rightarrow \text{CH}_3\text{CH}_2\text{OH} + \text{NAD}^+ + \text{H}_2\text{O}
\]

Adding the reactions for the decarboxylation of pyruvate and the reduction of acetaldehyde to the net reaction of glycolysis gives the overall reaction of alcoholic fermentation:

\[
\text{C}_6\text{H}_{12}\text{O}_6 + 2\text{HPO}_4^{2-} + 2\text{ADP} + 2\text{H}^+ \rightarrow 2\text{CH}_3\text{CH}_2\text{OH} + 2\text{CO}_2 + 2\text{ATP}
\]

\[
\text{Glucose} \rightarrow \text{Ethanol}
\]
C. Oxidation and Decarboxylation to Acetyl-CoA

Under aerobic conditions, pyruvate undergoes oxidative decarboxylation in which the carboxylate group is converted to carbon dioxide and the remaining two carbons are converted to the acetyl group of acetyl-CoA:

\[
\text{Oxidative decarboxylation} \\
\begin{array}{c}
\text{Pyruvate} \\
\text{CH}_3\text{COO}^- + \text{NAD}^+ + \text{CoASH} \\
\rightarrow \\
\text{Acetyl-CoA} \\
\text{CH}_3\text{CSCoA} + \text{CO}_2 + \text{NADH}
\end{array}
\]

The oxidative decarboxylation of pyruvate is considerably more complex than is suggested by the preceding equation. In addition to utilizing \(\text{NAD}^+\) and coenzyme A, this transformation also requires FAD, thiamine pyrophosphate (which is derived from thiamine, vitamin B1), and lipoic acid:

Acetyl coenzyme A then becomes a fuel for the citric acid cycle, which results in oxidation of the two-carbon chain of the acetyl group to \(\text{CO}_2\), with the production of \(\text{NADH}\) and \(\text{FADH}_2\). These reduced coenzymes are, in turn, oxidized to \(\text{NAD}^+\) and \(\text{FAD}\) during respiration, with \(\text{O}_2\) as the oxidizing agent.

21.5 What Are the Reactions of the \(\beta\)-Oxidation of Fatty Acids?

The first phase in the catabolism of fatty acids involves their release from triglycerides, either those stored in adipose tissue or those ingested from the diet. The hydrolysis of triglycerides is catalyzed by a group of enzymes called lipases.

The free fatty acids then pass into the bloodstream and on to cells for oxidation. There are two major stages in the \(\beta\)-oxidation of fatty acids: (1) activation of a free fatty acid in the cytoplasm and its transport across the inner mitochondrial membrane, followed by (2) \(\beta\)-oxidation, a repeated sequence of four reactions.

A. Activation of Fatty Acids: The Formation of a Thioester with Coenzyme A

The process of \(\beta\)-oxidation begins in the cytoplasm with the formation of a thioester between the carboxyl group of a fatty acid and the sulphydryl group of coenzyme A. The
formation of this acyl-CoA derivative is coupled with the hydrolysis of ATP to AMP and pyrophosphate ion.

$$\text{Fatty acid Coenzyme A \rightarrow ATP + AMP + P}_{2}O_{7}^{-} \rightarrow \text{An acyl-CoA derivative}$$

The mechanism of this reaction involves attack by the fatty acid carboxylate anion on $P\equiv O$ of a phosphoric anhydride group of ATP to form an intermediate analogous to the tetrahedral carbonyl addition intermediate formed in $C\equiv O$ chemistry. In the intermediate formed in the fatty acid–ATP reaction, the phosphorus attacked by the carboxylate anion becomes bonded to five groups. The collapse of this intermediate gives an acyl-AMP, which is a highly reactive mixed anhydride of the carboxyl group of the fatty acid and the phosphate group of AMP:

$$\text{Fatty acid Coenzyme A \rightarrow ATP + AMP + P}_{2}O_{7}^{-} \rightarrow \text{An acyl-CoA derivative}$$

This mixed anhydride then undergoes a carbonyl addition reaction with the sulphydryl group of coenzyme A to form a tetrahedral carbonyl addition intermediate, which collapses to give AMP and an acyl-CoA (a fatty acid thioester of coenzyme A):

$$\text{CoA-SH + An acyl-AMP \rightarrow AMP + CoA-S-CO}$$

At this point, the activated fatty acid is transported into the mitochondrion, where its carbon chain is degraded by the reactions of $\beta$-oxidation.

## B. The Four Reactions of $\beta$-Oxidation

### Reaction 1: Oxidation of the Hydrocarbon Chain

The first reaction of $\beta$-oxidation is oxidation of the carbon chain between the alpha- and beta-carbons of the fatty acid chain. The oxidizing agent is FAD, which is reduced to FADH$_2$. This reaction is stereoselective: Only the trans alkene isomer is formed:
What Are the Reactions of the \( \beta \)-Oxidation of Fatty Acids?

**Reaction 2: Hydration of the Carbon–Carbon Double Bond**

Enzyme-catalyzed hydration of the carbon–carbon double bond gives a \( \beta \)-hydroxyacyl-CoA:

\[
\begin{align*}
\text{Enzyme-catalyzed hydration} & \quad \text{gives a } \beta\text{-hydroxyacyl-CoA:} \\
\text{Enol-CoA hydrazase} & \quad \text{Enol-CoA hydrazase}
\end{align*}
\]

Note that the hydration is regioselective: The \(-\text{OH} \) is added to carbon 3 of the chain. It is also stereoselective: Only the \( R \) enantiomer is formed.

**Reaction 3: Oxidation of the \( \beta \)-Hydroxyl Group**

In the second oxidation step of \( \beta \)-oxidation, the secondary alcohol is oxidized to a ketone. The oxidizing agent is NAD\(^+\), which is reduced to NADH:

\[
\begin{align*}
\text{NAD}\(^+\) & \quad \text{NAD}\(^+\) \\
\text{NADH} & \quad \text{NADH}
\end{align*}
\]

**Reaction 4: Cleavage of the Carbon Chain**

The final step of \( \beta \)-oxidation is cleavage of the carbon chain to give a molecule of acetyl coenzyme A and a new acyl-CoA, the hydrocarbon chain of which is shortened by two carbon atoms:

\[
\begin{align*}
\text{Coenzyme A} & \quad \text{Coenzyme A} \\
\text{Acetyl-CoA} & \quad \text{Acetyl-CoA}
\end{align*}
\]

**Mechanism**

**A Reverse Claisen Condensation in \( \beta \)-Oxidation of Fatty Acids**

**STEP 1:** A sulfhydryl group of the enzyme thiolase attacks the carbonyl carbon of the ketone to form a tetrahedral carbonyl addition intermediate.

**STEP 2:** The addition intermediate collapses to give the enolate anion of acetyl-CoA and an enzyme-bound thioester, which is now shortened by two carbons.

**STEP 3:** The enolate anion reacts with a proton donor to give acetyl-CoA.
STEP 4: The enzyme–thioester intermediate undergoes reaction with a molecule of coenzyme A to regenerate a sulfhydryl group on the surface of the enzyme and liberate the fatty acyl-CoA, now shortened by two carbon atoms.

\[
\begin{align*}
&\text{R–C–CH}_2\text{C–SCoA} \quad \text{(1)} \quad \text{R–C–CH}_2\text{C–SCoA} \quad \text{Enz}\text{–S} \\
&\text{Enz}\text{–S} \quad \text{Tetrahedral carbonyl addition intermediate} \\
&\text{R–C–CH}_2\text{C–SCoA} \quad \text{(2)} \quad \text{R–C–S–Enz} + \text{CH}_2\text{C–SCoA} \\
&\text{An enzyme–thioester} \quad \text{Enolate anion of acetyl-CoA} \\
&\text{CoA–SH} \quad \text{(4)} \quad \text{(5) proton donor} \\
&\text{Enz}\text{–SH} + \text{R–C–S–CoA} \quad \text{A fatty acyl-CoA} \\
&\text{CH}_3\text{C–SCoA} \quad \text{Acetyl-CoA}
\end{align*}
\]

If Steps 1–3 of this mechanism are read in reverse, it is seen as an example of a Claisen condensation (Section 15.3A)—the attack by the enolate anion of acetyl-CoA on the carbonyl group of a thioester to form a tetrahedral carbonyl addition intermediate, followed by its collapse to give a β-ketothioester.

The four steps in β-oxidation are summarized in Figure 21.2.

C. Repetition of the β-Oxidation Spiral Yields Additional Acetate Units

The series of four reactions of β-oxidation then repeats on the shortened fatty acyl-CoA chain and continues until the entire fatty acid chain is degraded to acetyl-CoA. Seven cycles
of $\beta$-oxidation of palmitic acid, for example, give eight molecules of acetyl-CoA and involve seven oxidations by FAD and seven oxidations by NAD$^+$:

$$
\text{CH}_3\text{CH}(\text{CH}_2)_{14}\text{COH} + 8\text{CoA} \rightarrow \text{SH} + 7\text{NAD}^+ + 7\text{FAD} \rightarrow 8\text{CH}_3\text{CSCoA} + 7\text{NADH} + 7\text{FADH}_2
$$

Hexadecanoic acid (Palmitic acid)

### 21.6 What Are the Reactions of the Citric Acid Cycle?

Under aerobic conditions, the central metabolic pathway for the oxidation of the carbon skeletons not only of carbohydrates, but also of fatty acids and amino acids, to carbon dioxide is the citric acid cycle, also known as the tricarboxylic acid (TCA) cycle and Krebs cycle. The last-mentioned name is in honor of Sir Adolph Krebs, the biochemist who first proposed the cyclic nature of this pathway in 1937.

**A. Overview of the Cycle**

Through the reactions of the citric acid cycle, the carbon atoms of the acetyl group of acetyl-CoA are oxidized to carbon dioxide. There are four separate oxidations in the cycle, three involving NAD$^+$ and one involving FAD. Figure 21.3 gives an overview of the cycle, showing the four steps.

**B. Reactions of the Citric Acid Cycle**

1. **Formation of Citrate**

The two-carbon acetyl group of acetyl coenzyme A enters the cycle by an enzyme-catalyzed aldol reaction (Section 15.2) between the alpha carbon of acetyl-CoA and the ketone group of oxaloacetate. The product of this reaction is citrate, the tricarboxylic acid from which
the cycle derives its name. In the reaction, the carbonyl condensation is coupled with the hydrolysis of the thioester to give free coenzyme A:

\[
\begin{align*}
\text{Oxaloacetate} & \quad \text{Citrate} \\
\text{citrate synthase} & \quad \text{CoA} \\
\end{align*}
\]

2. Isomerization of Citrate to Isocitrate

In the second step of the cycle, citrate is converted to the constitutional isomer isocitrate. This isomerization occurs in two steps, both catalyzed by aconitase. First, in a reaction analogous to the acid-catalyzed dehydration of an alcohol (Section 8.2E), citrate undergoes enzyme-catalyzed dehydration of aconitate. Then, in a reaction analogous to acid-catalyzed hydration of an alkene (Section 5.3B), aconitate undergoes enzyme-catalyzed hydration to give isocitrate.

There are several important features to note about this transformation:

- The dehydration of citrate is completely regioselective: Dehydration is in the direction of the \(-\text{CH}_2-\) group from the original molecule of oxaloacetate.
- The dehydration of citrate is completely stereoselective: It gives only the cis isomer of aconitate.
- The hydration of aconitate is completely regioselective: It gives only isocitrate.
- The hydration of aconitate is completely stereoselective: Isocitrate has two stereocenters, and four possible stereoisomers (two pairs of enantiomers). Only one of the four stereoisomers is produced in this enzyme-catalyzed hydration.

3. Oxidation and Decarboxylation of Isocitrate

In Step 3, the secondary alcohol of isocitrate is oxidized to a ketone by NAD\(^+\) in a reaction catalyzed by the enzyme isocitrate dehydrogenase. The product, oxalosuccinate, is a \(\beta\)-ketoacid and undergoes decarboxylation (Section 13.8) to produce \(\alpha\)-ketoglutarate:

Note that only one of the three carboxyl groups in oxalosuccinate is beta to the ketone carbonyl; it is this carboxyl group that undergoes the decarboxylation.

4. Oxidation and Decarboxylation of \(\alpha\)-Ketoglutarate

The second molecule of carbon dioxide is generated in the cycle by the same type of oxidative decarboxylation as that for the conversion of pyruvate (also an \(\alpha\)-ketoacid) to acetyl-CoA.
and carbon dioxide (Section 21.4C). In the oxidative decarboxylation of \( \alpha \)-ketoglutarate, the carboxyl group is converted to carbon dioxide and the adjacent ketone is oxidized to a carboxyl group in the form of a thioester with coenzyme A:

\[
\begin{align*}
\text{CH}_2\text{---COO}^- & \quad \text{CH}_2\text{---COO}^- \\
\text{CH}_2 & + \text{NAD}^+ + \text{CoA} \quad \longrightarrow \\
\text{O=C---COO}^- & \quad \text{O=C---S---CoA} \\
\alpha\text{-Ketoglutarate} & \quad \text{Succinyl CoA}
\end{align*}
\]

*Note.* Of the two molecules of carbon dioxide given off in this turn of the citric acid cycle, both carbons are from the carbon skeleton of oxaloacetate; neither is from the acetyl group of acetyl coenzyme A.

5. **Conversion of Succinyl CoA to Succinate**

Next, in coupled reactions catalyzed by succinyl CoA synthetase, succinyl CoA, HPO\(_4\)^{2-}, and guanosine diphosphate (GDP) react to form succinate, guanosine triphosphate (GTP), and coenzyme A:

\[
\begin{align*}
\text{CH}_2\text{---COO}^- & \quad \text{COO}^- \\
\text{CH}_2 & + \text{GDP} + \text{HPO}_4^{2-} \quad \longrightarrow \\
\text{O=C---S---CoA} & \quad \text{CH}_2 + \text{GTP} + \text{CoA} \quad \longrightarrow \\
\text{Succinyl CoA} & \quad \text{Succinate}
\end{align*}
\]

Observe that to this point in the cycle, the two carbons of the original acetyl group of acetyl-CoA have remained differentiated from the carbon atoms of oxaloacetate. With the production of succinate, however, the two \(-\text{CH}_2\) groups, as well as the two \(-\text{COO}^-\) groups, are now indistinguishable.

6. **Oxidation of Succinate**

In the third oxidation of the cycle, succinate is oxidized to fumarate. The oxidizing agent is FAD, which is reduced to FADH\(_2\):

\[
\begin{align*}
\text{COO}^- & \quad \text{H} \quad \text{COO}^- \\
\text{CH}_2 & + \text{FAD} \quad \longrightarrow \\
\text{CH}_2 & \quad \text{COO}^- \quad \longrightarrow \\
\text{Succinate} & \quad \text{Fumarate}
\end{align*}
\]

This oxidation is completely stereoselective: Only the *trans* isomer is formed.

7. **Hydration of Fumarate**

In the second hydration step of the cycle, fumarate is converted to malate:

\[
\begin{align*}
\text{H} \quad \text{COO}^- & \quad \text{HO---CH---COO}^- \\
\text{C} & + \text{H}_2\text{O} \quad \longrightarrow \\
\text{Fumarate} & \quad \text{Malate}
\end{align*}
\]

Fumarase, the enzyme catalyzing this hydration, recognizes only fumarate (and not its *cis* isomer) and gives malate as a single enantiomer.
8. Oxidation of Malate

In the fourth oxidation of the cycle, the secondary alcohol of malate is oxidized to a ketone by NAD⁺:

\[
\text{HO} - \text{CH} - \text{COO}^- + \text{NAD}^+ \xrightarrow{\text{malate dehydrogenase}} \text{O} = \text{C} - \text{COO}^- + \text{NADH} + \text{H}^+
\]

Malate  Oxaloacetate

With the production of oxaloacetate, the reactions of the citric acid cycle are complete. Continued operation of the cycle requires two things: (1) a supply of carbon atoms in the form of acetyl groups from acetyl-CoA and (2) a supply of oxidizing agents in the form of NAD⁺ and FAD. For a continuing supply of these two oxidizing agents, the operation of the cycle depends on the reactions of respiration and electron transport, a series of reactions in which the reduced coenzymes NADH and FADH₂ are reoxidized by molecular oxygen, O₂.

Another important feature of the cycle is best seen by examining the balanced equation for the cycle:

\[
\text{CH}_3\text{CSCoA} + 3\text{NAD}^+ + \text{FAD} + \text{HPO}_4^{2-} + \text{ADP} \xrightarrow{\text{citric acid cycle}} 2\text{CO}_2 + 3\text{NADH} + \text{FADH}_2 + \text{ATP} + \text{CoA} - \text{SH}
\]

The cycle is truly catalytic: Its intermediates do not enter into the balanced equation for this pathway; they are neither destroyed nor synthesized in the net reaction. The only function of the cycle is to accept acetyl groups from acetyl-CoA, oxidize them to carbon dioxide, and at the same time produce a supply of reduced coenzymes as fuel for electron transport and oxidative phosphorylation. In fact, if any of the intermediates of the cycle are removed, the cycle ceases because there is no way to regenerate oxaloacetate. Fortunately, the cycle is connected to other metabolic pathways through several of its intermediates. In practice, certain intermediates can be used to synthesize other biomolecules, provided that another intermediate is supplied, which in turn can be converted to oxaloacetate, thus making up for the intermediate withdrawn.

**SUMMARY OF KEY QUESTIONS**

**Section 21.1  What Are the Key Participants in Glycolysis, the β-Oxidation of Fatty Acids, and the Citric Acid Cycle?**

ATP, ADP, and AMP are agents for the storage and transfer of phosphate groups. Nicotinamide adenine dinucleotide (NAD⁺) and flavin adenine dinucleotide (FAD) are agents for the storage and transport of electrons in metabolic oxidations and reductions. NAD⁺ is a two-electron oxidizing agent and is reduced to NADH, which, in turn, is a two-electron reducing agent and is oxidized to NAD⁺. In the reactions of FAD that are involved in the β-oxidation of fatty acids, FAD is a two-electron oxidizing agent and is reduced to FADH₂.

Coenzyme A is a carrier of acetyl groups.

**Section 21.2  What Is Glycolysis?**

Glycolysis is a series of ten enzyme-catalyzed reactions that oxidizes glucose to two molecules of pyruvate.
Section 21.3 What Are the Ten Reactions of Glycolysis?

The ten reactions of glycolysis can be grouped in the following way:

- Oxidation of an aldehyde group to the mixed anhydride of a carboxylic acid and phosphoric acid (Reaction 6).
- Transfer of a phosphate group from a monosaccharide intermediate to ADP to form ATP (Reactions 7 and 10).
- Transfer of a phosphate group from a 1° alcohol to a 2° alcohol (Reaction 8).
- Dehydration of a 1° alcohol to form a carbon–carbon double bond (Reaction 9).

Section 21.4 What Are the Fates of Pyruvate?

Pyruvate, the product of anaerobic glycolysis, does not accumulate in cells but rather undergoes one of three possible enzyme-catalyzed reactions, depending on the state of oxygenation and the type of cell in which the pyruvate is produced.

- In lactate fermentation, pyruvate is reduced to lactate by NADH.
- In alcoholic fermentation, pyruvate is converted to acetaldehyde, which is reduced to ethanol by NADH.
- Under aerobic conditions, pyruvate is oxidized to acetyl coenzyme A by NAD⁺.

Section 21.5 What Are the Reactions of the β-Oxidation of Fatty Acids?

There are two major stages in the metabolism of fatty acids:

- (1) Activation of free fatty acids in the cytoplasm through the formation of thioesters with coenzyme A and transport of the activated fatty acids across the inner mitochondrial membrane followed by

Section 21.6 What Are the Reactions of the Citric Acid Cycle?

The citric acid cycle accepts the two-carbon acetyl group from acetyl-CoA and in a series of steps oxidizes it to two molecules of carbon dioxide. Oxidizing agents are NAD⁺ and FAD.

QUICK QUIZ

Answer true or false to the following questions to assess your general knowledge of the concepts in this chapter. If you have difficulty with any of them, you should review the appropriate section in the chapter (shown in parentheses) before attempting the more challenging end-of-chapter problems.

1. Fatty acids are metabolized through the process of β-oxidation. (21.5)
2. NAD⁺, NADH, FAD, and FADH₂ are coenzymes that undergo oxidation and reduction during metabolism. (21.1)
3. An end product of glycolysis is pyruvate. (21.2–21.4)
4. The starting point of the citric acid cycle involves acetyl CoA, which can be formed from carbohydrates, triglycerides, or proteins. (21.6)
5. The conversion of a –CH₂–CH₂– unit to –CH=CH– by FAD is a reduction reaction. (21.1, 21.5)
6. Lactate fermentation allows pyruvate to be metabolized under aerobic conditions. (21.4)
7. All carbohydrates are directly metabolized through the process of glycolysis. (21.1–21.4)
8. Keto–enol tautomerism is an important reaction in glycolysis. (21.3)
9. Coenzyme A is used in metabolism to store and transfer hydroxyl groups. (21.1)
10. Coenzyme A contains a terminal —OH group, which forms a new bond to acetyl groups in the degradation of monosaccharides, fatty acids, glycerol, and amino acids. (21.1, 21.4–21.6)
11. Alcoholic fermentation is one possible means of pyruvate metabolism in humans. (21.4)
12. ATP, ADP, and AMP are involved in the storage and transfer of adenine groups. (21.1)
13. NAD⁺ acts to oxidize both hydroxyl and carbonyl groups. (21.2)
KEY REACTIONS

1. **Glycolysis (Sections 21.2 and 21.3)**
   Glycolysis is a series of ten enzyme-catalyzed reactions that converts glucose to pyruvate:
   \[
   \text{Glucose} \rightarrow 2\text{CH}_3\text{COO}^- + 2\text{NAD}^+ + 2\text{ATP} + 2\text{H}_3\text{O}^+ \quad \text{glycolysis}
   \]

2. **Reduction of Pyruvate to Lactate: Lactate Fermentation (Section 21.4A)**
   \[
   \text{CH}_3\text{COO}^- + \text{NAD}^+ + \text{H}^+ \rightarrow \text{OH} \rightarrow \text{CH}_3\text{CHCOO}^- + \text{NAD}^+ \quad \text{lactate dehydrogenase}
   \]

3. **Reduction of Pyruvate to Ethanol: Alcohol Fermentation (Section 21.4B)**
   The carbon dioxide formed in this reaction is responsible for the foam on beer and the carbonation of naturally fermented wines and champagnes:
   \[
   \text{CH}_3\text{CH}_2\text{OH} + \text{CO}_2 + \text{NAD}^+ \rightarrow \text{CH}_3\text{CSCO}_2^- + \text{HPO}_4^{2-} + \text{ADP} + 2\text{H}_2\text{O}^+ \quad \text{alcoholic fermentation}
   \]

4. **Oxidative Decarboxylation of Pyruvate to Acetyl-CoA (Section 21.4C)**
   \[
   \text{CH}_3\text{COO}^- + 7\text{NAD}^+ + 7\text{FADH}_2 + 8\text{CoA} \rightarrow 8\text{CH}_3\text{CSCO}_2^- + 7\text{NADH} + 7\text{FAD} + 2\text{CoA} \quad \text{oxidative decarboxylation}
   \]

5. **β-Oxidation of Fatty Acids (Section 21.5)**
   In this series of four enzyme-catalyzed reactions, the carbon chain of a fatty acid is shortened by two carbon atoms at a time:
   \[
   \text{CH}_3(\text{CH}_2)_{14}\text{COH} \rightarrow 8\text{NAD}^+ + 8\text{FAD} + 8\text{ATP} + 8\text{AMP} + 8\text{HPO}_4^{2-} \rightarrow 8\text{CH}_3\text{CSCO}_2^- + 7\text{NADH} + 7\text{FADH}_2 + 8\text{CoA} \quad \text{Acetyl coenzyme A}
   \]

6. **Citric Acid Cycle (Section 21.6)**
   Through the reactions of the citric acid cycle, the carbon atoms of the acetyl group of acetyl-CoA are oxidized to carbon dioxide:
   \[
   \text{CH}_3\text{CSCO}_2^- \rightarrow 2\text{CO}_2 + 3\text{NAD}^+ + \text{FADH}_2 + \text{ATP} + \text{CoA} \quad \text{citric acid cycle}
   \]
   There are four separate oxidation steps in the cycle, three involving NAD\(^+\) and one involving FAD.

PROBLEMS

A problem marked with an asterisk indicates an applied “real-world” problem. Answers to problems whose numbers are printed in blue are given in Appendix D.

Sections 21.2 and 21.3  **Glycolysis**

*21.3* In many enzyme-catalyzed reactions, a group on the enzyme functions as a proton donor. List several amino acid side chains that might function as proton acceptors.

*21.4* In many other enzyme-catalyzed reactions, a group on the enzyme surface functions as a proton acceptor.

21.5 Name one coenzyme required for glycolysis. From what vitamin is the coenzyme derived?
21.6 Number the carbons of glucose 1 through 6. Which carbons of glucose become the carboxyl groups of the two pyruvates?

21.7 How many moles of lactate are produced from 3 moles of glucose?

*21.8 Although glucose is the principal source of carbohydrates for glycolysis, fructose and galactose are also metabolized for energy.
(a) What is the main dietary source of fructose? Of galactose?
(b) Propose a series of reactions by which fructose might enter glycolysis.
(c) Propose a series of reactions by which galactose might enter glycolysis.

*21.9 How many moles of ethanol are produced per mole of sucrose through the reactions of glycolysis and alcoholic fermentation? How many moles of CO₂ are produced?

*21.10 Glycerol that is derived from the hydrolysis of triglycerides and phospholipids is also metabolized for energy. Propose a series of reactions by which the carbon skeleton of glycerol might enter glycolysis and be oxidized to pyruvate.

21.11 Write a mechanism to show the role of NADH in the reduction of acetaldehyde to ethanol.

*21.12 Ethanol is oxidized in the liver to acetate ion by NAD⁺. 
(a) Write a balanced equation for this oxidation.
(b) Do you expect the pH of blood plasma to increase, decrease, or remain the same as a result of the metabolism of a significant amount of ethanol?

21.13 When pyruvate is reduced to lactate by NADH, two hydrogens are added to pyruvate: one to the carbonyl carbon, the other to the carbonyl oxygen. Which of these hydrogens is derived from NADH?

21.14 Why is glycolysis called an anaerobic pathway?

21.15 Which carbons of glucose end up in CO₂ as a result of alcoholic fermentation?

21.16 Which steps in glycolysis require ATP? Which steps produce ATP?

Section 21.5 β-Oxidation

21.17 Write structural formulas for palmitic, oleic, and stearic acids, the three most abundant fatty acids.

21.18 A fatty acid must be activated before it can be metabolized in cells. Write a balanced equation for the activation of palmitic acid.

21.19 Name three coenzymes that are necessary for the β-oxidation of fatty acids. From what vitamin is each derived?

*21.20 We have examined β-oxidation of saturated fatty acids, such as palmitic acid and stearic acid. Oleic acid, an unsaturated fatty acid, is also a common component of dietary fats and oils. This unsaturated fatty acid is degraded by β-oxidation, but, at one stage in its degradation, requires an additional enzyme named enoyl-CoA isomerase. Why is this enzyme necessary, and what isomerization does it catalyze? (Hint: Consider both the configuration of the carbon-carbon double bond in oleic acid and its position in the carbon chain.)

Section 21.6 Citric Acid Cycle

21.21 What is the main function of the citric acid cycle?

21.22 Which steps in the citric acid cycle involve
(a) The formation of new carbon–carbon bonds
(b) The breaking of carbon–carbon bonds
(c) Oxidation by NAD⁺
(d) Oxidation by FAD
(e) Decarboxylation
(f) The creation of new stereocenters

21.23 What does it mean to say that the citric acid cycle is catalytic—that is, that it does not produce any new compounds?

Additional Problems

21.24 Review the oxidation reactions of glycolysis, β-oxidation, and the citric acid cycle, and compare the types of functional groups oxidized by NAD⁺ with those oxidized by FAD.

*21.25 The respiratory quotient (RQ), used in studies of energy metabolism and exercise physiology, is defined as the ratio of the volume of carbon dioxide produced to the volume of oxygen used:
\[ RQ = \frac{\text{Volume CO}_2}{\text{Volume O}_2} \]
(a) Show that RQ for glucose is 1.00. (Hint: Look at the balanced equation for the complete
oxidation of glucose to carbon dioxide and water.)

(b) Calculate RQ for triolein, a triglyceride with the molecular formula C_{57}H_{104}O_{6}.

(c) For an individual on a normal diet, RQ is approximately 0.85. Would this value increase or decrease if ethanol were to supply an appreciable portion of the person's caloric needs?

**21.26** Acetoacetate, β-hydroxybutyrate, and acetone are commonly referred to within the health sciences as "ketone bodies," in spite of the fact that one of them is not a ketone at all. All are products of human metabolism and are always present in blood plasma. Most tissues (with the notable exception of the brain) have the enzyme systems necessary to use ketone bodies as energy sources. Ketone bodies are synthesized by the enzyme-catalyzed reactions shown below. Describe the type of reaction involved in each step.

**21.27** A connecting point between anaerobic glycolysis and β-oxidation is the formation of acetyl-CoA. Which carbon atoms of glucose appear as methyl groups of acetyl-CoA? Which carbon atoms of palmitic acid appear as methyl groups of acetyl-CoA?

**21.28** Which of the steps in the following biochemical pathways use molecular oxygen as the oxidizing agent?

(a) Glycolysis  
(b) β-Oxidation  
(c) The citric acid cycle

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GROUP LEARNING ACTIVITIES

21.29 Compare biological (enzyme-catalyzed) reactions with laboratory reactions in terms of

(a) Efficiency of yields  
(b) Regiochemical outcome of products  
(c) Stereochemical outcome of products

21.30 Comment on the importance of stereochemistry in the synthesis of new drugs.

*21.31 Of the functional groups that we have studied, which are affected by the acidity of biological environments (biological pH)?

21.32 Can you think of any aspect of your day-to-day life that does not involve or is not affected by organic chemistry? Explain.