# 29 The Organic Chemistry of Metabolic Pathways

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Anyone who wants to understand or contribute to the revolution now taking place in the biological sciences must first understand life processes at the molecular level. This understanding, in turn, must be based on a detailed knowledge of the chemical reactions and paths used by living organisms. Just knowing *what* occurs is not enough; it's also necessary to understand *how* and *why* organisms use the chemistry they do.

Biochemical reactions are not mysterious. It's true that many of the biological reactions occurring in even the simplest living organism are more complex than those carried out in any laboratory, yet they follow the same rules of reactivity as laboratory reactions and they take place by the same mechanisms. In past chapters, we've seen many biological reactions used as examples, but it's now time to focus specifically on biological reactions, with particular attention to some typical metabolic pathways that organisms use to synthesize and degrade biomolecules.

A word of warning: biological molecules are often larger and more complex than the substances we've been dealing with thus far. As always, though, keep your focus on the functional groups in those parts of the molecules where changes occur. The reactions themselves are the same sorts of additions, eliminations, substitutions, carbonyl condensations, and so forth, that we've been dealing with all along. By the end of this chapter, a fundamental conclusion should be clear: the chemistry of living organisms *is* organic chemistry.

## WHY THIS CHAPTER?

In this chapter, we'll look at some of the pathways by which organisms carry out their chemistry, focusing primarily on how they metabolize fats and carbohydrates. The treatment will be far from complete, but it should give you an idea of the kinds of processes that occur.

Sean Duggan

## 29.1 An Overview of Metabolism and Biochemical Energy

The many reactions that go on in the cells of living organisms are collectively called **metabolism**. The pathways that break down larger molecules into smaller ones are called **catabolism**, and the pathways that synthesize larger biomolecules from smaller ones are known as **anabolism**. Catabolic reaction pathways are usually exergonic and release energy, while anabolic pathways are often endergonic and absorb energy. Catabolism can be divided into the four stages shown in Figure 29.1.

**Stage 1** Bulk food is digested in the stomach and small intestine to give small molecules.

**Stage 2** Fatty acids, monosaccharides, and amino acids are degraded in cells to yield acetyl CoA.

**Stage 3** Acetyl CoA is oxidized in the citric acid cycle to give $CO_2$.

**Stage 4** The energy released in the citric acid cycle is used by the electron-transport chain to oxidatively phosphorylate ADP and produce ATP.

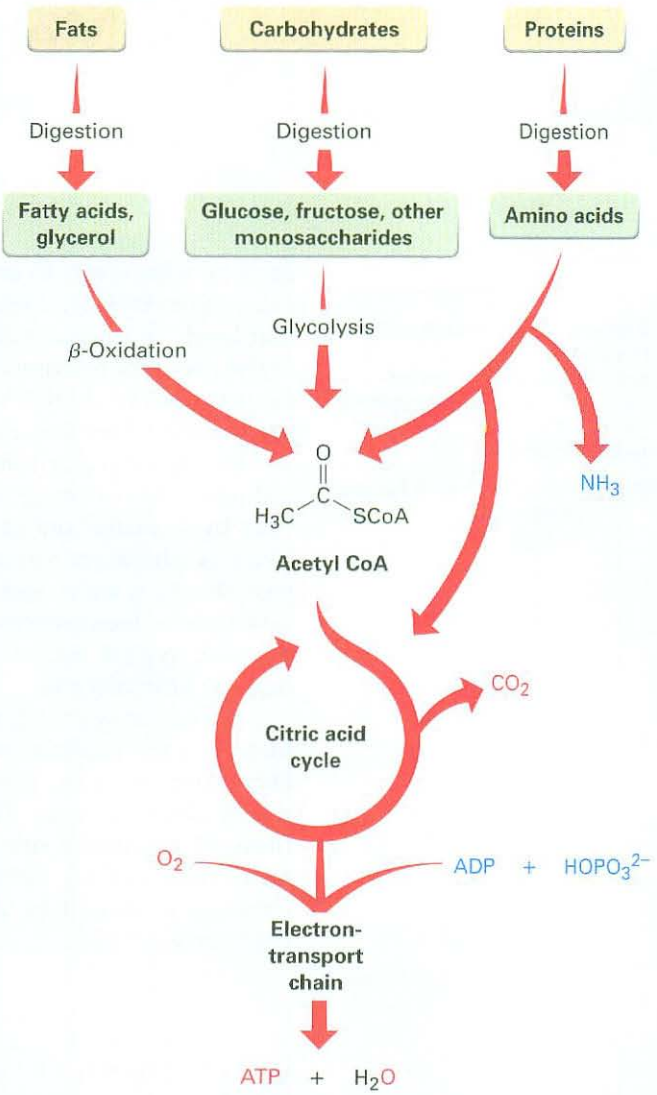
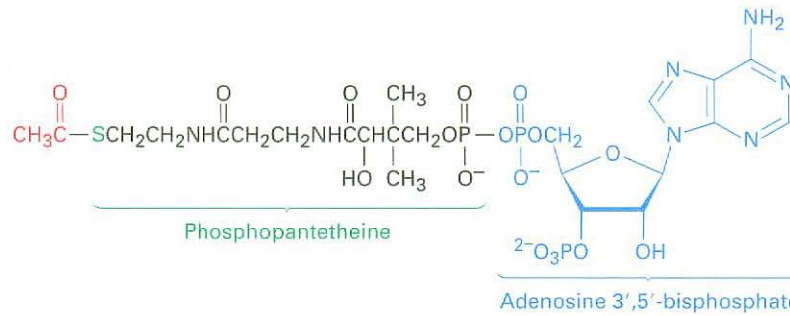


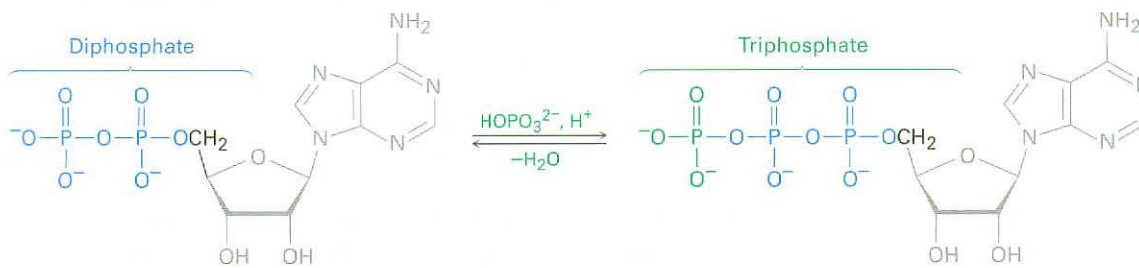

**Figure 29.1** An overview of catabolic pathways for the degradation of food and the production of biochemical energy. The ultimate products of food catabolism are $CO_2$ and $H_2O$, with the energy released in the citric acid cycle used to drive the endergonic synthesis of adenosine triphosphate (ATP) from adenosine diphosphate (ADP) plus phosphate ion, $HOPO_3^{2-}$.

In *digestion*, the first catabolic stage, food is broken down in the mouth, stomach, and small intestine by hydrolysis of ester, glycoside (acetal), and peptide (amide) bonds to yield primarily fatty acids plus glycerol, simple sugars, and amino acids. These smaller molecules are further degraded in the cytoplasm of cells in the second stage of catabolism to yield acetyl groups attached by a thioester bond to the large carrier molecule coenzyme A. The resultant compound, acetyl coenzyme A (acetyl CoA), is a key substance both in the metabolism of food molecules and in numerous other biological pathways. As noted in Section 21.8, the acetyl group in acetyl CoA is linked to the sulfur atom of phosphopantetheine, which is itself linked to adenosine 3′,5′-bisphosphate.




**Acetyl CoA—a thioester**

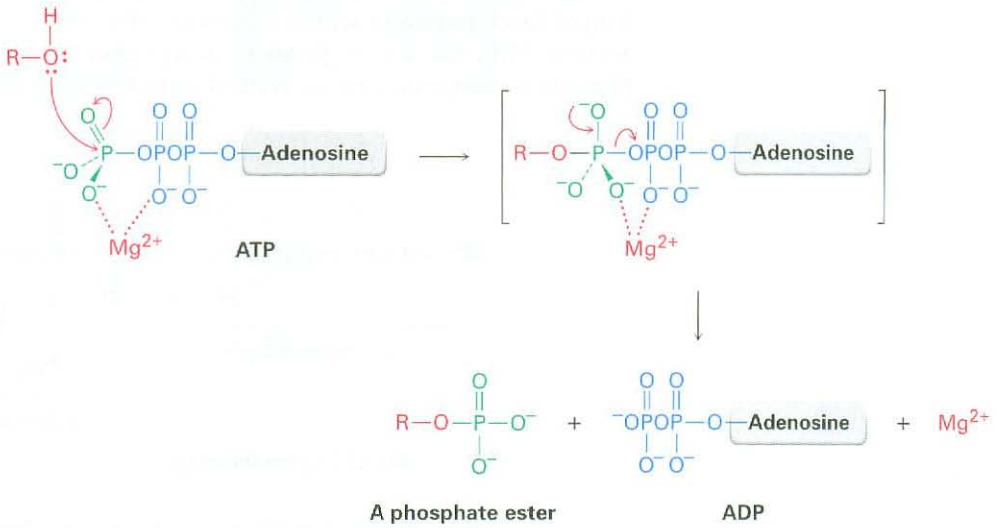
Acetyl groups are oxidized inside cellular mitochondria in the third stage of catabolism, the *citric acid cycle*, to yield $CO_2$. (We'll see the details of the process in Section 29.7.) Like many oxidations, this stage releases a large amount of energy, which is used in the fourth stage, the *electron-transport chain*, to accomplish the endergonic phosphorylation of ADP with hydrogen phosphate ion ($HOPO_3^{2-}$, abbreviated $P_i$) to give ATP. The final result of food catabolism, ATP has been called the "energy currency" of the cell. Catabolic reactions "pay off" in ATP by synthesizing it from ADP plus phosphate ion, and anabolic reactions "spend" ATP by transferring a phosphate group to another molecule, thereby regenerating ADP. Energy production and use in living organisms thus revolves around the ATP ⇄ ADP interconversion.




**Adenosine diphosphate (ADP)** **Adenosine triphosphate (ATP)**

ADP and ATP are both phosphoric acid anhydrides, which contain $-\overset{O}{\overset{\|}{P}}-O-\overset{O}{\overset{\|}{P}}-$ linkages analogous to the $-\overset{O}{\overset{\|}{C}}-O-\overset{O}{\overset{\|}{C}}-$ linkage in carboxylic acid anhydrides. Just as carboxylic anhydrides react with alcohols by breaking a

C–O bond and forming a carboxylic ester (Section 21.5), phosphoric anhydrides react with alcohols by breaking a P–O bond and forming a phosphate ester, $ROPO_3^{2-}$. Note that phosphorylation reactions with ATP generally require the presence of a divalent metal cation in the enzyme, usually $Mg^{2+}$, to form a Lewis acid/base complex with the phosphate oxygen atoms and neutralize some negative charge.




How does the body use ATP? Recall from Section 5.7 that the free-energy change $\Delta G$ must be negative and energy must be released for a reaction to occur spontaneously. If $\Delta G$ is positive, the reaction is unfavorable and the process can't occur spontaneously.

What typically happens for an energetically unfavorable reaction to occur is that it is "coupled" to an energetically favorable reaction so that the overall free-energy change for the two reactions together is favorable. To understand what it means for reactions to be coupled, imagine that reaction 1 does not occur to any reasonable extent because it has a small equilibrium constant and is energetically unfavorable; that is, the reaction has $\Delta G > 0$.

$$(1)\ \mathrm{A} + m \rightleftarrows \mathrm{B} + n \qquad \Delta G > 0$$

where A and B are the biochemically "interesting" substances undergoing transformation, while $m$ and $n$ are enzyme cofactors, $H_2O$, or various other substances.

Imagine also that product $n$ can react with substance $o$ to yield $p$ and $q$ in a second, strongly favorable reaction that has a large equilibrium constant and $\Delta G << 0$.

$$(2)\ n + o \rightleftarrows p + q \qquad \Delta G < 0$$

Considering the two reactions together, they share, or are coupled through, the common intermediate $n$, which is a product in the first reaction and a

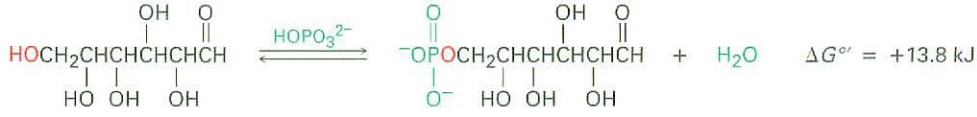
reactant in the second. When even a tiny amount of *n* is formed in reaction 1, it undergoes essentially complete conversion in reaction 2, thereby removing it from the first equilibrium and forcing reaction 1 to continually replenish *n* until the reactant A is gone. That is, the two reactions added together have a favorable $\Delta G < 0$, and we say that the favorable reaction 2 "drives" the unfavorable reaction 1. Because the two reactions are coupled through *n*, the transformation of **A** to **B** becomes possible.

(1) A + *m* ⟷ B + ~~*n*~~ $\Delta G > 0$

(2) ~~*n*~~ + *o* ⟷ *p* + *q* $\Delta G << 0$

Net: A + *m* + *o* ⟷ B + *p* + *q* $\Delta G < 0$

As an example of two reactions that are coupled, look at the phosphorylation reaction of glucose to yield glucose 6-phosphate plus water, an important step in the breakdown of dietary carbohydrates. The reaction of glucose with $HOPO_3^{2-}$ does not occur spontaneously because it is energetically unfavorable, with $\Delta G^{\circ\prime} = +13.8$ kJ/mol. (The standard free-energy change for a biological reaction is denoted $\Delta G^{\circ\prime}$ and refers to a process in which reactants and products have a concentration of 1.0 M in a solution with pH = 7.)




**Glucose** **Glucose 6-phosphate**

With ATP, however, glucose undergoes an energetically favorable reaction to yield glucose 6-phosphate plus ADP. The overall effect is as if $HOPO_3^{2-}$ reacted with glucose and ATP then reacted with the water by-product, making the coupled process favorable by about 16.7 kJ/mol (4.0 kcal/mol). That is, ATP drives the phosphorylation reaction of glucose.

Glucose + ~~$HOPO_3^{2-}$~~ ⟶ Glucose 6-phosphate + ~~$H_2O$~~ $\Delta G^{\circ\prime} = +13.8$ kJ/mol

ATP + ~~$H_2O$~~ ⟶ ADP + ~~$HOPO_3^{2-}$~~ + $H^+$ $\Delta G^{\circ\prime} = -30.5$ kJ/mol

Net: Glucose + ATP ⟶ Glucose 6-phosphate + ADP + $H^+$ $\Delta G^{\circ\prime} = -16.7$ kJ/mol

It's this ability to drive otherwise unfavorable phosphorylation reactions that makes ATP so useful. The resultant phosphates are much more reactive as leaving groups in nucleophilic substitutions and eliminations than the corresponding alcohols they're derived from and are therefore more likely to be chemically useful.

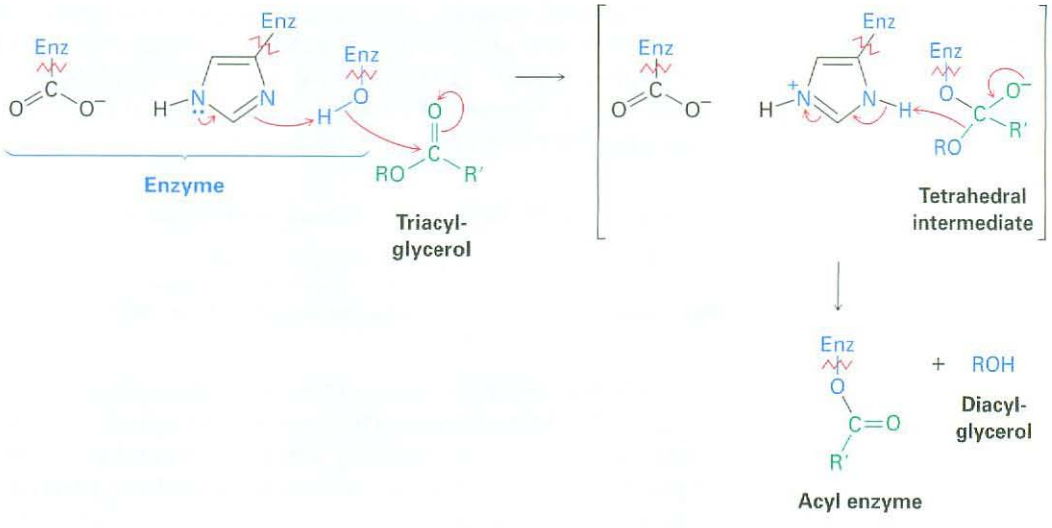
**Problem 29.1** One of the steps in fat metabolism is the reaction of glycerol (1,2,3-propanetriol) with ATP to yield glycerol 1-phosphate. Write the reaction, and draw the structure of glycerol 1-phosphate.

## 29.2 Catabolism of Triacylglycerols: The Fate of Glycerol

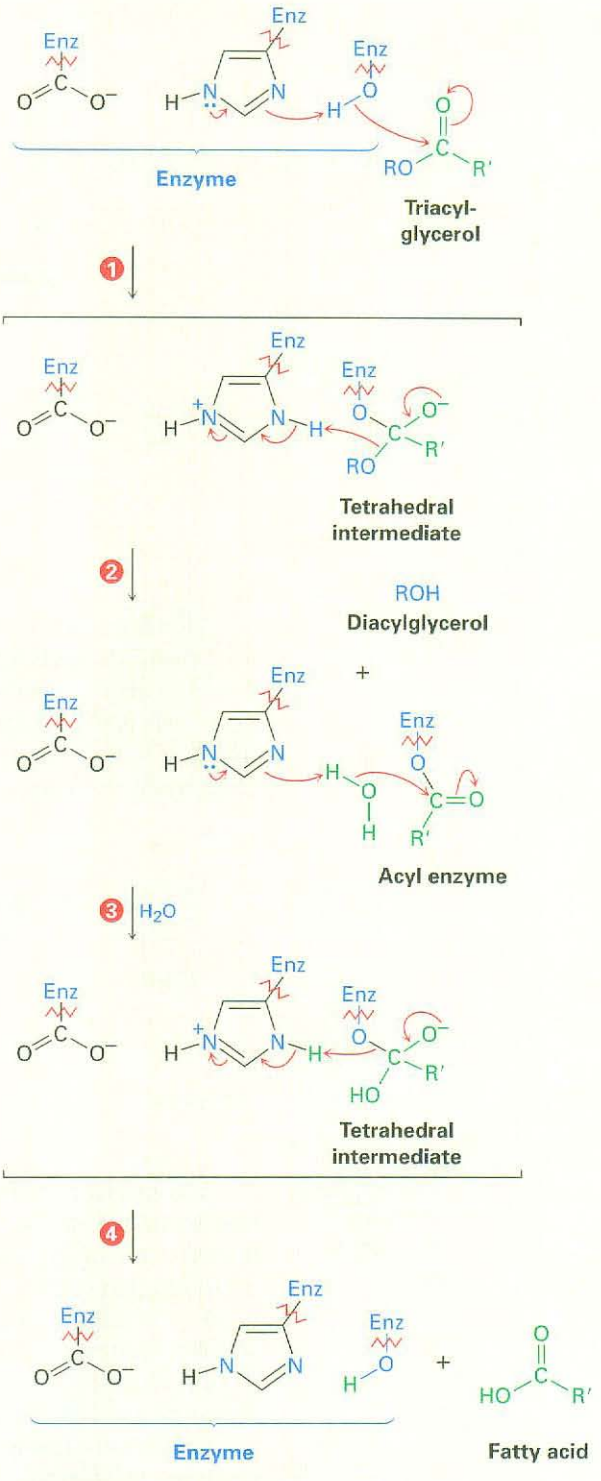
The metabolic breakdown of triacylglycerols begins with their hydrolysis to yield glycerol plus fatty acids. The reaction is catalyzed by a lipase, whose mechanism of action is shown in Figure 29.2. The active site of the enzyme contains a catalytic triad of aspartic acid, histidine, and serine residues, which act cooperatively to provide the necessary acid and base catalysis for the individual steps. Hydrolysis is accomplished by two sequential nucleophilic acyl substitution reactions, one that covalently binds an acyl group to the side chain –OH of a serine residue on the enzyme and a second that frees the fatty acid from the enzyme.

**Steps 1–2 of Figure 29.2: Acyl Enzyme Formation** The first nucleophilic acyl substitution step—reaction of the triacylglycerol with the active-site serine to give an acyl enzyme—begins with deprotonation of the serine alcohol by histidine to form the more strongly nucleophilic alkoxide ion. This proton transfer is facilitated by a nearby side-chain carboxylate anion of aspartic acid, which makes the histidine more basic and stabilizes the resultant histidine cation by electrostatic interactions. The deprotonated serine adds to a carbonyl group of a triacylglycerol to give a tetrahedral intermediate.

The tetrahedral intermediate expels a diacylglycerol as the leaving group and produces an acyl enzyme. The step is catalyzed by a proton transfer from histidine to make the leaving group a neutral alcohol.




**Steps 3–4 of Figure 29.2: Hydrolysis** The second nucleophilic acyl substitution step hydrolyzes the acyl enzyme and gives the free fatty acid by a mechanism analogous to that of the first two steps. Water is deprotonated by *histidine* to give hydroxide ion, which adds to the enzyme-bound acyl group. The tetrahedral




1. The enzyme active site contains an aspartic acid, a histidine, and a serine. First, histidine acts as a base to deprotonate the –OH group of serine, with the negatively charged carboxylate of aspartic acid stabilizing the nearby histidine cation that results. Serine then adds to the carbonyl group of the triacylglycerol, yielding a tetrahedral intermediate.

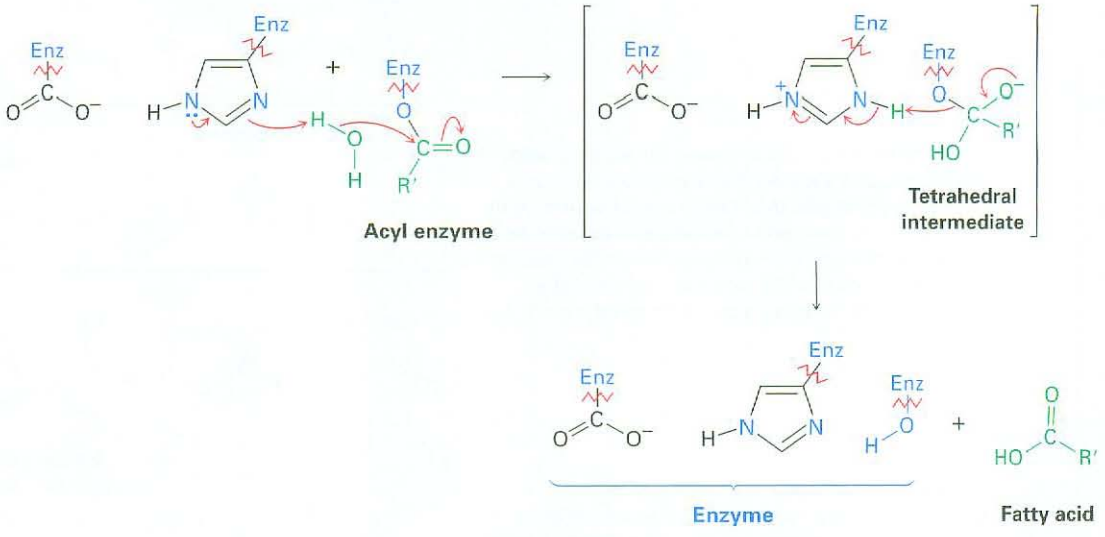
2. This intermediate expels a diacylglycerol as leaving group in a nucleophilic acyl substitution reaction, giving an acyl enzyme. The diacylglycerol is protonated by the histidine cation.

3. Histidine deprotonates a water molecule, which adds to the acyl group. A tetrahedral intermediate is again formed, and the histidine cation is again stabilized by the nearby carboxylate.

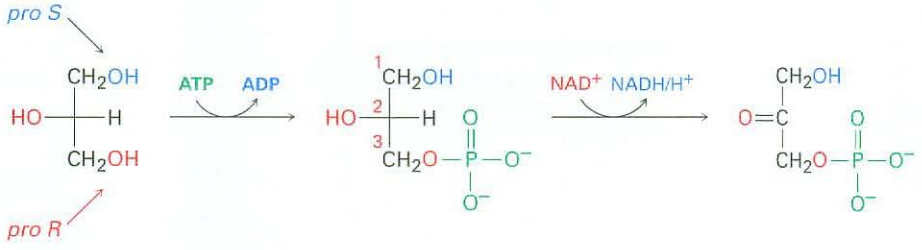
4. The tetrahedral intermediate expels the serine as leaving group in a second nucleophilic acyl substitution reaction, yielding a free fatty acid. The serine accepts a proton from histidine, and the enzyme has now returned to its starting structure.

**Figure 29.2 MECHANISM:** Mechanism of action of lipase. The active site of the enzyme contains a catalytic triad of aspartic acid, histidine, and serine, which react cooperatively to carry out two nucleophilic acyl substitution reactions. Individual steps are explained in the text.

intermediate then expels the neutral serine residue as the leaving group, freeing the fatty acid and returning the enzyme to its active form.

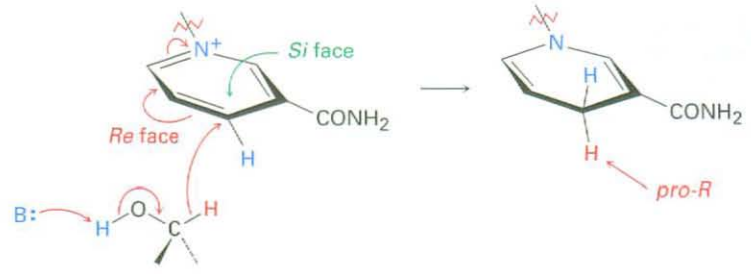



The fatty acids released on triacylglycerol hydrolysis are transported to mitochondria and degraded to acetyl CoA, while the glycerol is carried to the liver for further metabolism. In the liver, glycerol is first phosphorylated by reaction with ATP. Oxidation by $NAD^+$ then yields dihydroxyacetone phosphate (DHAP), which enters the carbohydrate metabolic pathway. We'll discuss this carbohydrate pathway in more detail in Section 29.5.




You might note that C2 of glycerol is a prochiral center (Section 9.13) with two identical "arms." As is typical for enzyme-catalyzed reactions, the phosphorylation of glycerol is selective. Only the *pro-R* arm undergoes reaction, although this can't be predicted in advance. Note also that the phosphorylation product is named *sn*-glycerol 3-phosphate, where the *sn*- prefix means "stereospecific numbering." In this convention, the molecule is drawn in Fischer projection with the –OH group at C2 pointing to the left and the glycerol carbon atoms numbered beginning at the top.

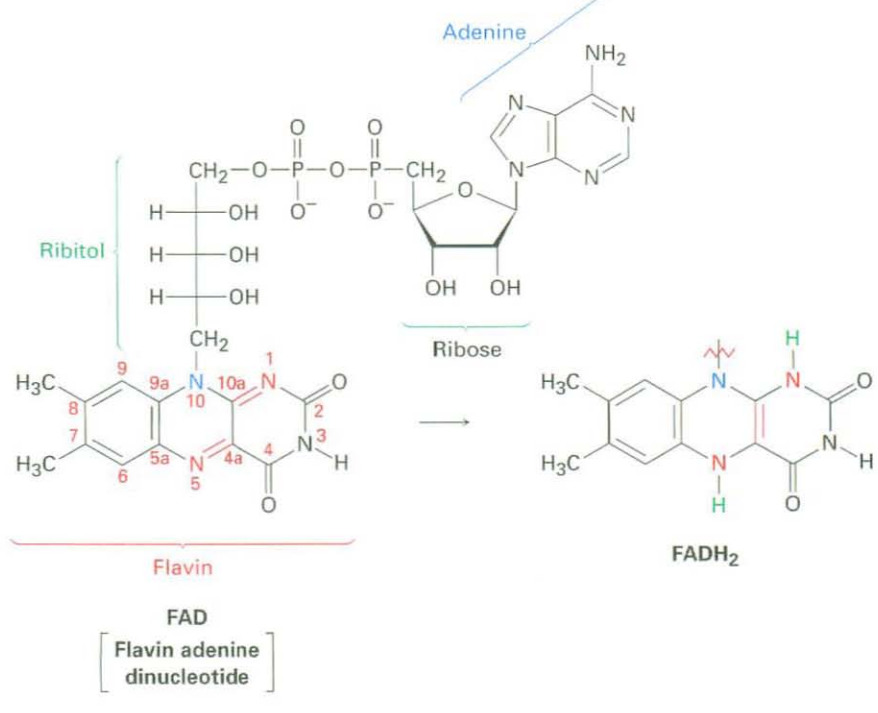
Oxidation of *sn*-glycerol 3-phosphate to give dihydroxyacetone phosphate is catalyzed by *sn*-glycerol-3-phosphate dehydrogenase, with $NAD^+$ as cofactor. The reaction is stereospecific, occurring exclusively on the *Re* face of the nicotinamide ring and adding a hydrogen with *pro-R* stereochemistry. All alcohol dehydrogenases are stereospecific, although their specificity differs depending on the enzyme.




## 29.3 Catabolism of Triacylglycerols: β-Oxidation

The fatty acids that result from triacylglycerol hydrolysis are catabolized by a repetitive four-step sequence of enzyme-catalyzed reactions called the **β-oxidation pathway**, shown in Figure 29.3 on page 1134. Each passage along the pathway results in the cleavage of an acetyl group from the end of the fatty-acid chain, until the entire molecule is ultimately degraded. As each acetyl group is produced, it enters the citric acid cycle and is further degraded, as we'll see in Section 29.7.

**Step 1 of Figure 29.3: Introduction of a Double Bond** The β-oxidation pathway begins when a fatty acid forms a thioester with coenzyme A to give a fatty acyl CoA. Two hydrogen atoms are then removed from C2 and C3 of the fatty acyl CoA by one of a family of acyl-CoA dehydrogenases to yield an α,β-unsaturated acyl CoA. This kind of oxidation—the introduction of a conjugated double bond into a carbonyl compound—occurs frequently in biochemical pathways and usually involves the coenzyme flavin adenine dinucleotide (FAD). Reduced $FADH_2$ is the by-product.



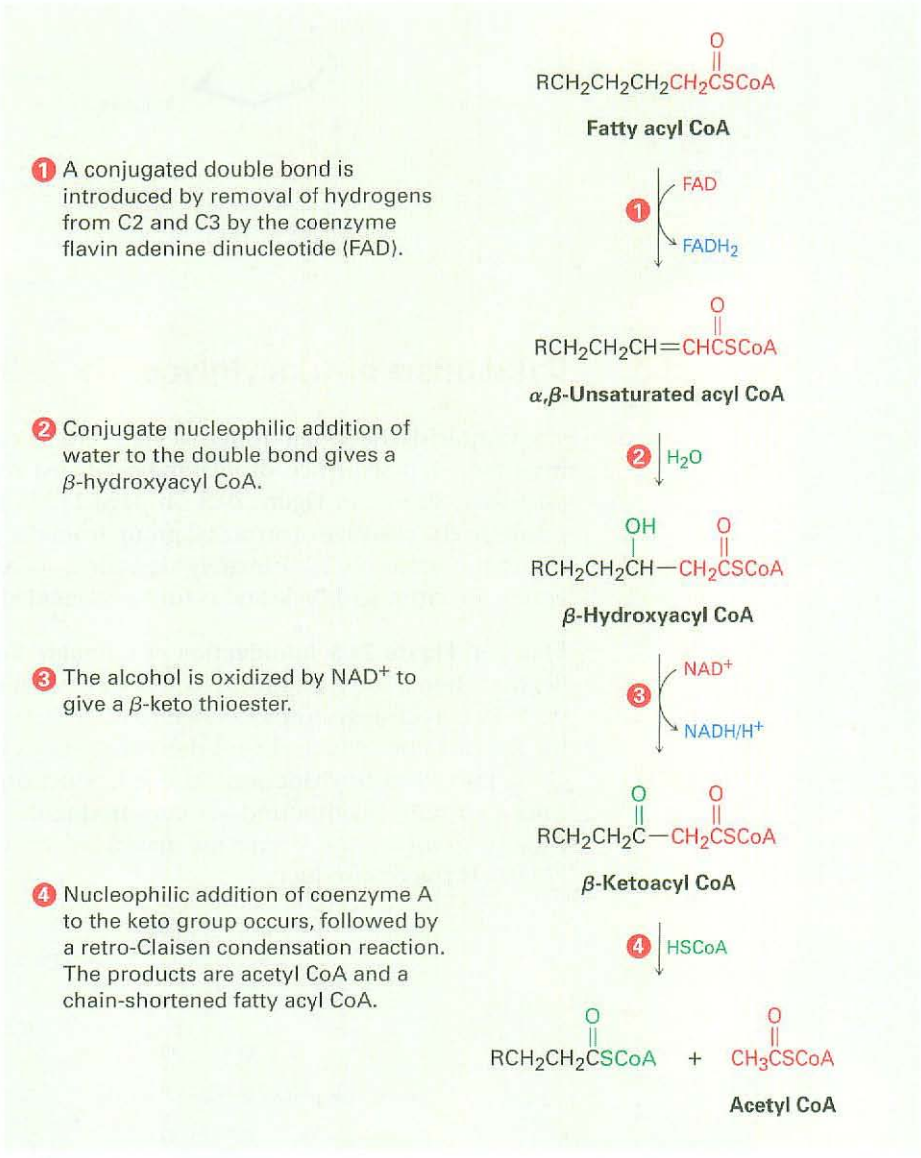
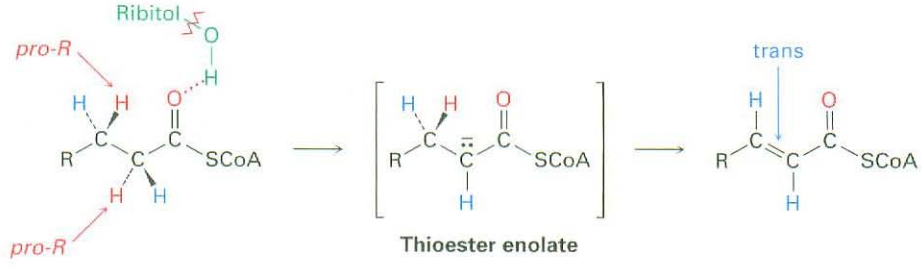



**Figure 29.3 MECHANISM:** The four steps of the β-oxidation pathway, resulting in the cleavage of an acetyl group from the end of the fatty-acid chain. The key chain-shortening step is a retro-Claisen reaction of a β-keto thioester. Individual steps are explained in the text.

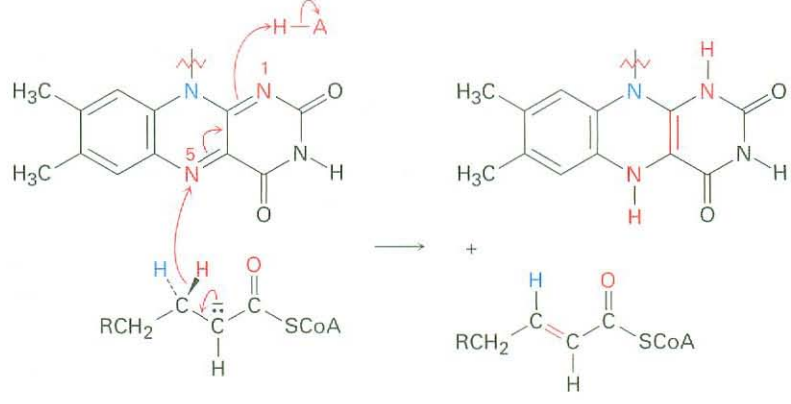


The mechanisms of FAD-catalyzed reactions are often difficult to establish because flavin coenzymes can operate by both two-electron (polar) and one-electron (radical) pathways. As a result, extensive studies of the family of acyl-CoA dehydrogenases have not yet provided a clear picture of how these enzymes function. What is known is that: (1) The first step is abstraction of the *pro-R* hydrogen from the acidic α position of the acyl CoA to give a thioester enolate ion. Hydrogen-bonding between the acyl carbonyl group and the ribitol hydroxyls of FAD increases the acidity of the acyl group. (2) The *pro-R* hydrogen

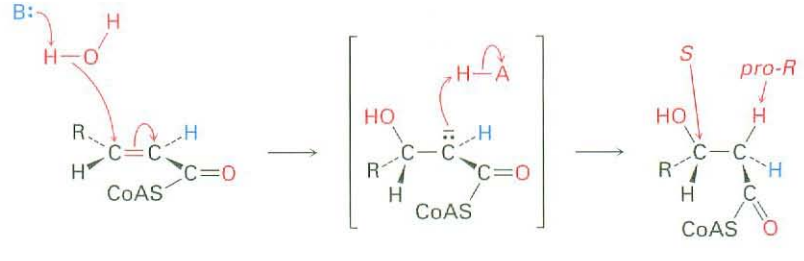
at the β position is transferred to FAD. (3) The α,β-unsaturated acyl CoA that results has a trans double bond.



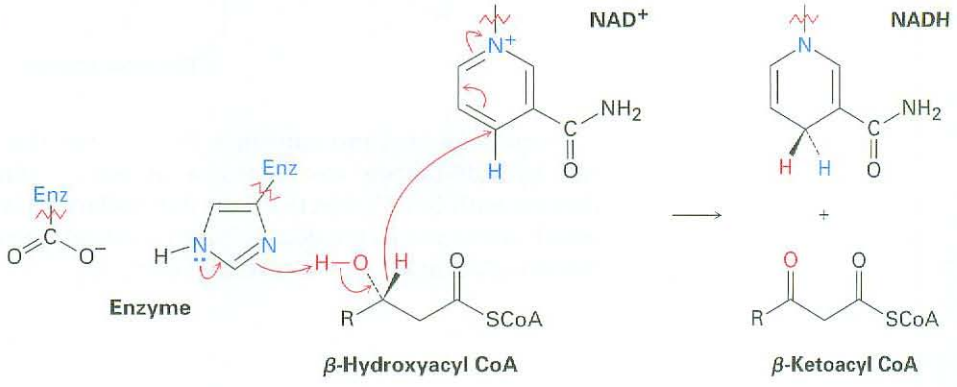

One suggested mechanism is that the reaction may take place by a conjugate hydride-transfer mechanism, analogous to what occurs during alcohol oxidations with $NAD^+$. Electrons on the enolate ion might expel a β hydride ion, which could add to the doubly bonded N5 nitrogen on FAD. Protonation of the intermediate at N1 would give the product.



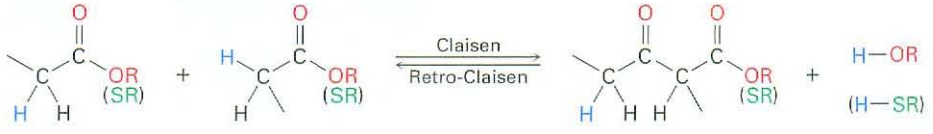

**Step 2 of Figure 29.3: Conjugate Addition of Water** The α,β-unsaturated acyl CoA produced in step 1 reacts with water by a conjugate addition pathway (Section 19.13) to yield a β-hydroxyacyl CoA in a process catalyzed by enoyl CoA hydratase. Water as nucleophile adds to the β carbon of the double bond, yielding an enolate ion intermediate that is protonated on the α position.



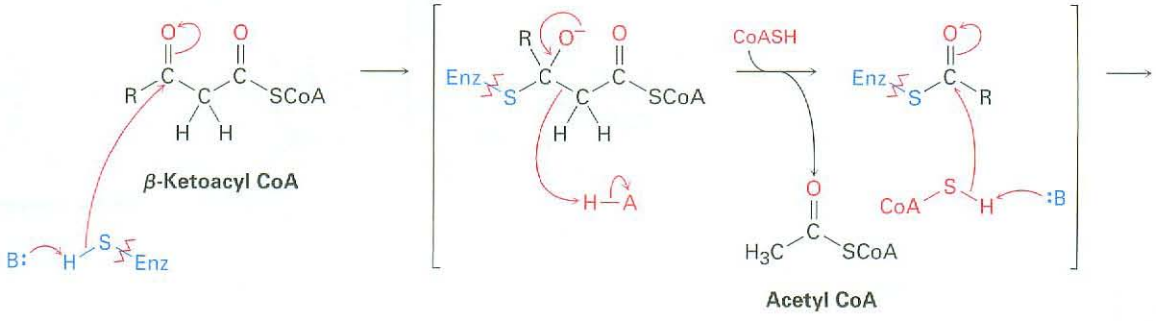
**Step 3 of Figure 29.3: Alcohol Oxidation** The β-hydroxyacyl CoA from step 2 is oxidized to a β-ketoacyl CoA in a reaction catalyzed by one of a family of L-3-hydroxyacyl-CoA dehydrogenases, which differ in substrate specificity according to the chain length of the acyl group. As in the oxidation of *sn*-glycerol 3-phosphate to dihydroxyacetone phosphate mentioned at the end of Section 29.2, this alcohol oxidation requires $NAD^+$ as a coenzyme and yields reduced $NADH/H^+$ as by-product. Deprotonation of the hydroxyl group is carried out by a histidine residue at the active site.

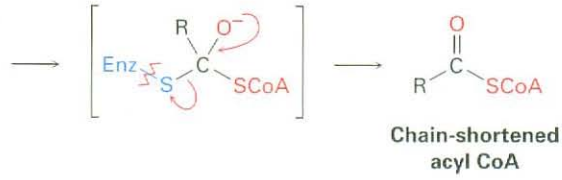



**Step 4 of Figure 29.3: Chain Cleavage** Acetyl CoA is split off from the chain in the final step of β-oxidation, leaving an acyl CoA that is two carbon atoms shorter than the original. The reaction is catalyzed by β-ketoacyl-CoA thiolase and is mechanistically the reverse of a Claisen condensation reaction (Section 23.7). In the *forward* direction, a Claisen condensation joins two esters together to form a β-keto ester product. In the *reverse* direction, a retro-Claisen reaction splits a β-keto ester (or β-keto thioester) apart to form two esters (or two thioesters).




The retro-Claisen reaction occurs by initial nucleophilic addition of a cysteine –SH group on the enzyme to the keto group of the β-ketoacyl CoA to yield an alkoxide ion intermediate. Cleavage of the C2–C3 bond then follows, with expulsion of an acetyl CoA enolate ion. Protonation of the enolate ion gives acetyl CoA, and the enzyme-bound acyl group undergoes nucleophilic acyl substitution by reaction with a molecule of coenzyme A. The chain-shortened acyl CoA that results then enters another round of the β-oxidation pathway for further degradation.






**Chain-shortened acyl CoA**

Look at the catabolism of myristic acid shown in Figure 29.4 to see the overall results of the β-oxidation pathway. The first passage converts the 14-carbon myristoyl CoA into the 12-carbon lauroyl CoA plus acetyl CoA, the second passage converts lauroyl CoA into the 10-carbon caproyl CoA plus acetyl CoA, the third passage converts caproyl CoA into the 8-carbon capryloyl CoA, and so on. Note that the final passage produces *two* molecules of acetyl CoA because the precursor has four carbons.

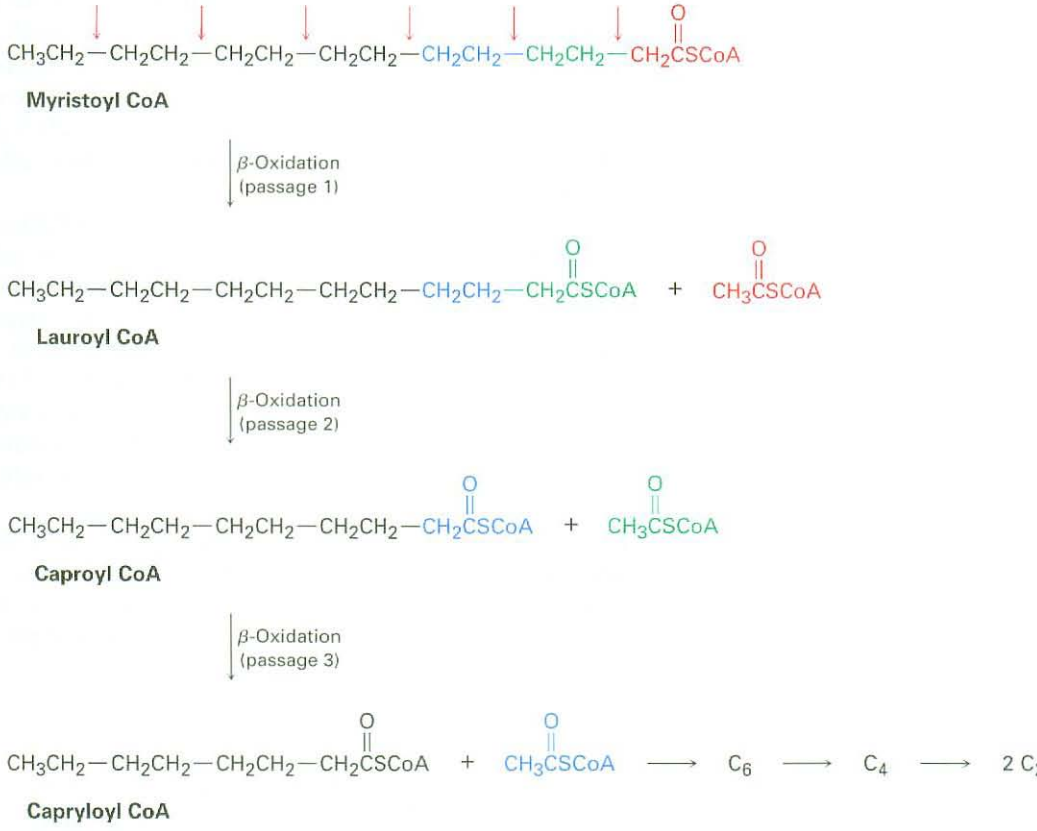



**Figure 29.4** Catabolism of the 14-carbon myristic acid by the β-oxidation pathway yields seven molecules of acetyl CoA after six passages.

Most fatty acids have an even number of carbon atoms, so none are left over after β-oxidation. Those fatty acids with an odd number of carbon atoms yield the three-carbon propionyl CoA in the final β-oxidation. Propionyl CoA is then converted to succinate by a multistep radical pathway, and succinate enters the citric acid cycle (Section 29.7). Note that the three-carbon propionyl group should properly be called *propanoyl*, but biochemists generally use the nonsystematic name.

**Problem 29.2** Write the equations for the remaining passages of the β-oxidation pathway following those shown in Figure 29.4.

**Problem 29.3** How many molecules of acetyl CoA are produced by catabolism of the following fatty acids, and how many passages of the β-oxidation pathway are needed?

(a) Palmitic acid, $CH_3(CH_2)_{14}CO_2H$

(b) Arachidic acid, $CH_3(CH_2)_{18}CO_2H$

## 29.4 Biosynthesis of Fatty Acids

One of the most striking features of the common fatty acids is that they have an even number of carbon atoms (Table 27.1, p. 1062). This even number results because all fatty acids are derived biosynthetically from acetyl CoA by sequential addition of two-carbon units to a growing chain. The acetyl CoA, in turn, arises primarily from the metabolic breakdown of carbohydrates in the glycolysis pathway that we'll see in Section 29.5. Thus, dietary carbohydrates consumed in excess of immediate energy needs are turned into fats for storage.

As a rule, the anabolic pathway by which a substance is made is not the reverse of the catabolic pathway by which the same substance is degraded. The two paths *must* differ in some respects for both to be energetically favorable. Thus, the β-oxidation pathway for converting fatty acids *into* acetyl CoA and the biosynthesis of fatty acids *from* acetyl CoA are related but are not exact opposites. Differences include the identity of the acyl-group carrier, the stereochemistry of the β-hydroxyacyl reaction intermediate, and the identity of the redox coenzyme. FAD is used to introduce a double bond in β-oxidation, while NADPH is used to reduce the double bond in fatty-acid biosynthesis.

In bacteria, each step in fatty-acid synthesis is catalyzed by separate enzymes. In vertebrates, however, fatty-acid synthesis is catalyzed by a large, multienzyme complex called a *synthase* that contains two identical subunits of 2505 amino acids each and catalyzes all steps in the pathway. An overview of fatty-acid biosynthesis is shown in Figure 29.5.

**Steps 1–2 of Figure 29.5: Acyl Transfers** The starting material for fatty-acid synthesis is the thioester acetyl CoA, the ultimate product of carbohydrate breakdown, as we'll see in Section 29.6. The synthetic pathway begins with several *priming reactions,* which transport acetyl CoA and convert it into more reactive species. The first priming reaction is a nucleophilic acyl substitution reaction that converts acetyl CoA into acetyl ACP (acyl carrier protein). The reaction is catalyzed by ACP transacylase.

Notice that the mechanism of the nucleophilic acyl substitution step can be given in an abbreviated form that saves space by not explicitly showing the tetrahedral reaction intermediate. Instead, electron movement is shown as a heart-shaped path around the carbonyl oxygen to imply the full mechanism.

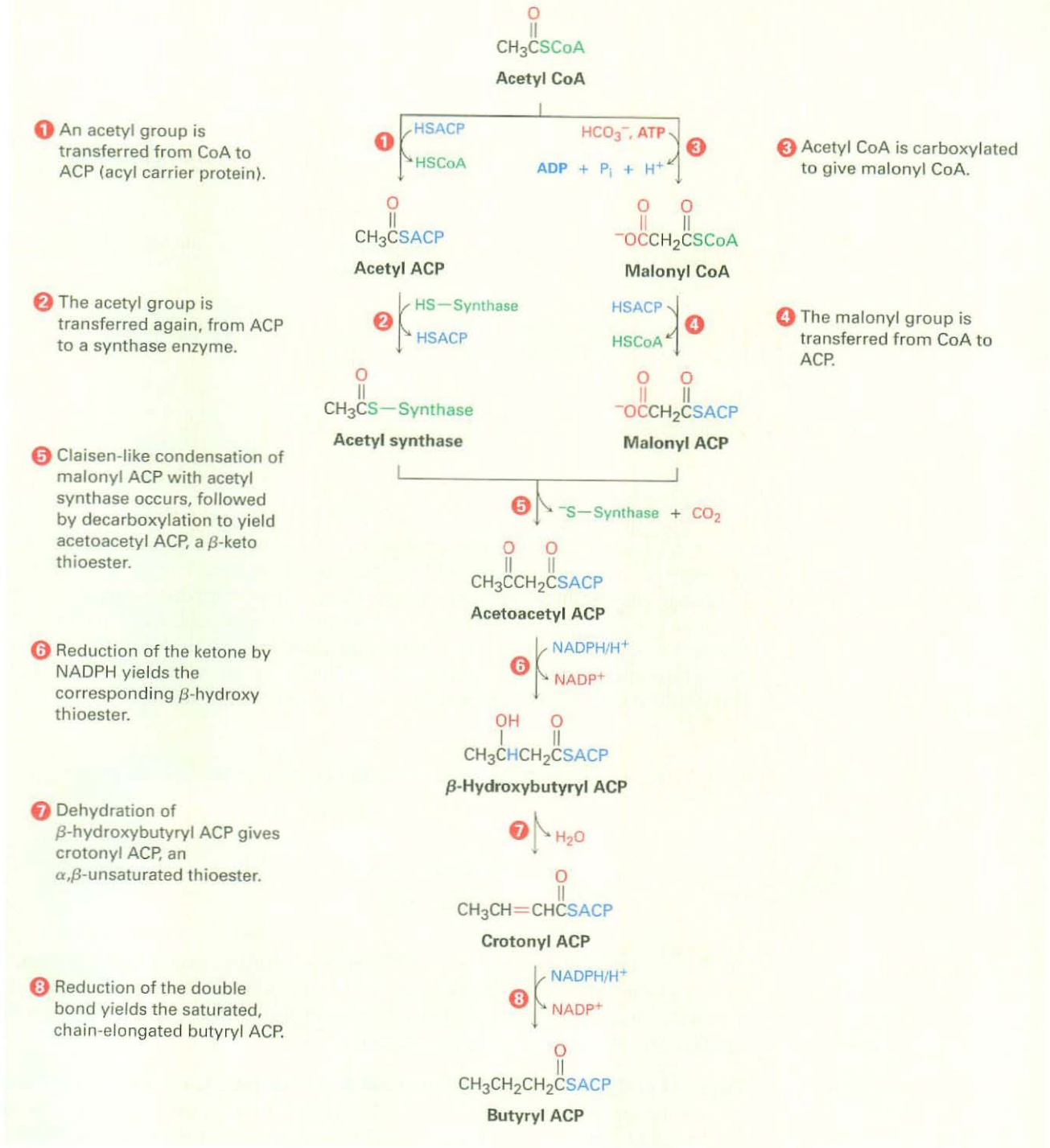
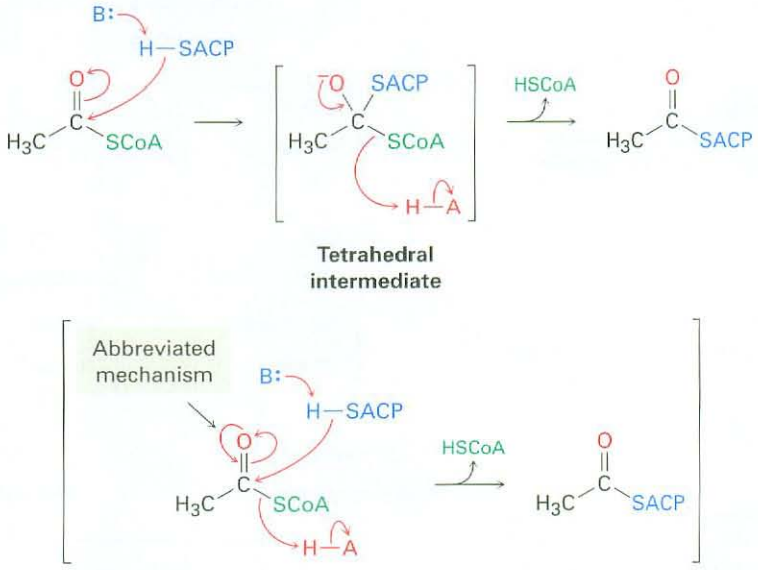
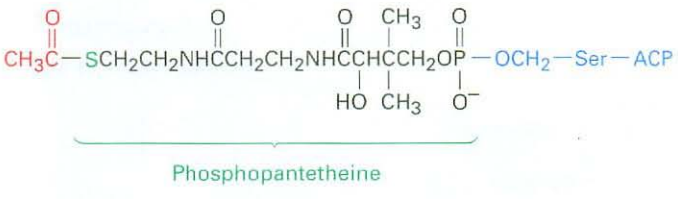



**Figure 29.5 MECHANISM:** The pathway for fatty-acid biosynthesis from the two-carbon precursor, acetyl CoA. Individual steps are explained in the text.

Biochemists use this kind of format commonly, and we'll also use it on occasion in the remainder of this chapter.




In bacteria, ACP is a small protein of 77 residues that transports an acyl group from enzyme to enzyme. In vertebrates, however, ACP appears to be a long arm on a multienzyme synthase complex, whose apparent function is to shepherd an acyl group from site to site within the complex. As in acetyl CoA, the acyl group in acetyl ACP is linked by a thioester bond to the sulfur atom of phosphopantetheine. The phosphopantetheine is in turn linked to ACP through the side-chain —OH group of a serine residue in the enzyme.




**Acetyl ACP**

Step 2, another priming reaction, involves a further exchange of thioester linkages by another nucleophilic acyl substitution and results in covalent bonding of the acetyl group to a cysteine residue in the synthase complex that will catalyze the upcoming condensation step.

**Steps 3–4 of Figure 29.5: Carboxylation and Acyl Transfer** The third step is a *loading* reaction in which acetyl CoA is carboxylated by reaction with $HCO_3^-$ and ATP to yield malonyl CoA plus ADP. This step requires the coenzyme biotin, which is bonded to the lysine residue of acetyl CoA carboxylase and acts as a carrier of $CO_2$. Biotin first reacts with bicarbonate ion to give *N*-carboxybiotin, which then reacts with the enolate ion of acetyl CoA and transfers the $CO_2$ group. Thus, biotin acts as a carrier of $CO_2$, binding it in one step and releasing it in another.

The mechanism of the $CO_2$ transfer reaction with acetyl CoA to give malonyl CoA is thought to involve $CO_2$ as the reactive species. One proposal is that loss of $CO_2$ is favored by hydrogen-bond formation between the *N*-carboxybiotin carbonyl group and a nearby acidic site in the enzyme. Simultaneous deprotonation of acetyl CoA by a basic site in the enzyme gives a thioester enolate ion that can react with $CO_2$ as it is formed (Figure 29.6).

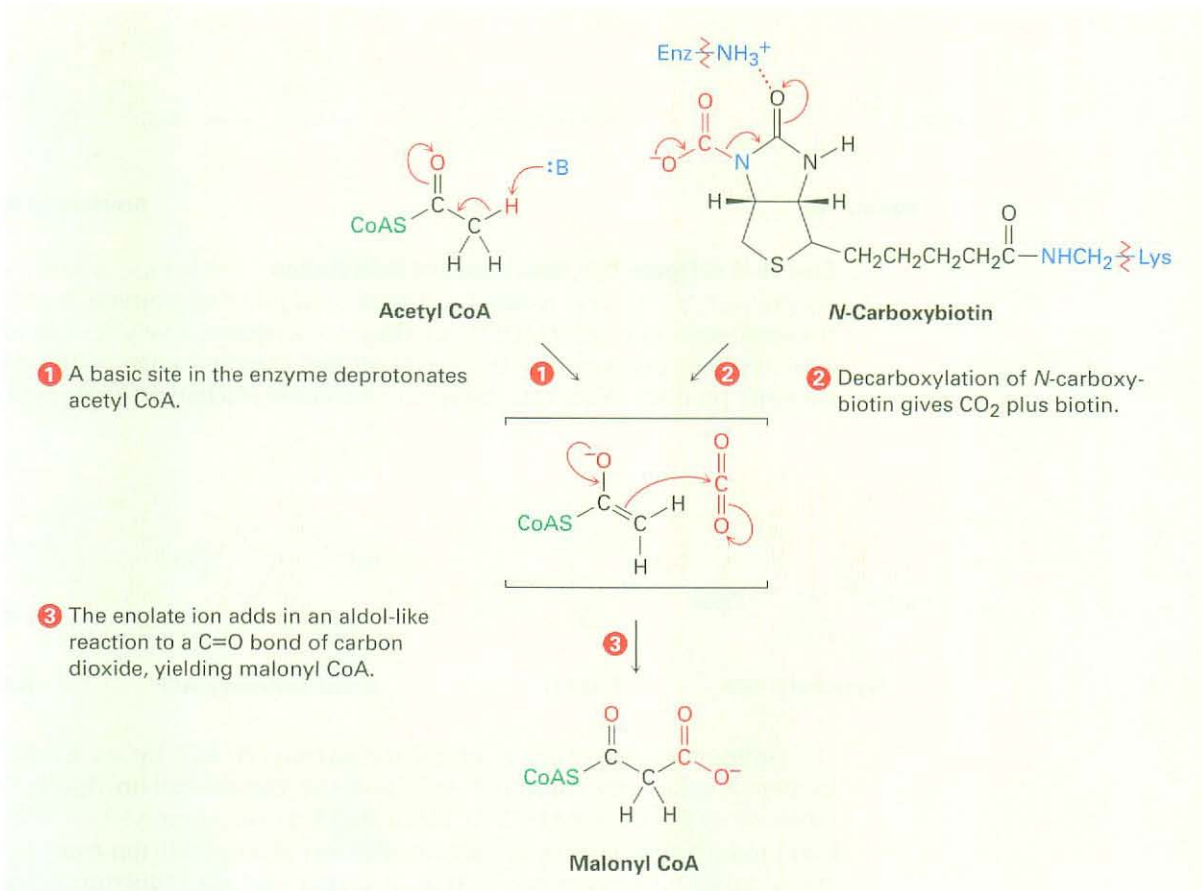
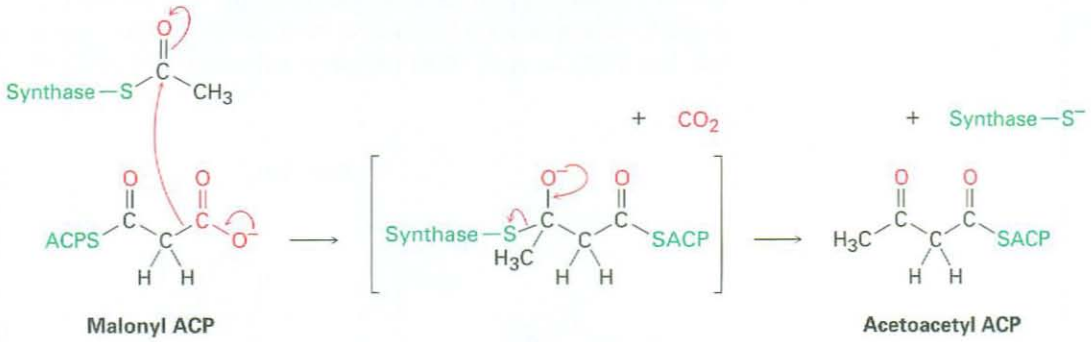



**Figure 29.6 MECHANISM:** Mechanism of step 3 in Figure 29.5, the biotin-dependent carboxylation of acetyl CoA to yield malonyl CoA.

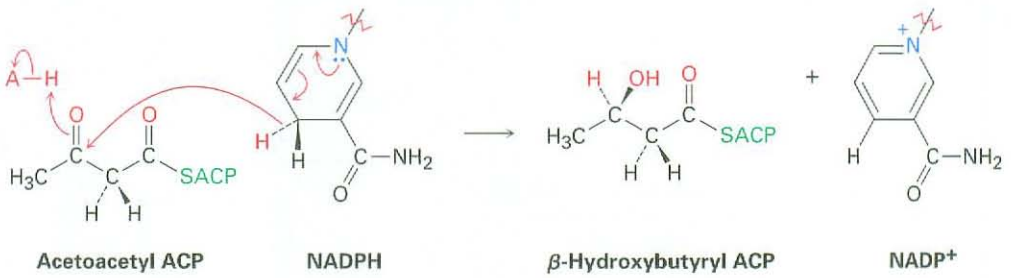
Following the formation of malonyl CoA, another nucleophilic acyl substitution reaction occurs in step 4 to form the more reactive malonyl ACP, thereby binding the malonyl group to an ACP arm of the multienzyme synthase. At this point, both acetyl and malonyl groups are bound to the enzyme, and the stage is set for their condensation.

**Step 5 of Figure 29.5: Condensation** The key carbon–carbon bond-forming reaction that builds the fatty-acid chain occurs in step 5. This step is simply a Claisen condensation between acetyl synthase as the electrophilic acceptor and malonyl ACP as the nucleophilic donor. The mechanism of the condensation is thought to involve decarboxylation of malonyl ACP to give an enolate ion, followed by immediate addition of the enolate ion to the carbonyl group of acetyl

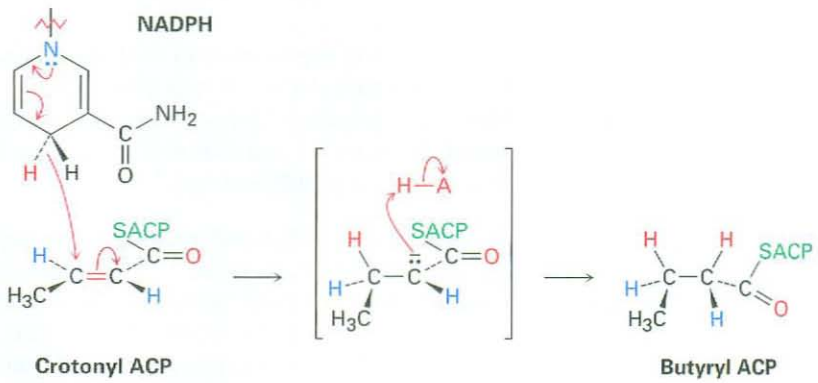
synthase. Breakdown of the tetrahedral intermediate gives the four-carbon condensation product acetoacetyl ACP and frees the synthase binding site for attachment of the chain-elongated acyl group at the end of the sequence.



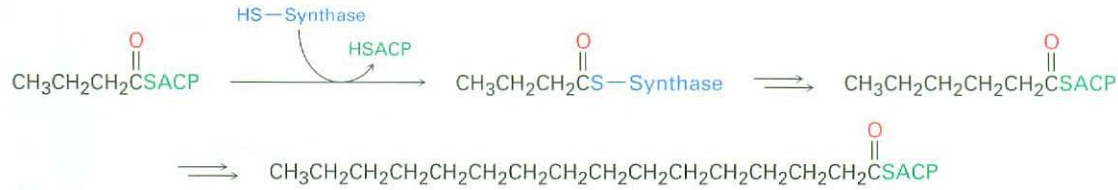

**Steps 6–8 of Figure 29.5: Reduction and Dehydration** The ketone carbonyl group in acetoacetyl ACP is next reduced to the alcohol β-hydroxybutyryl ACP by β-keto thioester reductase and NADPH, a reducing coenzyme closely related to NADH. *R* Stereochemistry results at the newly formed chirality center in the β-hydroxy thioester product. (Note that the systematic name of a butyryl group is *butanoyl*.)




Subsequent dehydration of β-hydroxybutyryl ACP by an E1cB reaction in step 7 yields *trans*-crotonyl ACP, and the carbon–carbon double bond of crotonyl ACP is reduced by NADPH in step 8 to yield butyryl ACP. The double-bond reduction occurs by conjugate addition of a hydride ion from NADPH to the β carbon of *trans*-crotonyl ACP. In vertebrates, the reduction occurs by an overall syn addition, but other organisms carry out similar chemistry with different stereochemistry.



The net effect of the eight steps in the fatty-acid biosynthesis pathway is to take two 2-carbon acetyl groups and combine them into a 4-carbon butyryl group. Further condensation of the butyryl group with another malonyl ACP yields a 6-carbon unit, and still further repetitions of the pathway add two more carbon atoms to the chain each time until the 16-carbon palmitoyl ACP is reached.




**Palmitoyl ACP**

Further chain elongation of palmitic acid occurs by reactions similar to those just described, but CoA rather than ACP is the carrier group, and separate enzymes are needed for each step rather than a multienzyme synthase complex.

**Problem 29.4** Write a mechanism for the dehydration reaction of β-hydroxybutyryl ACP to yield crotonyl ACP in step 7 of fatty-acid synthesis.

**Problem 29.5** Evidence for the role of acetate in fatty-acid biosynthesis comes from isotope-labeling experiments. If acetate labeled with $^{13}C$ in the methyl group ($^{13}CH_3CO_2H$) were incorporated into fatty acids, at what positions in the fatty-acid chain would you expect the $^{13}C$ label to appear?

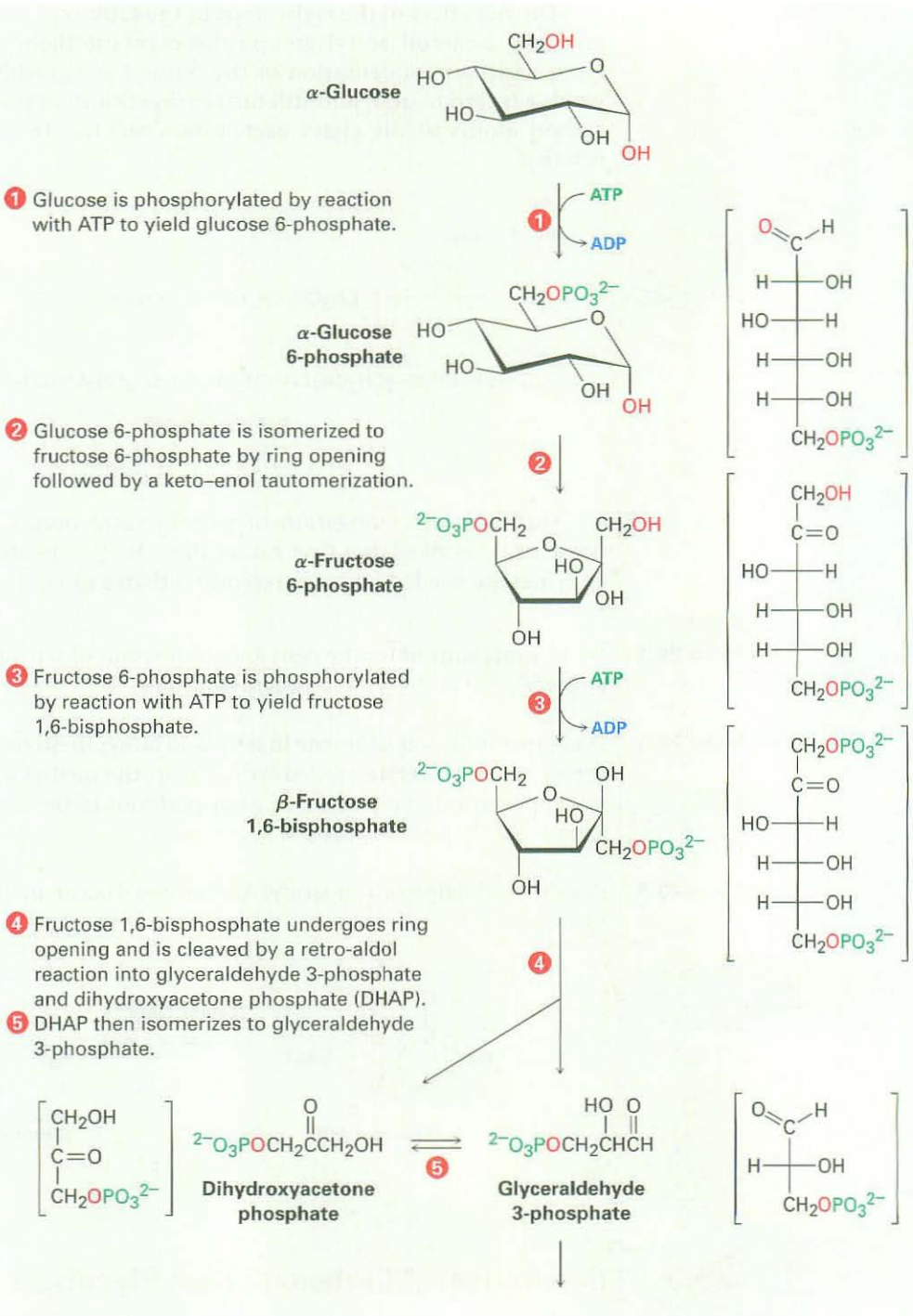
**Problem 29.6** Does the reduction of acetoacetyl ACP in step 6 occur on the *Re* face or the *Si* face of the molecule?


**Acetoacetyl ACP** **β-Hydroxybutyryl ACP**

## 29.5 Catabolism of Carbohydrates: Glycolysis

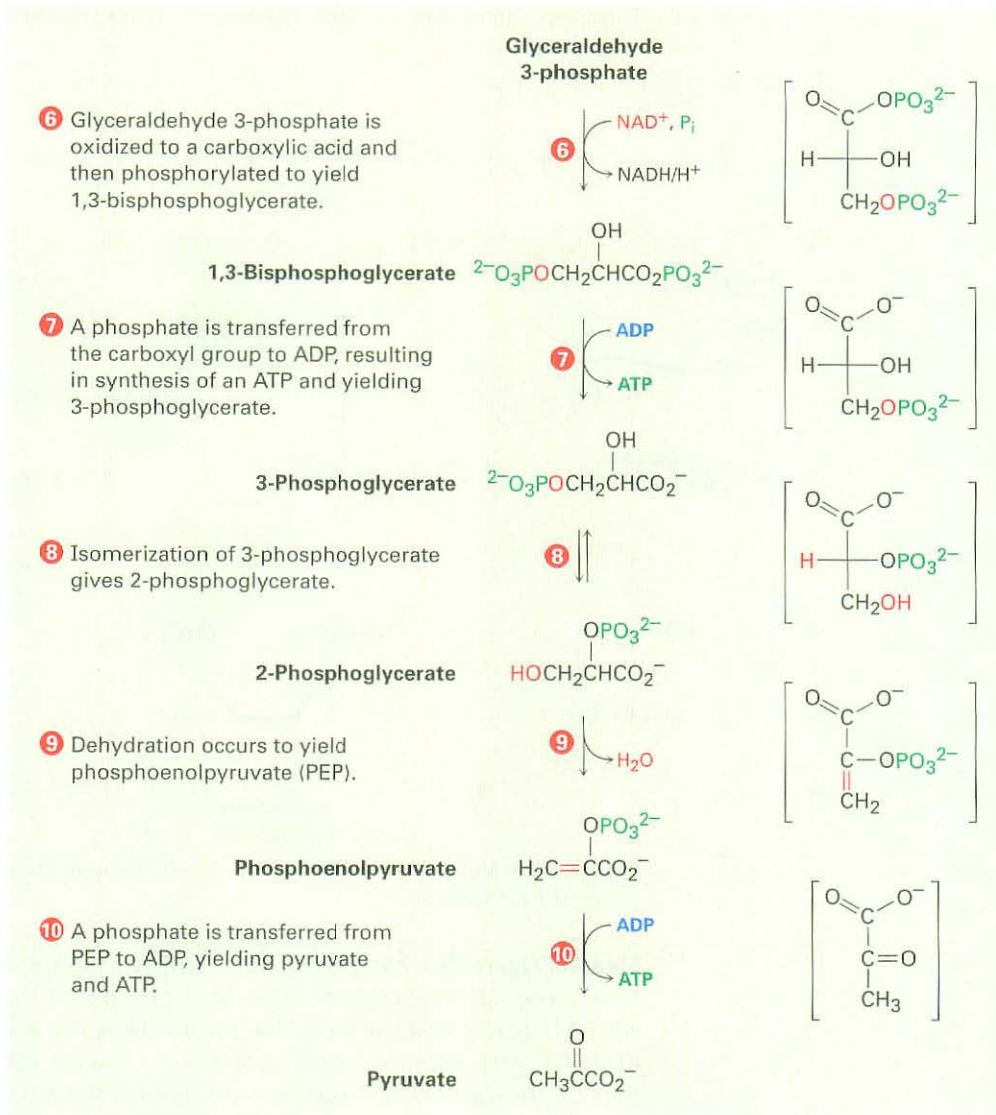
ThomsonNOW Click *Organic Interactive* for **a tutorial linking metabolic pathways with their underlying organic reaction mechanisms.**

Glucose is the body's primary short-term energy source. Its catabolism begins with **glycolysis**, a series of ten enzyme-catalyzed reactions that break down glucose into 2 equivalents of pyruvate, $CH_3COCO_2^-$. The steps of glycolysis, also called the *Embden–Meyerhoff pathway* after its discoverers, are summarized in Figure 29.7.






**Active Figure 29.7 MECHANISM:** The 10-step glycolysis pathway for catabolizing glucose to two molecules of pyruvate. Individual steps are described in the text. *Sign in at* **www.thomsonedu.com** *to see a simulation based on this figure and to take a short quiz.*




**Active Figure 29.7** *(continued)*

**Steps 1–2 of Figure 29.7: Phosphorylation and Isomerization** Glucose, produced by the digestion of dietary carbohydrates, is first phosphorylated at the C6 hydroxyl group by reaction with ATP in a process catalyzed by hexokinase. As noted in Section 29.1, the reaction requires $Mg^{2+}$ as a cofactor to complex with the negatively charged phosphate oxygens. The glucose 6-phosphate that results is isomerized in step 2 by glucose 6-phosphate isomerase to give fructose 6-phosphate. The isomerization takes place by initial opening of the glucose hemiacetal ring to the open-chain form, followed by keto–enol tautomerization to a cis enediol, HO—C=C—OH. But because glucose and fructose share a common enediol, further tautomerization to a different keto

form produces open-chain fructose, and cyclization completes the process (Figure 29.8).

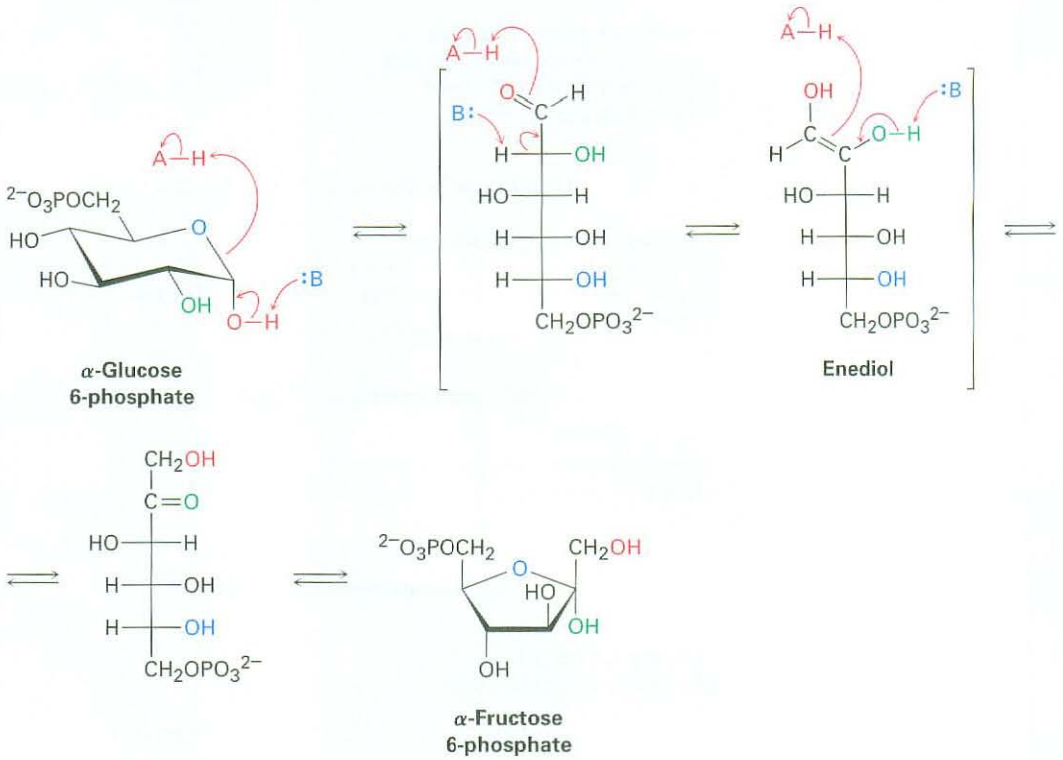
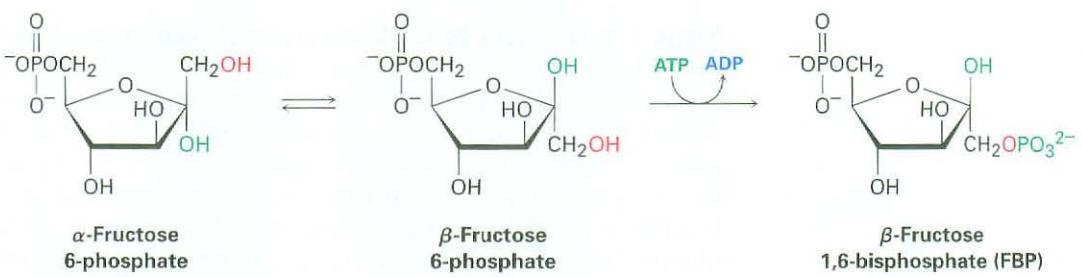


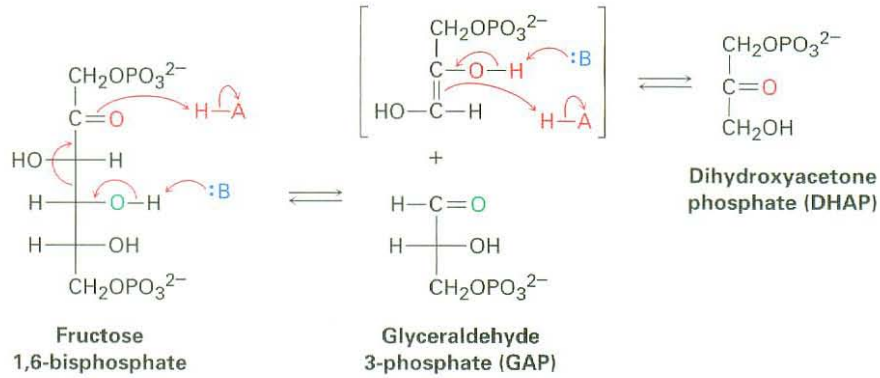

**Figure 29.8** Mechanism of step 2 in glycolysis, the isomerization of glucose 6-phosphate to fructose 6-phosphate.

**Step 3 of Figure 29.7: Phosphorylation** Fructose 6-phosphate is converted in step 3 to fructose 1,6-bisphosphate (FBP) by a phosphofructokinase-catalyzed reaction with ATP (recall that the prefix *bis-* means two). The mechanism is similar to that in step 1, with $Mg^{2+}$ ion again required as cofactor. Interestingly, the product of step 2 is the $\alpha$ anomer of fructose 6-phosphate, but it is the $\beta$ anomer that is phosphorylated in step 3, implying that the two anomers equilibrate rapidly through the open-chain form. The result of step 3 is a molecule ready to be split into the two three-carbon intermediates that will ultimately become two molecules of pyruvate.



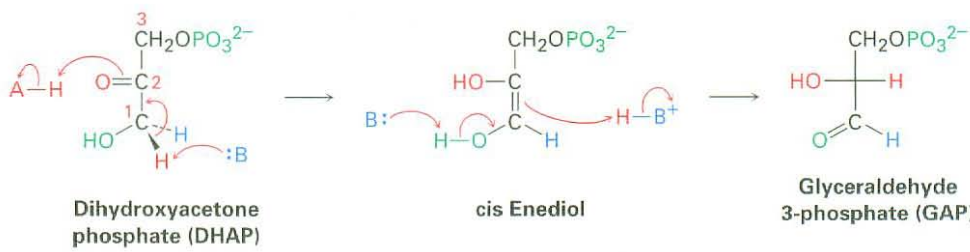

**Step 4 of Figure 29.7: Cleavage** Fructose 1,6-bisphosphate is cleaved in step 4 into two 3-carbon pieces, dihydroxyacetone phosphate (DHAP) and glyceraldehyde 3-phosphate (GAP). The bond between C3 and C4 of fructose 1,6-bisphosphate

breaks, and a C=O group is formed at C4. Mechanistically, the cleavage is the reverse of an aldol reaction (Section 23.1) and is catalyzed by an aldolase. A forward aldol reaction joins two aldehydes or ketones to give a β-hydroxy carbonyl compound, while a retro aldol reaction such as that occurring here cleaves a β-hydroxy carbonyl compound into two aldehydes or ketones.




Two classes of aldolases are used by organisms for catalysis of the retro-aldol reaction. In fungi, algae, and some bacteria, the retro-aldol reaction is catalyzed by class II aldolases, which function by coordination of the fructose carbonyl group with $Zn^{2+}$ as Lewis acid. In plants and animals, however, the reaction is catalyzed by class I aldolases and does not take place on the free ketone. Instead, fructose 1,6-bisphosphate undergoes reaction with the side-chain $-NH_2$ group of a lysine residue on the aldolase to yield a protonated enzyme-bound imine (Section 19.8), often called a **Schiff base** in biochemistry. Because of its positive charge, the iminium ion is a better electron acceptor than a ketone carbonyl group. Retro-aldol reaction ensues, giving glyceraldehyde 3-phosphate and an enamine, which is protonated to give another iminium ion that is hydrolyzed to yield dihydroxyacetone phosphate (Figure 29.9 on page 1148).

**Step 5 of Figure 29.7: Isomerization** Dihydroxyacetone phosphate is isomerized in step 5 by triose phosphate isomerase to form a second equivalent of glyceraldehyde 3-phosphate. As in the conversion of glucose 6-phosphate to fructose 6-phosphate in step 2, the isomerization takes place by keto–enol tautomerization through a common enediol intermediate. A base deprotonates of C1 and then reprotonates C2 using the same hydrogen. The net result of steps 4 and 5 is the production of two glyceraldehyde 3-phosphate molecules, both of which pass down the rest of the pathway. Thus, each of the remaining five steps of glycolysis takes place twice for every glucose molecule that enters at step 1.



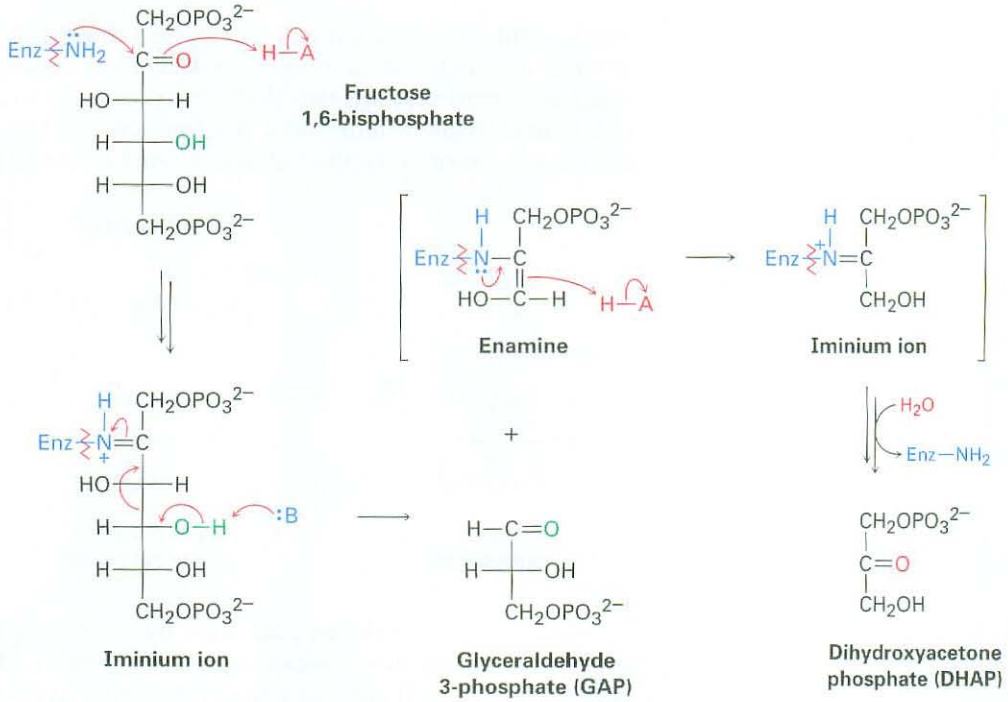
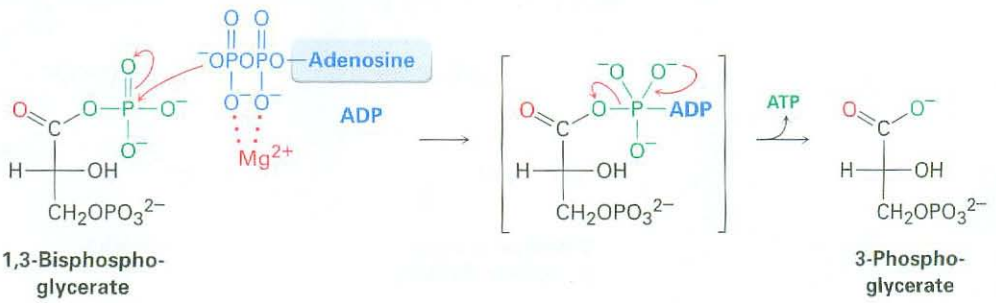



**Figure 29.9** Mechanism of step 4 in Figure 29.7, the cleavage of fructose 1,6-bisphosphate to yield glyceraldehyde 3-phosphate and dihydroxyacetone phosphate.

**Steps 6–7 of Figure 29.7: Oxidation, Phosphorylation, and Dephosphorylation** Glyceraldehyde 3-phosphate is oxidized and phosphorylated in step 6 to give 1,3-bisphosphoglycerate (Figure 29.10). The reaction is catalyzed by glyceraldehyde 3-phosphate dehydrogenase and begins by nucleophilic addition of the –SH group of a cysteine residue in the enzyme to the aldehyde carbonyl group to yield a *hemithioacetal*, the sulfur analog of a hemiacetal. Oxidation of the hemithioacetal –OH group by $NAD^+$ then yields a thioester, which reacts with phosphate ion in a nucleophilic acyl substitution step to yield 1,3-bisphosphoglycerate, a mixed anhydride between a carboxylic acid and phosphoric acid.

Like all anhydrides (Section 21.5), the mixed carboxylic–phosphoric anhydride is a reactive substrate in nucleophilic acyl (or phosphoryl) substitution reactions. Reaction of 1,3-bisphosphoglycerate with ADP occurs in step 7 by substitution on phosphorus, resulting in transfer of a phosphate group to ADP and giving ATP plus 3-phosphoglycerate. The process is catalyzed by phosphoglycerate kinase and requires $Mg^{2+}$ as cofactor. Together, steps 6 and 7 accomplish the oxidation of an aldehyde to a carboxylic acid.



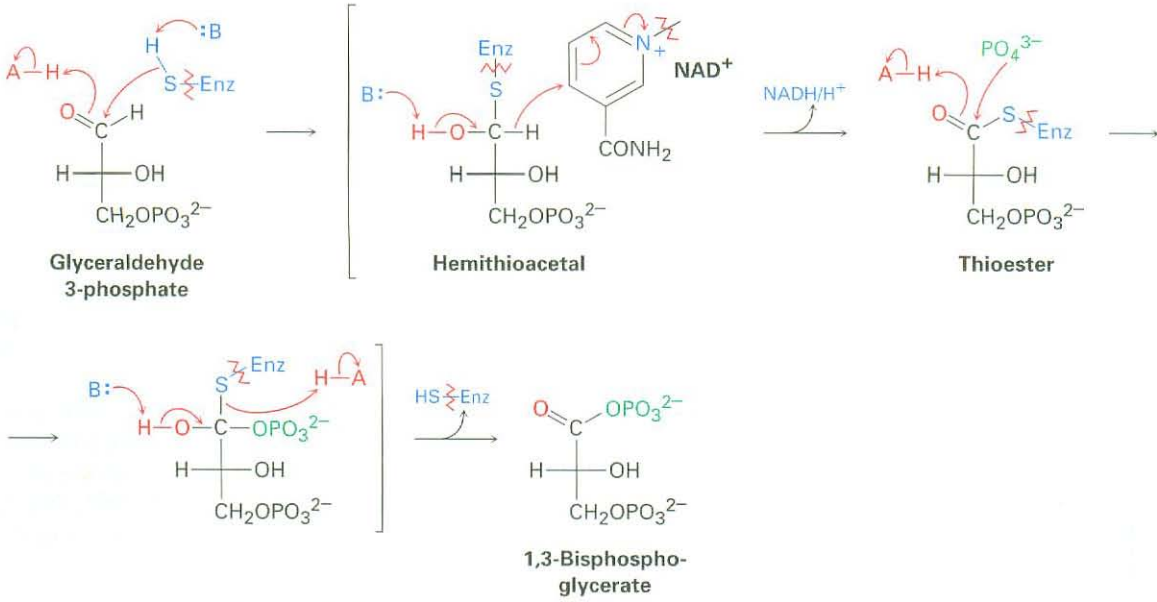
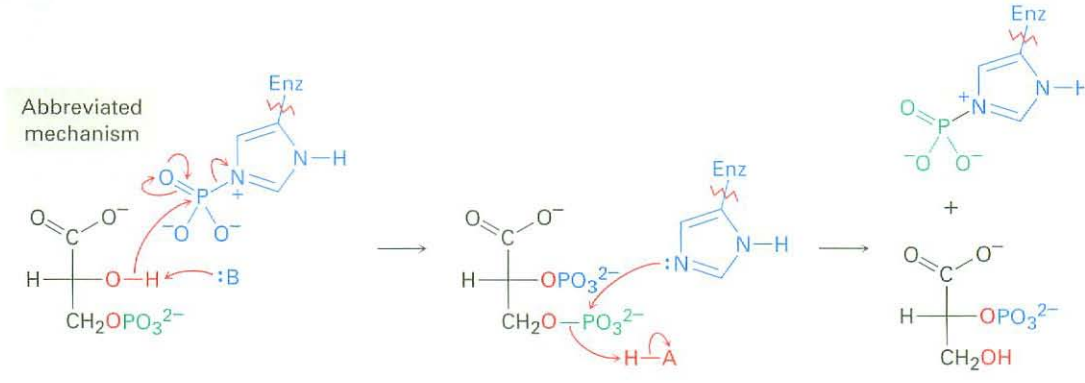


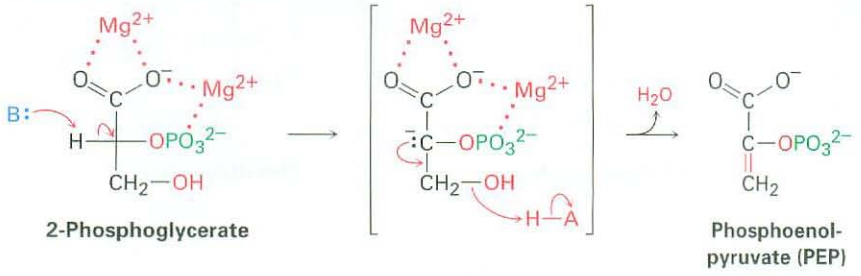

**Figure 29.10** Mechanism of step 6 in Figure 29.7, the oxidation and phosphorylation of glyceraldehyde 3-phosphate to give 1,3-bisphosphoglycerate.

**Step 8 of Figure 29.7: Isomerization** 3-Phosphoglycerate isomerizes to 2-phospho-glycerate in a step catalyzed by phosphoglycerate mutase. In plants, 3-phos-phoglycerate transfers its phosphoryl group from its C3 oxygen to a histidine residue on the enzyme in one step and then accepts the same phosphoryl group back onto the C2 oxygen in a second step. In animals and yeast, however, the enzyme contains a phosphorylated histidine, which transfers its phosphoryl group to the C2 oxygen of 3-phosphoglycerate and forms 2,3-bisphospho-glycerate as intermediate. The same histidine then accepts a phosphoryl group from the C3 oxygen to yield the isomerized product plus regenerated enzyme.

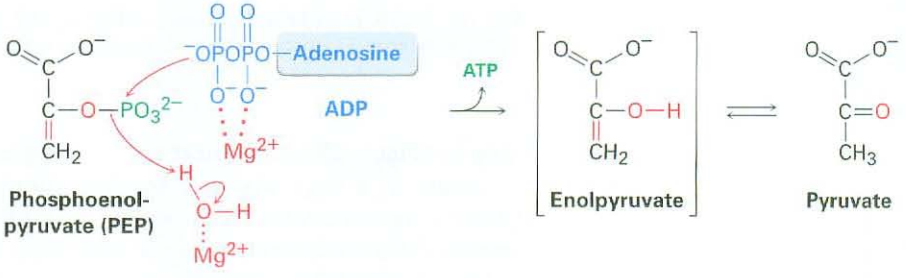



**Steps 9–10 of Figure 29.7: Dehydration and Dephosphorylation** Like most $\beta$-hydroxy carbonyl compounds produced in aldol reactions, 2-phospho-glycerate undergoes a ready dehydration in step 9 by an E1cB mechanism (Section 23.3). The process is catalyzed by enolase, and the product i

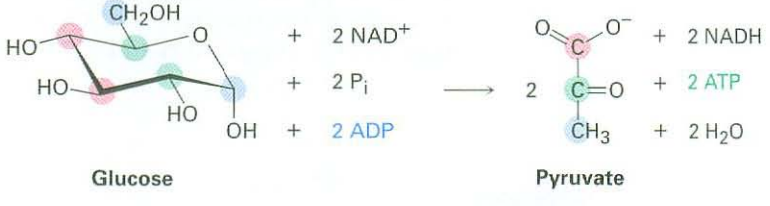
phosphoenolpyruvate, abbreviated PEP. Two $Mg^{2+}$ ions are associated with the 2-phosphoglycerate to neutralize the negative charges.




Transfer of the phosphoryl group to ADP in step 10 then generates ATP and gives enolpyruvate, which undergoes tautomerization to pyruvate. The reaction is catalyzed by pyruvate kinase and requires that a molecule of fructose 1,6-bisphosphate also be present, as well as 2 equivalents of $Mg^{2+}$. One $Mg^{2+}$ ion coordinates to ADP, and the other increases the acidity of a water molecule necessary for protonation of the enolate ion.




The overall result of glycolysis can be summarized by the following equation:




**Problem 29.7** Identify the two steps in glycolysis in which ATP is produced.

**Problem 29.8** Look at the entire glycolysis pathway and make a list of the kinds of organic reactions that take place—nucleophilic acyl substitutions, aldol reactions, E1cB reactions, and so forth.

## 29.6 Conversion of Pyruvate to Acetyl CoA

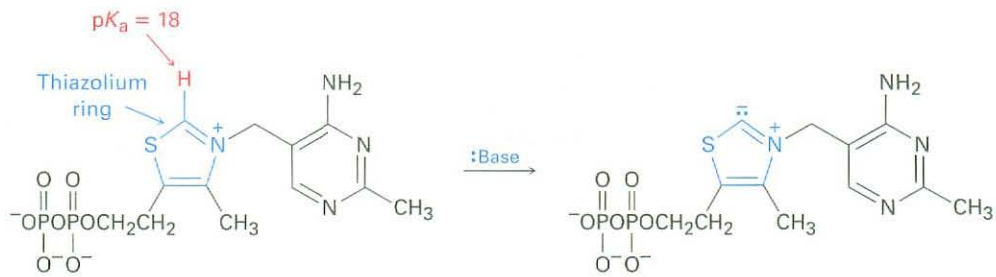
Pyruvate, produced by catabolism of glucose (and by degradation of several amino acids), can undergo several further transformations depending on the conditions and on the organism. In the absence of oxygen, pyruvate can be either reduced by NADH to yield lactate [$CH_3CH(OH)CO_2^-$] or, in yeast,

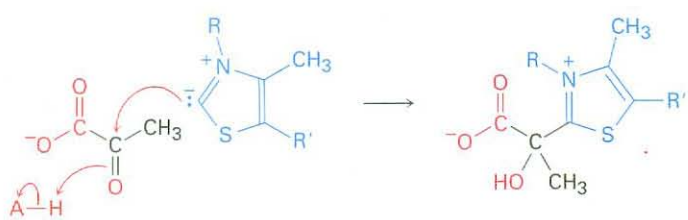
fermented to give ethanol. Under typical aerobic conditions in mammals, however, pyruvate is converted by a process called *oxidative decarboxylation* to give acetyl CoA plus $CO_2$. (*Oxidative* because the oxidation state of the carbonyl carbon rises from that of a ketone to that of a thioester.)

The conversion occurs through a multistep sequence of reactions catalyzed by a complex of enzymes and cofactors called the *pyruvate dehydrogenase complex*. The process occurs in three stages, each catalyzed by one of the enzymes in the complex, as outlined in Figure 29.11 on page 1152. Acetyl CoA, the ultimate product, then acts as fuel for the final stage of catabolism, the citric acid cycle. All the steps have laboratory analogies.

**Step 1 of Figure 29.11: Addition of Thiamin Diphosphate** The conversion of pyruvate to acetyl CoA begins by reaction of pyruvate with thiamin diphosphate, a derivative of vitamin $B_1$. Formerly called thiamin *pyro*phosphate, thiamin diphosphate is usually abbreviated as TPP. The spelling *thiamine* is also correct and frequently used.

The key structural feature in thiamin diphosphate is the presence of a thiazolium ring—a five-membered, unsaturated heterocycle containing a sulfur atom and a positively charged nitrogen atom. The thiazolium ring is weakly acidic, with a $pK_a$ of approximately 18 for the ring hydrogen between N and S. Bases can therefore deprotonate thiamin diphosphate, leading to formation of a nucleophilic ylide much like the phosphonium ylides used in Wittig reactions (Section 19.11). As in the Wittig reaction, the TPP ylide is a nucleophile and adds to the ketone carbonyl group of pyruvate to yield an alcohol addition product.




**Step 2 of Figure 29.11: Decarboxylation** The TPP addition product, which contains an iminium ion $\beta$ to a carboxylate anion, undergoes decarboxylation in much the same way that a $\beta$-keto acid decarboxylates in the acetoacetic ester synthesis (Section 22.7). The C=N$^+$ bond of the pyruvate addition product acts

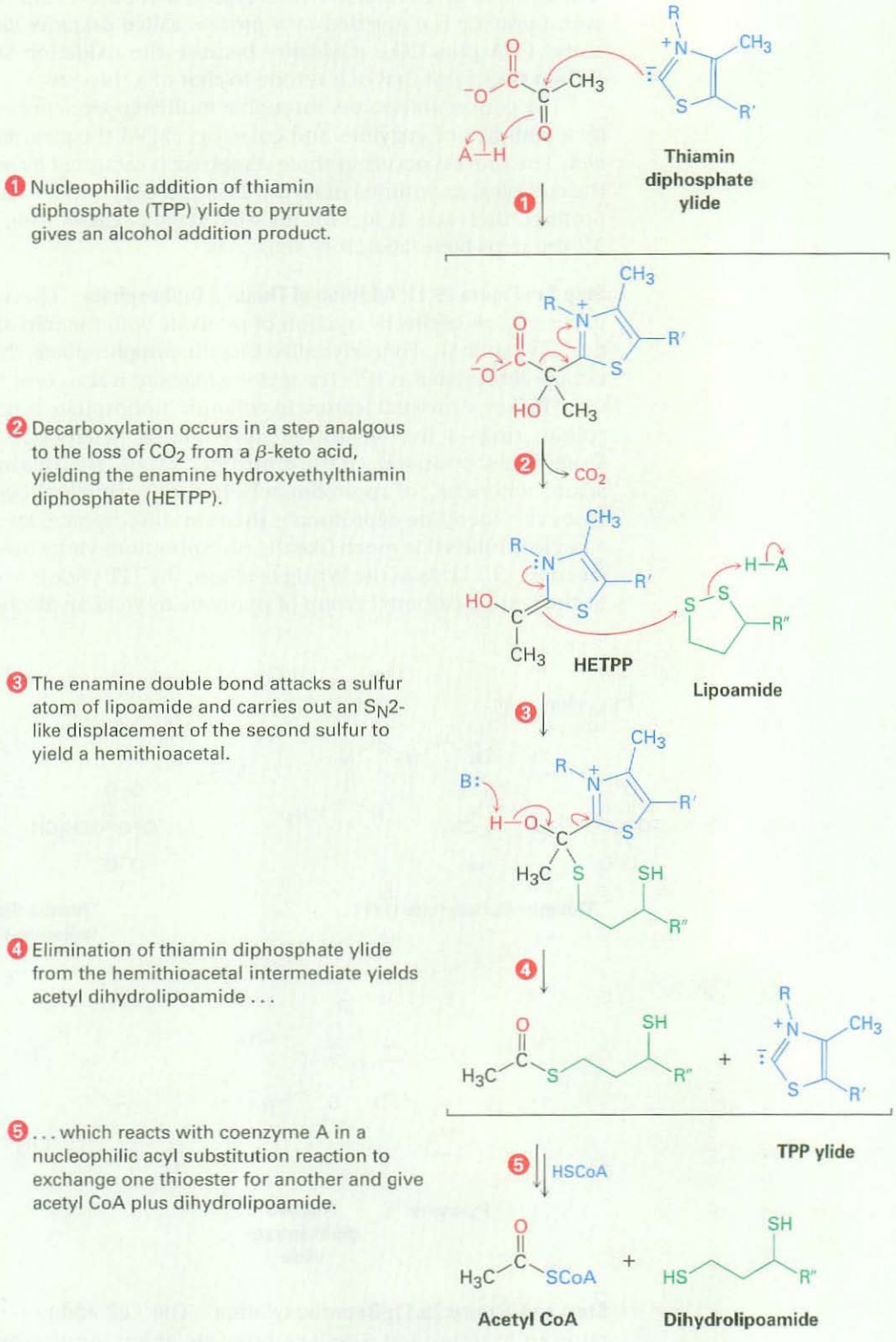
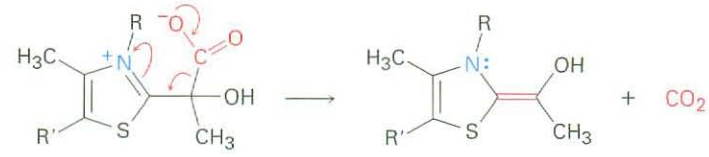


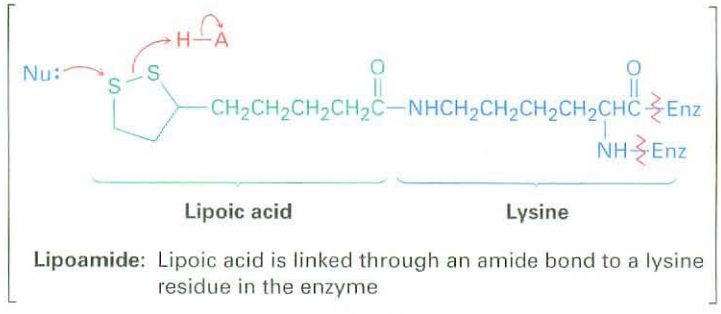


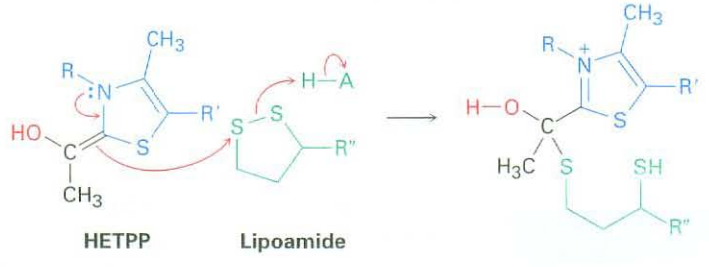

**Figure 29.11 MECHANISM:** Mechanism of the conversion of pyruvate to acetyl CoA through a multistep sequence of reactions that requires three different enzymes and four different coenzymes. The individual steps are explained in the text.

like the C=O bond of a β-keto acid to accept electrons as $CO_2$ leaves, giving hydroxyethylthiamin diphosphate (HETPP).

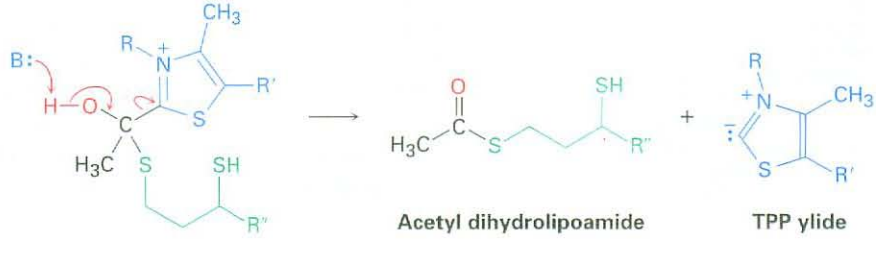



**Step 3 of Figure 29.11: Reaction with Lipoamide** Hydroxyethylthiamin diphosphate is an enamine ($R_2N-C=C$), which, like all enamines, is nucleophilic (Section 23.11). It therefore reacts with the enzyme-bound disulfide lipoamide by nucleophilic attack on a sulfur atom, displacing the second sulfur in an $S_N2$-like process.



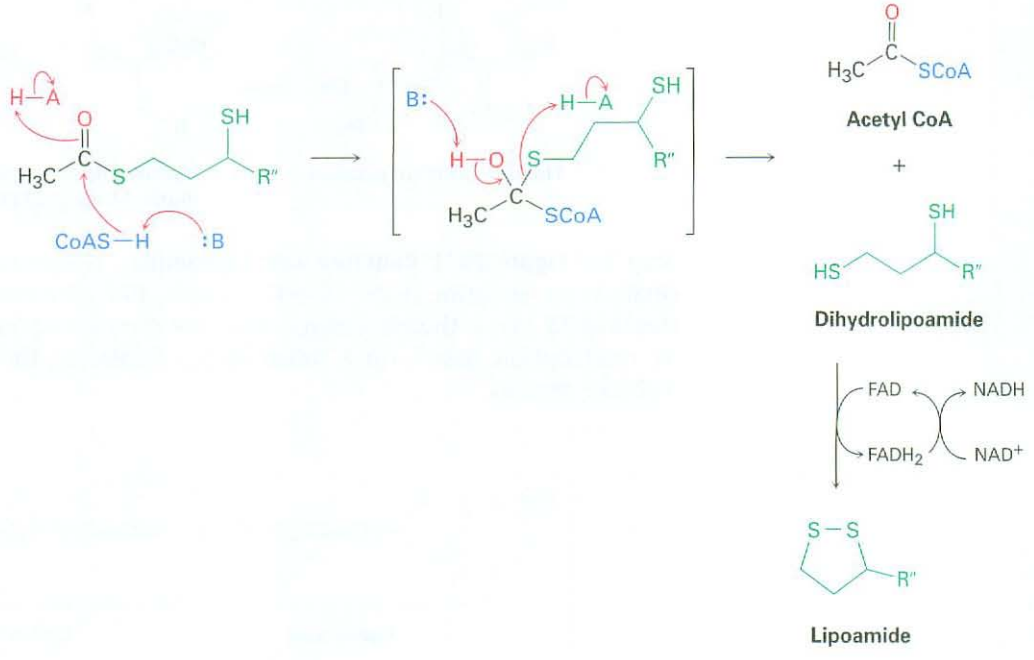




**Step 4 of Figure 29.11: Elimination of Thiamin Diphosphate** The product of the HETPP reaction with lipoamide is a hemithioacetal, which eliminates thiamin diphosphate ylide. This elimination is the reverse of the ketone addition in step 1 and generates acetyl dihydrolipoamide.




**Step 5 of Figure 29.11: Acyl Transfer** Acetyl dihydrolipoamide, a thioester, undergoes a nucleophilic acyl substitution reaction with coenzyme A to yield acetyl CoA plus dihydrolipoamide. The dihydrolipoamide is then oxidized back

to lipoamide by FAD (Section 29.3), and the $FADH_2$ that results is in turn oxidized back to FAD by $NAD^+$, completing the catalytic cycle.




**Problem 29.9** Which carbon atoms in glucose end up as $-CH_3$ carbons in acetyl CoA? Which carbons end up as $CO_2$?

## 29.7 The Citric Acid Cycle

**Sir Hans Adolf Krebs**

**Sir Hans Adolf Krebs** (1900–1981) was born in Hildesheim, Germany, and received an M.D. in 1925 from the University of Hamburg. In 1933, his appointment in Germany was terminated by the government, so he moved to England, first at the University of Cambridge, then at the University of Sheffield (1935–1954), and finally at the University of Oxford (1954–1967). He received the 1953 Nobel Prize in medicine for his work on elucidating pathways in intermediary metabolism.

The initial stages of catabolism result in the conversion of both fats and carbohydrates into acetyl groups that are bonded through a thioester link to coenzyme A. Acetyl CoA then enters the next stage of catabolism—the **citric acid cycle**, also called the *tricarboxylic acid (TCA) cycle*, or *Krebs cycle*, after Hans Krebs, who unraveled its complexities in 1937. The overall result of the cycle is the conversion of an acetyl group into two molecules of $CO_2$ plus reduced coenzymes by the eight-step sequence of reactions shown in Figure 29.12.

As its name implies, the citric acid *cycle* is a closed loop of reactions in which the product of the final step (oxaloacetate) is a reactant in the first step. The intermediates are constantly regenerated and flow continuously through the cycle, which operates as long as the oxidizing coenzymes $NAD^+$ and FAD are available. To meet this condition, the reduced coenzymes NADH and $FADH_2$ must be reoxidized via the electron-transport chain, which in turn relies on oxygen as the ultimate electron acceptor. Thus, the cycle is dependent on the availability of oxygen and on the operation of the electron-transport chain.

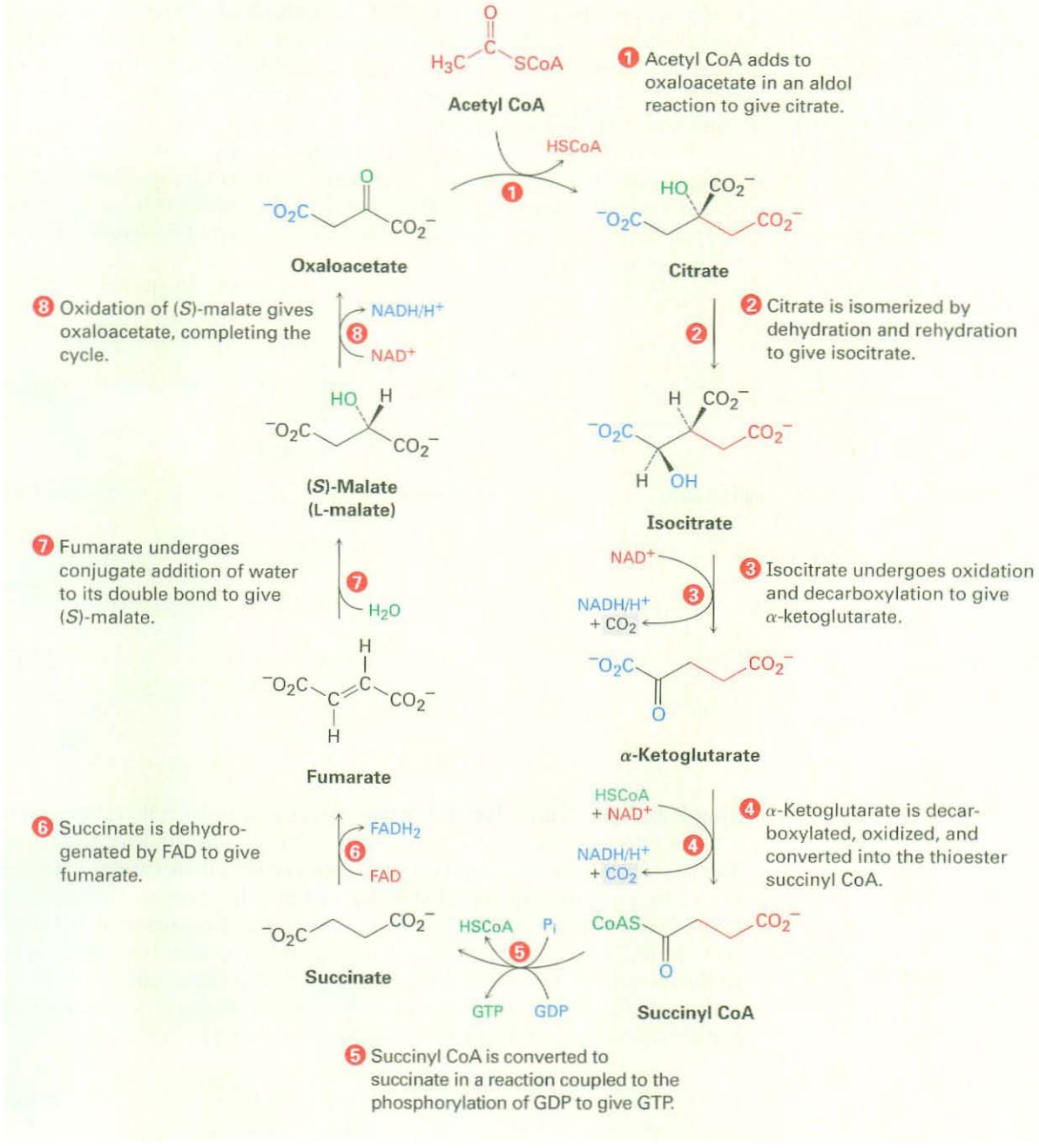
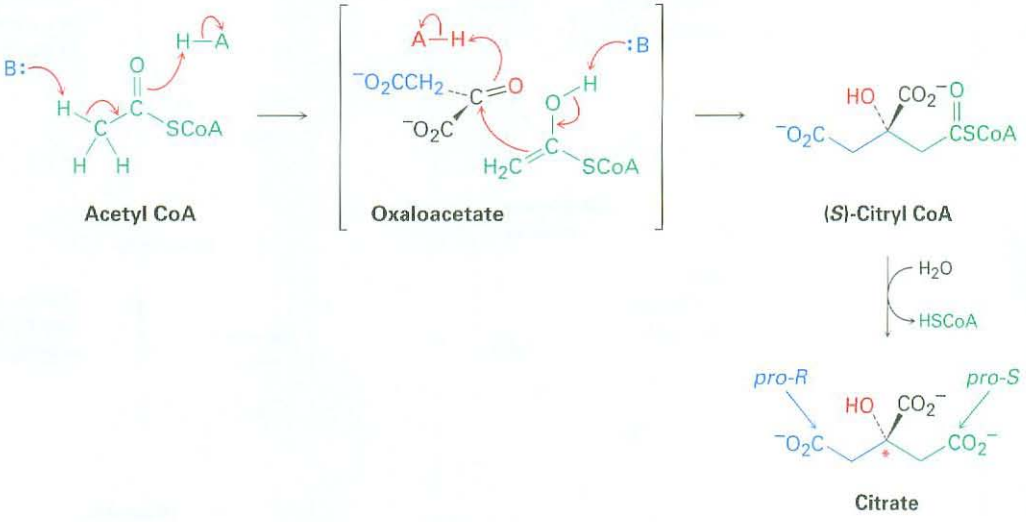




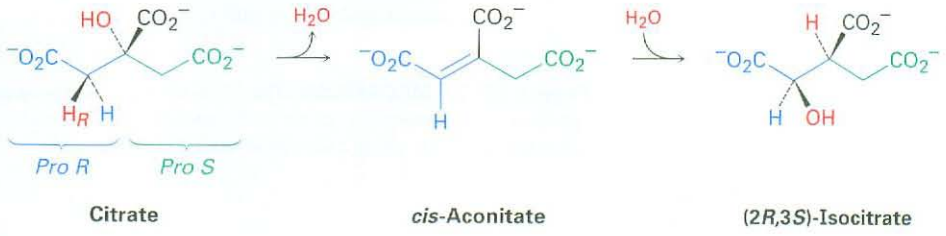

**Figure 29.12 MECHANISM:** The citric acid cycle is an eight-step series of reactions that results in the conversion of an acetyl group into two molecules of $CO_2$ plus reduced coenzymes. Individual steps are explained in the text.

**Step 1 of Figure 29.12: Addition to Oxaloacetate** Acetyl CoA enters the citric acid cycle in step 1 by nucleophilic addition to the oxaloacetate carbonyl group, to give (*S*)-citryl CoA. The addition is an aldol reaction and is catalyzed by citrate synthase, as discussed in Section 26.11. (*S*)-Citryl CoA is then hydrolyzed to citrate by a typical nucleophilic acyl substitution reaction, catalyzed by the same citrate synthase enzyme.

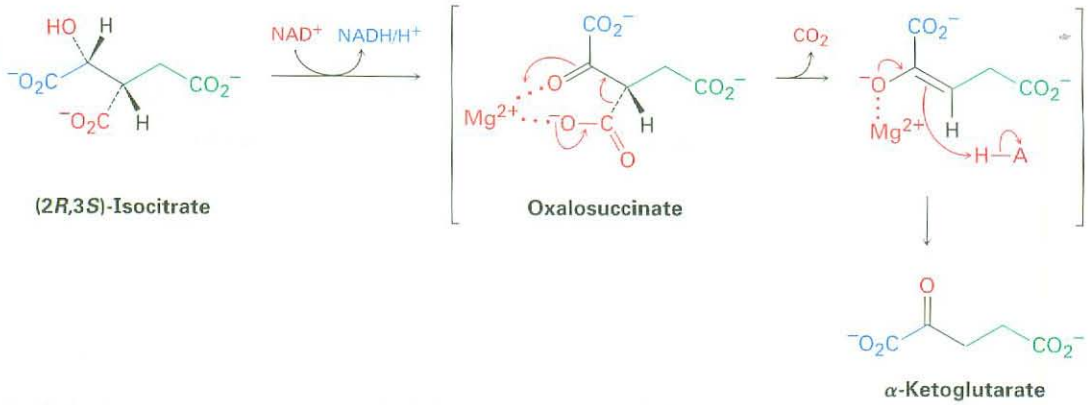
Note that the hydroxyl-bearing carbon of citrate is a prochirality center and contains two identical "arms." Because the initial aldol reaction of acetyl CoA to oxaloacetate occurs specifically from the *Si* face of the ketone carbonyl group, the *pro-S* arm of citrate is derived from acetyl CoA and the *pro-R* arm is derived from oxaloacetate.



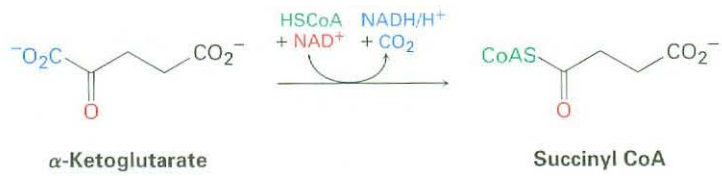

**Step 2 of Figure 29.12: Isomerization** Citrate, a prochiral tertiary alcohol, is next converted into its isomer, (2*R*,3*S*)-isocitrate, a chiral secondary alcohol. The isomerization occurs in two steps, both of which are catalyzed by the same aconitase enzyme. The initial step is an E1cB dehydration of a β-hydroxy acid to give *cis*-aconitate, the same sort of reaction that occurs in step 9 of glycolysis (Figure 29.7). The second step is a conjugate nucleophilic addition of water to the C=C bond (Section 19.13). The dehydration of citrate takes place specifically on the *pro-R* arm—the one derived from oxaloacetate—rather than on the *pro-S* arm derived from acetyl CoA.



**Step 3 of Figure 29.12: Oxidation and Decarboxylation** (2*R*,3*S*)-Isocitrate, a secondary alcohol, is oxidized by $NAD^+$ in step 3 to give the ketone oxalosuccinate, which loses $CO_2$ to give $\alpha$-ketoglutarate. Catalyzed by isocitrate dehydrogenase, the decarboxylation is a typical reaction of a $\beta$-keto acid, just like that in the acetoacetic ester synthesis (Section 22.7). The enzyme requires a divalent cation as cofactor, presumably to polarize the ketone carbonyl group.

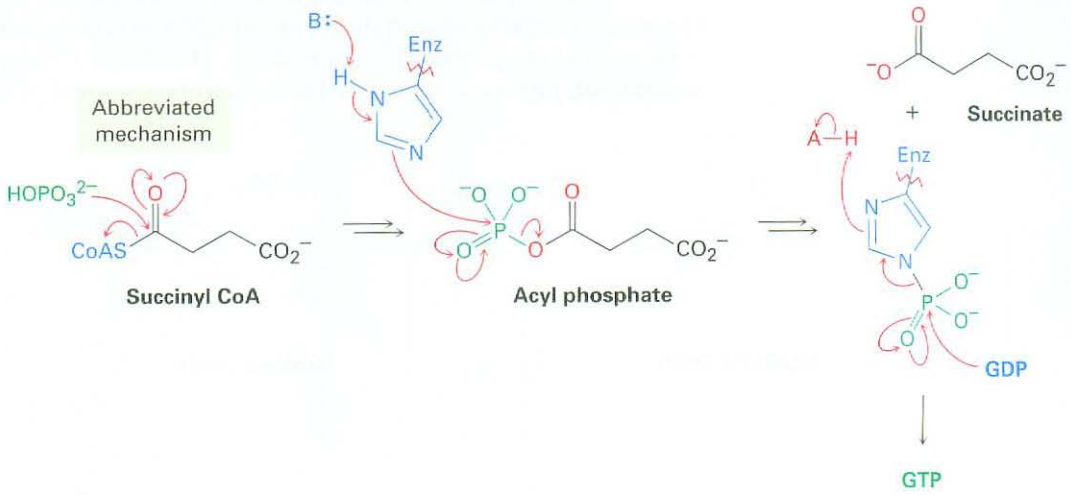



**Step 4 of Figure 29.12: Oxidative Decarboxylation** The transformation of $\alpha$-ketoglutarate to succinyl CoA in step 4 is a multistep process just like the transformation of pyruvate to acetyl CoA that we saw in Figure 29.11. In both cases, an $\alpha$-keto acid loses $CO_2$ and is oxidized to a thioester in a series of steps catalyzed by a multienzyme dehydrogenase complex. As in the conversion of pyruvate to acetyl CoA, the reaction involves an initial nucleophilic addition reaction to $\alpha$-ketoglutarate by thiamin diphosphate ylide, followed by decarboxylation, reaction with lipoamide, elimination of TPP ylide, and finally a transesterification of the dihydrolipoamide thioester with coenzyme A.

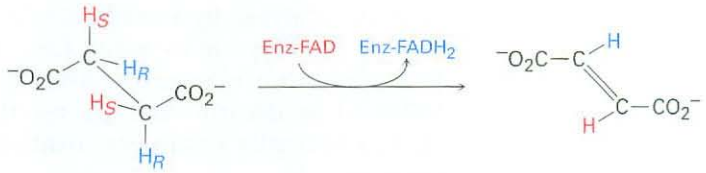



**Step 5 of Figure 29.12: Acyl CoA Cleavage** Succinyl CoA is converted to succinate in step 5. The reaction is catalyzed by succinyl CoA synthetase and is coupled with phosphorylation of guanosine diphosphate (GDP) to give guanosine triphosphate (GTP). The overall transformation is similar to that of steps 6 through 8 in glycolysis (Figure 29.7), in which a thioester is converted into an acyl phosphate and a phosphate group is then transferred to ADP.

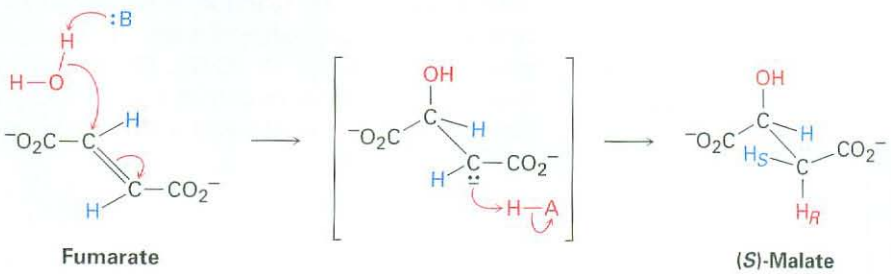
The overall result is a "hydrolysis" of the thioester group without involvement of water.



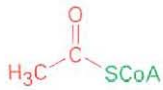

**Step 6 of Figure 29.12: Dehydrogenation** Succinate is dehydrogenated in step 6 by the FAD-dependent succinate dehydrogenase to give fumarate. The process is analogous to what occurs during the $\beta$-oxidation pathway of fatty-acid catabolism (Section 29.3). The reaction is stereospecific, removing the *pro-S* hydrogen from one carbon and the *pro-R* hydrogen from the other.




**Steps 7–8 of Figure 29.12: Hydration and Oxidation** The final two steps in the citric acid cycle are the conjugate nucleophilic addition of water to fumarate to yield (*S*)-malate (L-malate) and the oxidation of (*S*)-malate by $NAD^+$ to give oxaloacetate. The addition is catalyzed by fumarase and is mechanistically similar to the addition of water to *cis*-aconitate in step 2. The reaction occurs through an enolate-ion intermediate, which is protonated on the side opposite the OH, leading to a net anti addition.



The final step is the oxidation of (*S*)-malate by $NAD^+$ to give oxaloacetate, a reaction catalyzed by malate dehydrogenase. The citric acid cycle has now returned to its starting point, ready to revolve again. The overall result of the cycle is



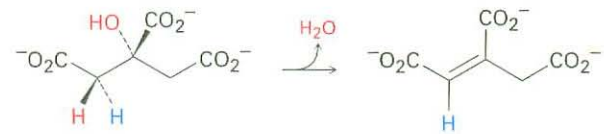
**Acetyl CoA**

$$+ \ 3\ NAD^+ + FAD + GDP + P_i + 2\ H_2O \longrightarrow 2\ CO_2 + HSCoA + 3\ NADH + 2\ H^+ + FADH_2 + GTP$$

**Problem 29.10** Which of the substances in the citric acid cycle are tricarboxylic acids, thus giving the cycle its alternative name?

**Problem 29.11** Write mechanisms for step 2 of the citric acid cycle, the dehydration of citrate and the addition of water to aconitate.

**Problem 29.12** Is the *pro-R* or *pro-S* hydrogen removed from citrate during the dehydration in step 2 of the citric acid cycle? Does the elimination reaction occur with syn or anti geometry?

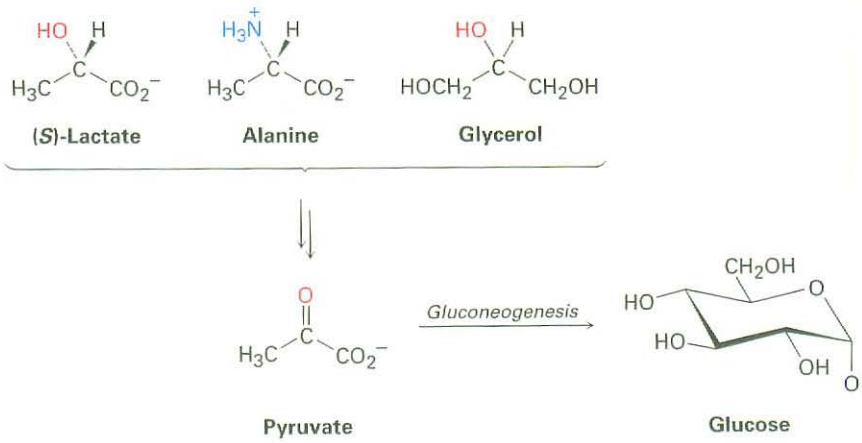


**Citrate** **cis-Aconitate**

## 29.8 Carbohydrate Biosynthesis: Gluconeogenesis

Glucose is the body's primary fuel when food is plentiful, but in times of fasting or prolonged exercise, glucose stores can become depleted. Most tissues then begin metabolizing fats as their source of acetyl CoA, but the brain is different. The brain relies almost entirely on glucose for fuel and is dependent on receiving a continuous supply in the blood. When the supply of glucose fails for even a brief time, irreversible damage can occur. Thus, a pathway for synthesizing glucose from simple precursors is needed.

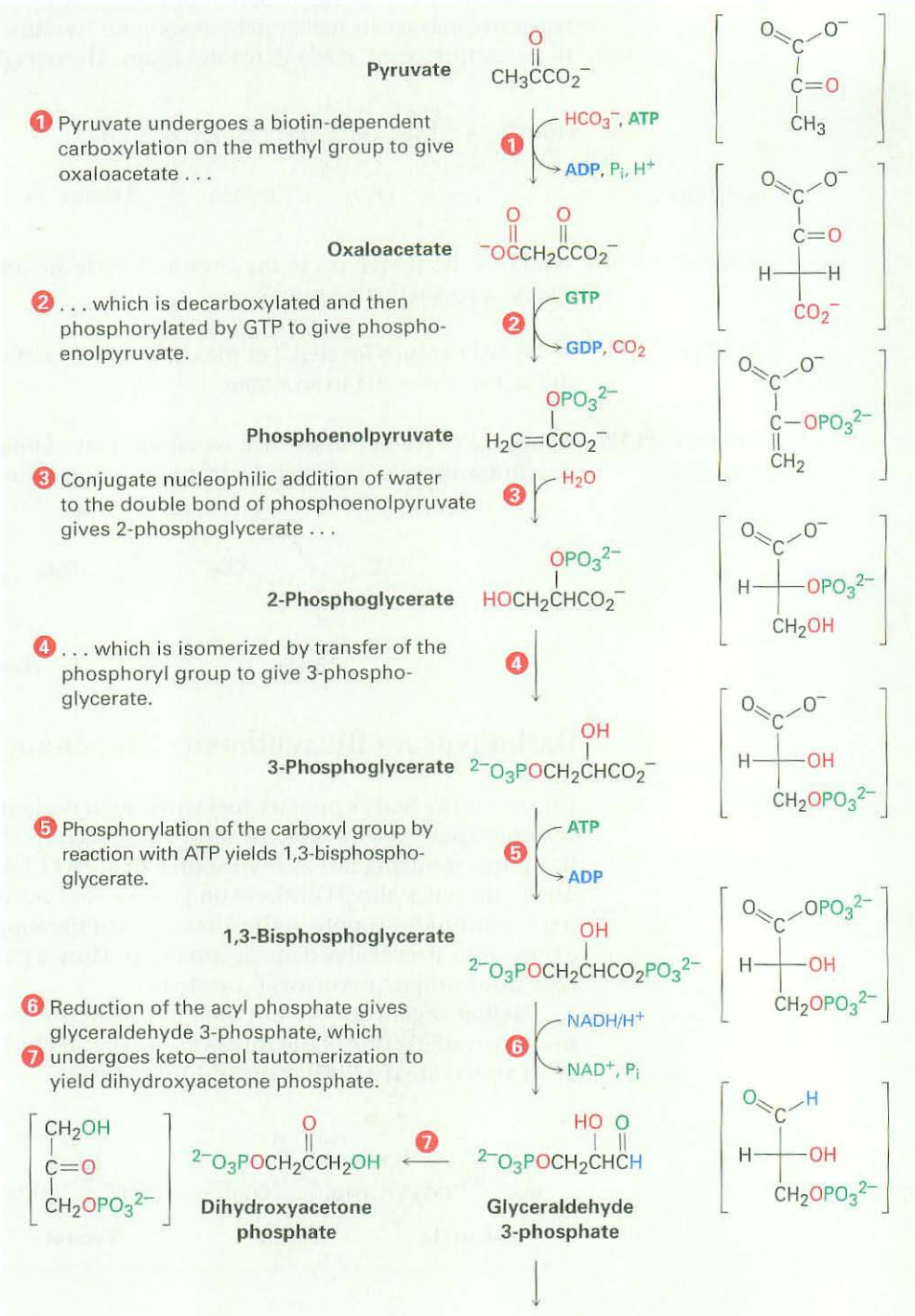
Higher organisms are not able to synthesize glucose from acetyl CoA but must instead use one of the three-carbon precursors lactate, glycerol, or alanine, all of which are readily converted into pyruvate.




**(*S*)-Lactate** **Alanine** **Glycerol**

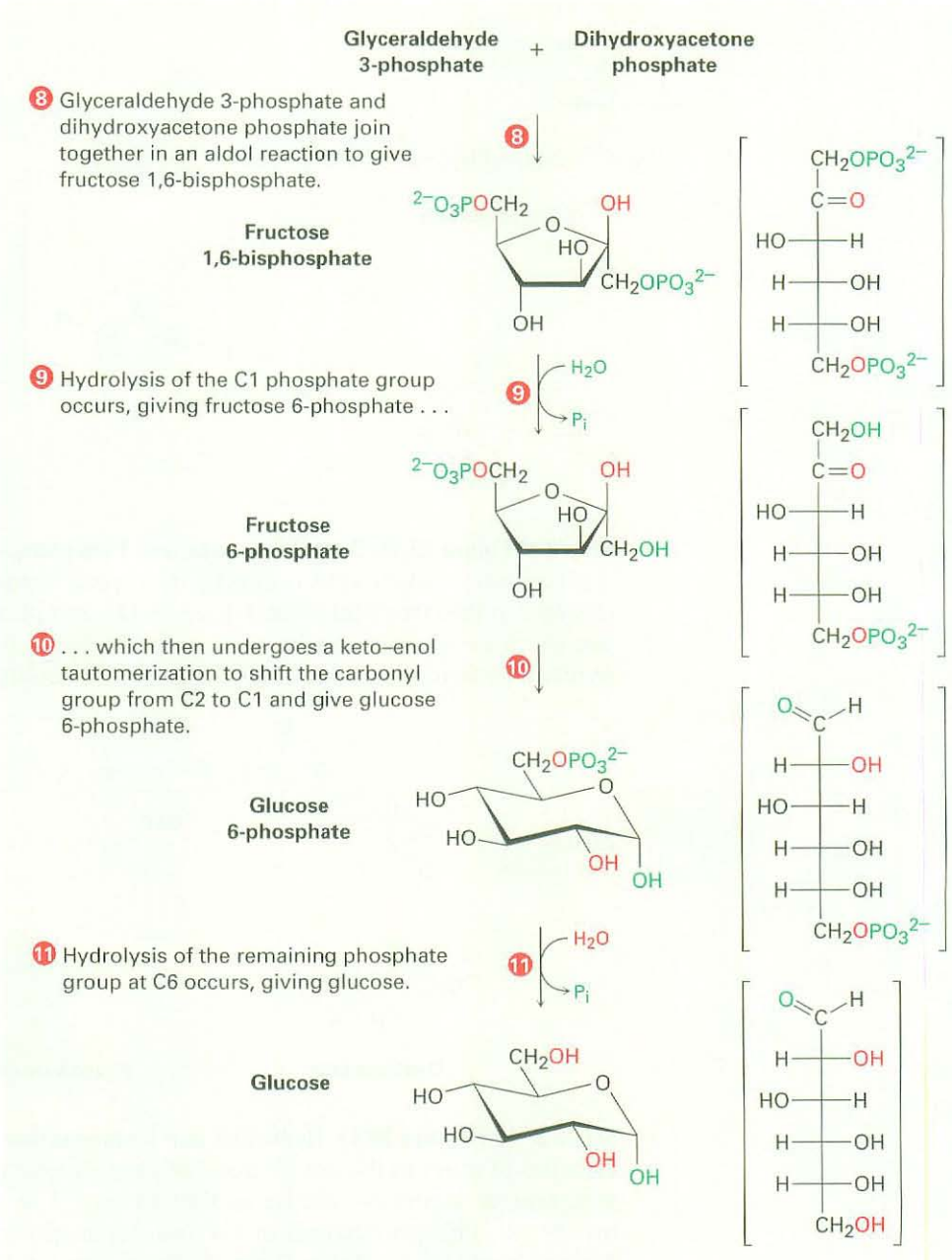
**Pyruvate** **Glucose**

**Figure 29.13**
**MECHANISM:** The gluconeogenesis pathway for the biosynthesis of glucose from pyruvate. Individual steps are explained in the text.

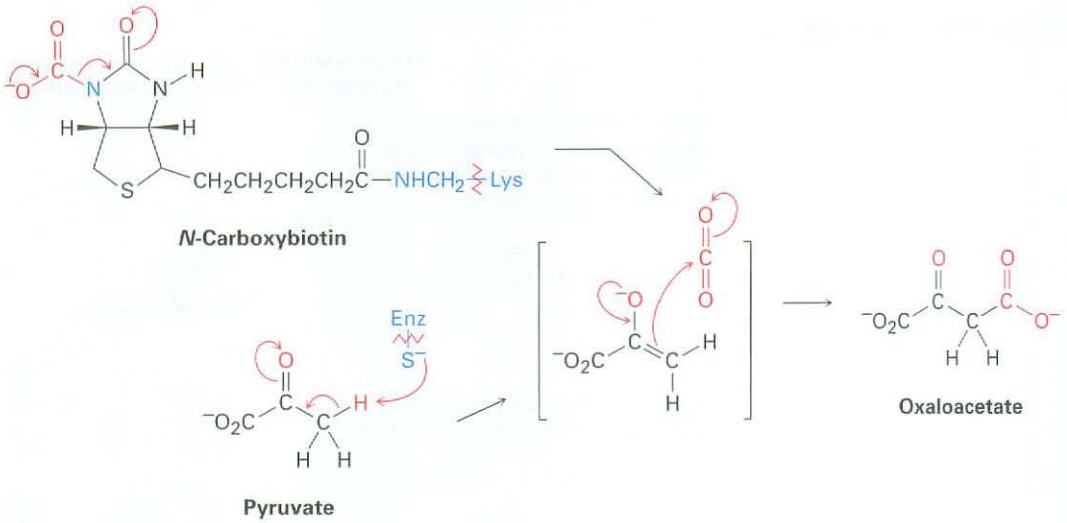


Pyruvate then becomes the starting point for **gluconeogenesis**, the 11-step biosynthetic pathway by which organisms make glucose (Figure 29.13). The gluconeogenesis pathway by which glucose is made, however, is not the reverse of the glycolysis pathway by which it is degraded. As with the catabolic and anabolic pathways for fatty acids (Sections 29.3 and 29.4), the catabolic and anabolic pathways for carbohydrates differ in some details so that both are energetically favorable.

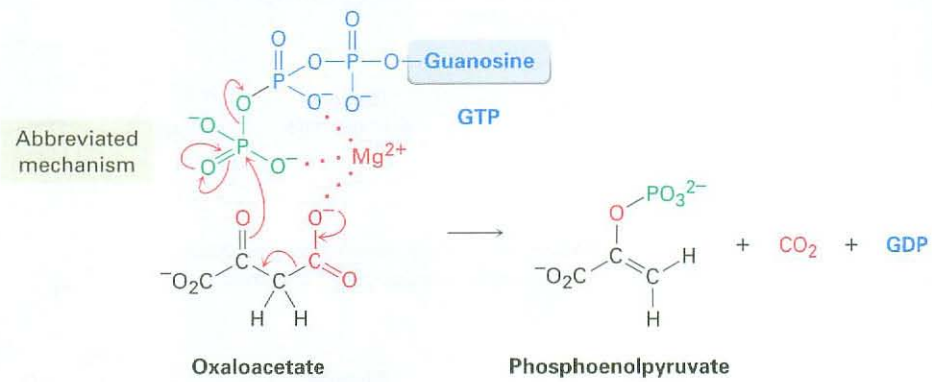
**Figure 29.13** *(continued)*



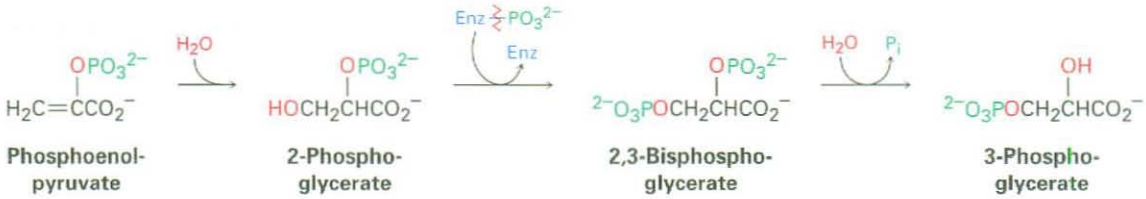
**Step 1 of Figure 29.13: Carboxylation** Gluconeogenesis begins with the carboxylation of pyruvate to yield oxaloacetate. The reaction is catalyzed by pyruvate carboxylase and requires ATP, bicarbonate ion, and the coenzyme biotin, which acts as a carrier to transport $CO_2$ to the enzyme active site. The mechanism is analogous to that of step 3 in fatty-acid biosynthesis (Figure 29.6), in which acetyl CoA is carboxylated to yield malonyl CoA.



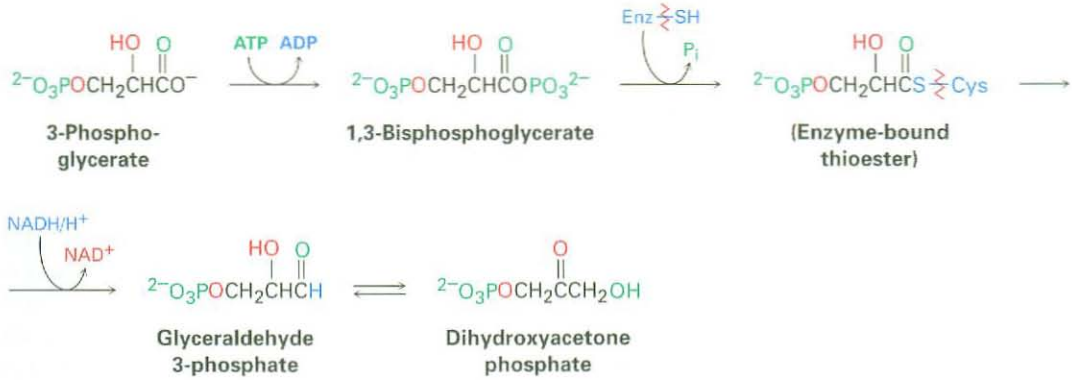

**Step 2 of Figure 29.13: Decarboxylation and Phosphorylation** Decarboxylation of oxaloacetate, a $\beta$-keto acid, occurs by the typical retro-aldol mechanism like that in step 3 in the citric acid cycle (Figure 29.12), and phosphorylation of the resultant pyruvate enolate ion by GTP occurs concurrently to give phosphoenolpyruvate. The reaction is catalyzed by phosphoenolpyruvate carboxykinase.



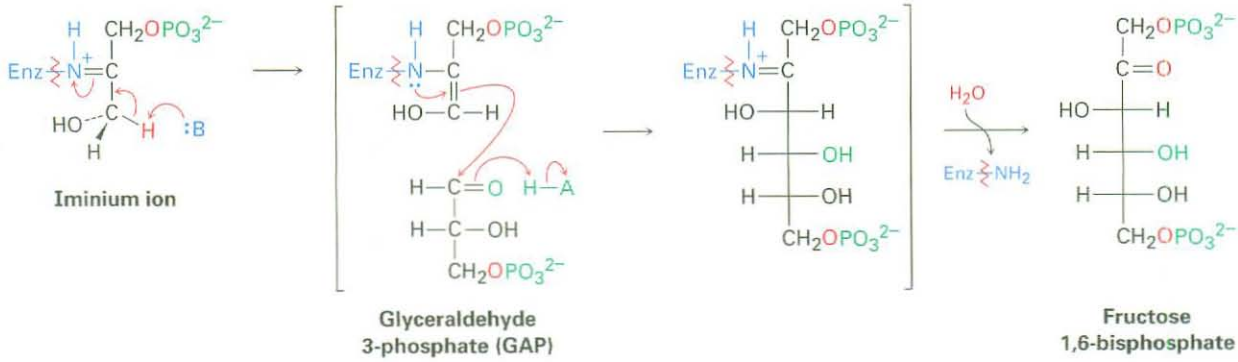

**Steps 3–4 of Figure 29.13: Hydration and Isomerization** Conjugate nucleophilic addition of water to the double bond of phosphoenolpyruvate gives 2-phosphoglycerate by a process similar to that of step 7 in the citric acid cycle (Figure 29.12). Phosphorylation of C3 and dephosphorylation of C2 then yields 3-phosphoglycerate. Mechanistically, these steps are the reverse of steps 9 and 8 in glycolysis (Figure 29.7), which have equilibrium constants near 1 so that substantial amounts of reactant and product are both present.




**Steps 5–7 of Figure 29.13: Phosphorylation, Reduction, and Tautomerization** Reaction of 3-phosphoglycerate with ATP generates the corresponding acyl phosphate, 1,3-bisphosphoglycerate, which binds to the glyceraldehyde 3-phosphate dehydrogenase by a thioester bond to a cysteine residue. Reduction by $NADH/H^+$ yields the aldehyde, and keto–enol tautomerization of the aldehyde gives dihydroxyacetone phosphate. All three steps are mechanistically the reverse of the corresponding steps 7, 6, and 5 of glycolysis and have equilibrium constants near 1.

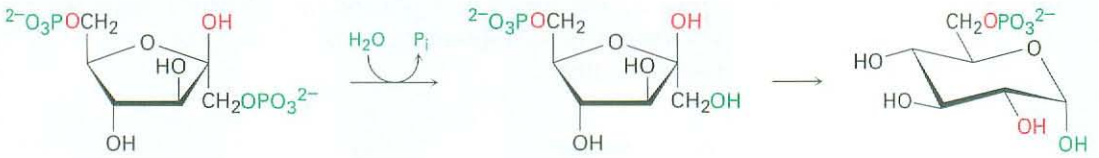



**Step 8 of Figure 29.13: Aldol Reaction** Dihydroxyacetone phosphate and glyceraldehyde 3-phosphate, the two 3-carbon units produced in step 7, join by an aldol reaction to give fructose 1,6-bisphosphate, the reverse of step 4 in glycolysis. As in glycolysis (Figure 29.9), the reaction is catalyzed in plants and animals by a class I aldolase and takes place on an iminium ion formed by reaction of dihydroxyacetone phosphate with a side-chain lysine $-NH_2$ group on the enzyme. Loss of a proton from the neighboring carbon then generates an enamine, an aldol-like reaction ensues, and the product is hydrolyzed.



**Steps 9–10 of Figure 29.13: Hydrolysis and Isomerization** Hydrolysis of the phosphate group at C1 of fructose 1,6-bisphosphate gives fructose 6-phosphate. Although the result of the reaction is the exact opposite of step 3 in glycolysis, the mechanism is not. In glycolysis, the phosphorylation is accomplished by reaction of the fructose with ATP. The reverse of that process, however—the reaction of fructose 1,6-bisphosphate with ADP to give fructose 6-phosphate and ATP—is energetically unfavorable because ATP is too high in energy. Thus, an alternative pathway is used in which the C1 phosphate group is removed by a direct hydrolysis reaction, catalyzed by fructose 1,6-bisphosphatase.

Following hydrolysis, keto–enol tautomerization of the carbonyl group from C2 to C1 gives glucose 6-phosphate. The isomerization is the reverse of step 2 in glycolysis.

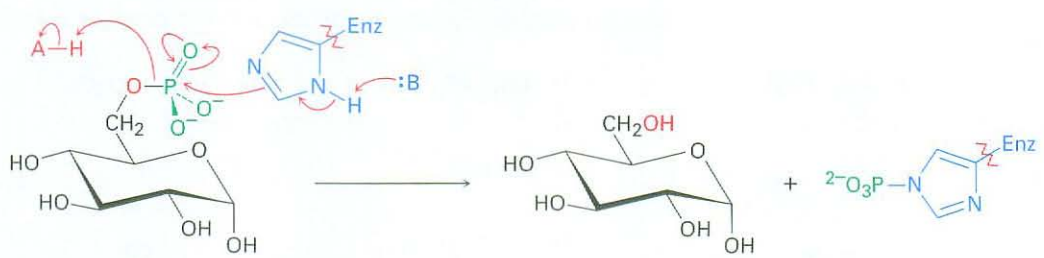



**Fructose 1,6-bisphosphate** **Fructose 6-phosphate** **Glucose 6-phosphate**

**Step 11 of Figure 29.13: Hydrolysis** The final step in gluconeogenesis is the conversion of glucose 6-phosphate to glucose by another phosphatase-catalyzed hydrolysis reaction. As just discussed for the hydrolysis of fructose 1,6-bisphosphate in step 9, and for the same energetic reasons, the mechanism of the glucose 6-phosphate hydrolysis is not the exact opposite of the corresponding step 1 in glycolysis.

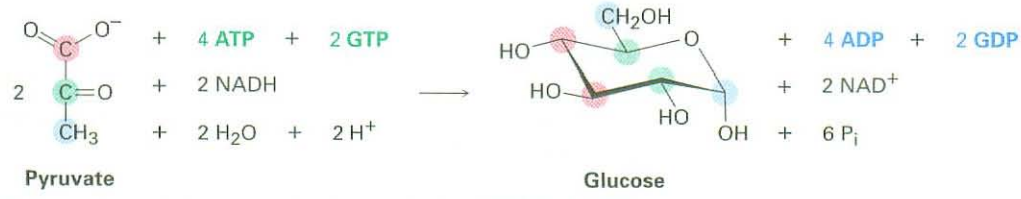
Interestingly, however, the mechanisms of the two phosphate hydrolysis reactions in steps 9 and 11 are not the same. In step 9, water is the nucleophile, but in the glucose 6-phosphate reaction of step 11, a histidine residue on the enzyme attacks phosphorus, giving a phosphoryl enzyme intermediate that subsequently reacts with water.

Abbreviated mechanism




**Glucose 6-phosphate** **Glucose**

The overall result of gluconeogenesis is summarized by the following equation:

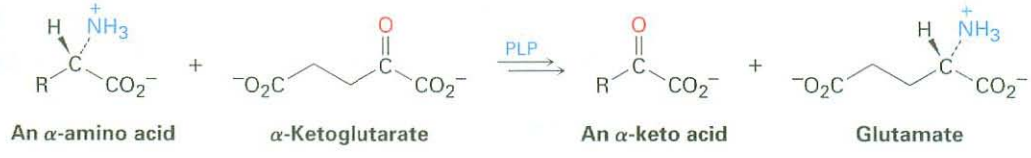


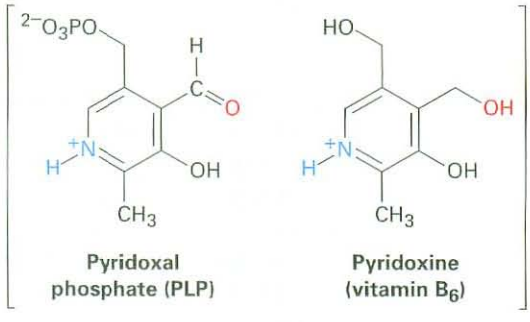

**Problem 29.13** Write a mechanism for step 6 of gluconeogenesis, the reduction of 3-phosphoglyceryl phosphate with $NADH/H^+$ to yield glyceraldehyde 3-phosphate.

## 29.9 Catabolism of Proteins: Transamination

The catabolism of proteins is much more complex than that of fats and carbohydrates because each of the 20 amino acids is degraded through its own unique pathway. The general idea, however, is that the amino nitrogen atom is removed and the substance that remains is converted into a compound that enters the citric acid cycle.

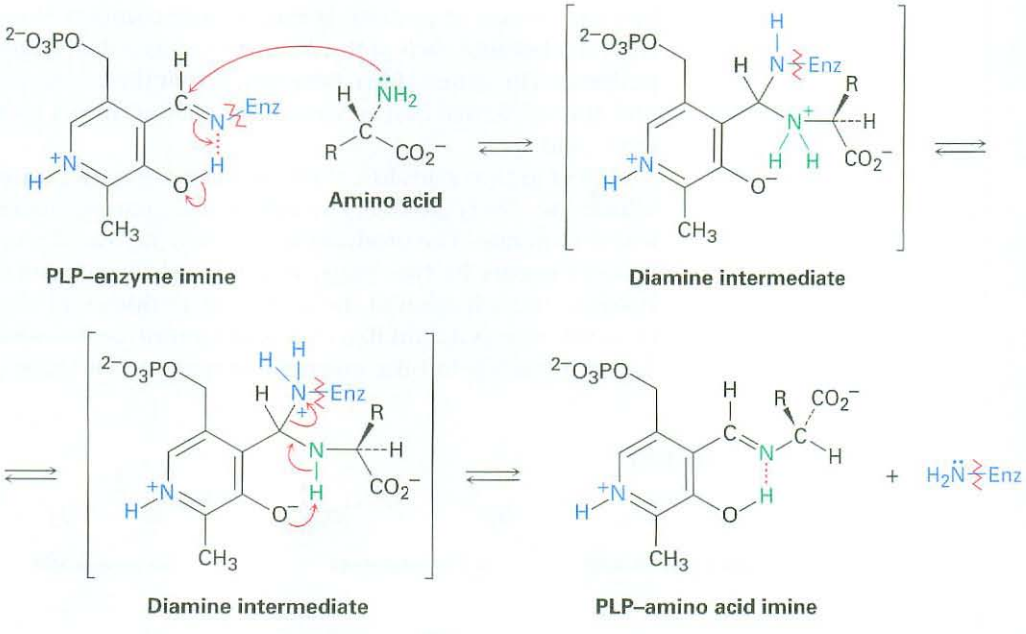
Most amino acids lose their nitrogen atom by a **transamination** reaction in which the $-NH_2$ group of the amino acid changes places with the keto group of $\alpha$-ketoglutarate. The products are a new $\alpha$-keto acid plus glutamate. The overall process occurs in two parts, is catalyzed by aminotransferase enzymes, and involves participation of the coenzyme pyridoxal phosphate (PLP), a derivative of pyridoxine (vitamin $B_6$). Different aminotransferases differ in their specificity for amino acids, but the mechanism remains the same.



The mechanism of the first part of transamination is shown in Figure 29.14. The process begins with reaction between the $\alpha$-amino acid and pyridoxal phosphate, which is covalently bonded to the aminotransferase by an imine linkage between the side-chain $-NH_2$ group of a lysine residue and the PLP aldehyde group. Deprotonation/reprotonation of the PLP–amino acid imine in steps 2 and 3 effects tautomerization of the imine C=N bond, and hydrolysis of the tautomerized imine in step 4 gives an $\alpha$-keto acid plus pyridoxamine phosphate (PMP).

**Step 1 of Figure 29.14: Transimination** The first step in trans*amination* is trans*imination*—the reaction of the PLP–enzyme imine with an $\alpha$-amino acid to give a PLP–amino acid imine plus expelled enzyme as the leaving group. The reaction occurs by nucleophilic addition of the amino acid $-NH_2$ group to the C=N bond of the PLP imine, much as an amine adds to the C=O bond of a ketone or aldehyde in a nucleophilic addition reaction (Section 19.8). The protonated diamine intermediate undergoes a proton transfer and expels the lysine amino group in the enzyme to complete the step.




**Steps 2–4 of Figure 29.14: Tautomerization and Hydrolysis** Following formation of the PLP–amino acid imine in step 1, a tautomerization of the C=N bond occurs in step 2. The basic lysine residue in the enzyme that was expelled as a leaving group during transimination deprotonates the acidic $\alpha$ position of the amino acid, with the protonated pyridine ring of PLP acting as the electron acceptor as shown in step 2 of Figure 29.2. Reprotonation occurs on the carbon atom next to the ring (step 3), generating a tautomeric product that is the imine of an $\alpha$-keto acid with pyridoxamine phosphate, abbreviated PMP (Figure 29.15).

Hydrolysis of this PMP–$\alpha$-keto acid imine in step 4 then completes the first part of the transamination reaction. The hydrolysis is the mechanistic reverse of

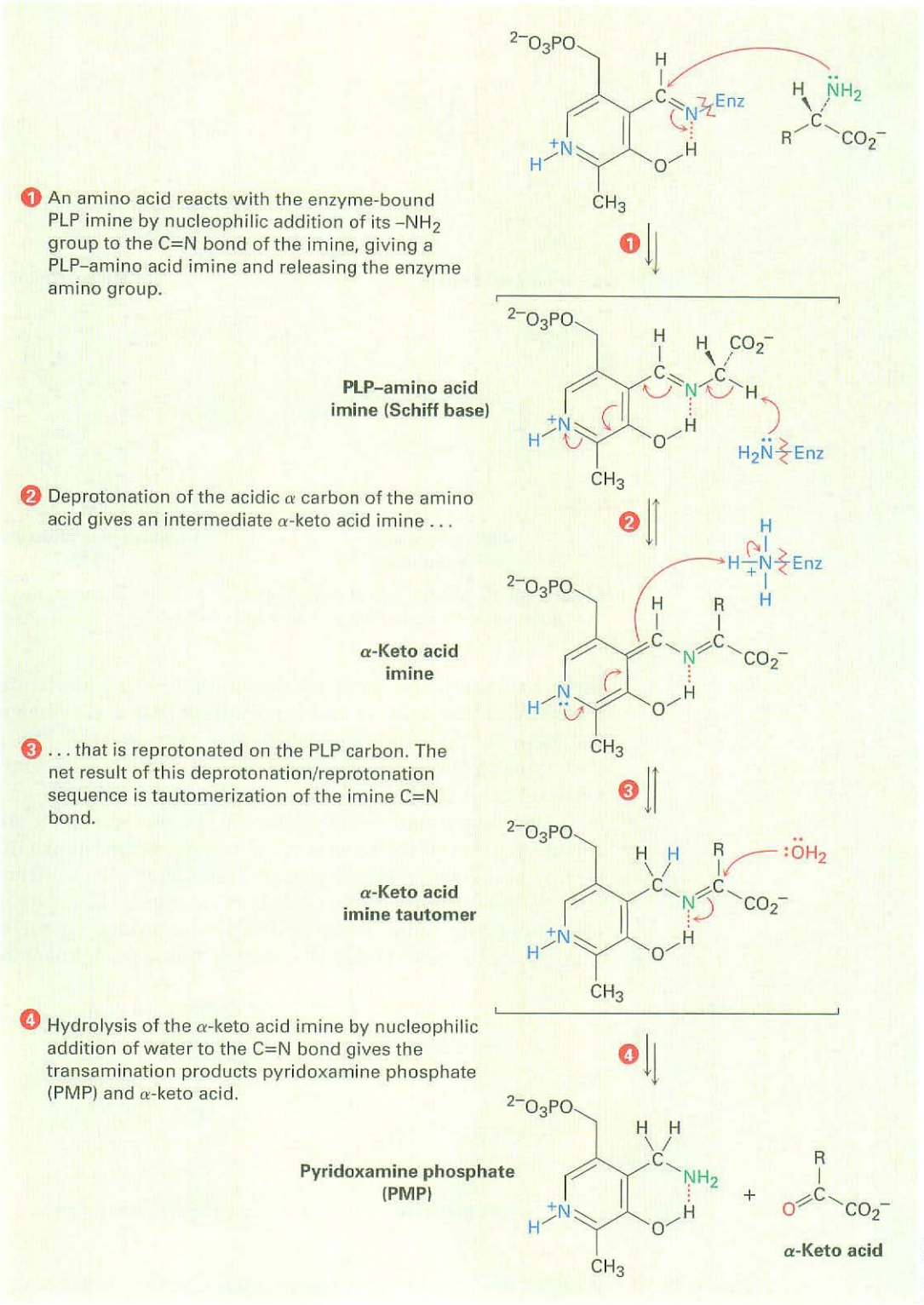



**Figure 29.14 MECHANISM:** Mechanism of the enzyme-catalyzed, PLP-dependent transamination of an α-amino acid to give an α-keto acid. Individual steps are explained in the text.

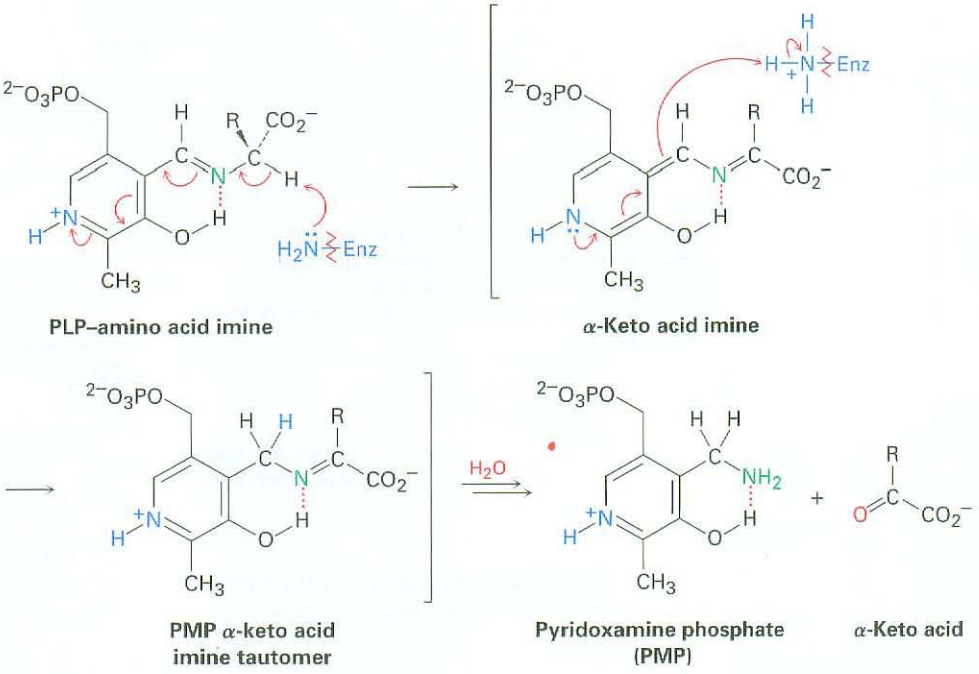
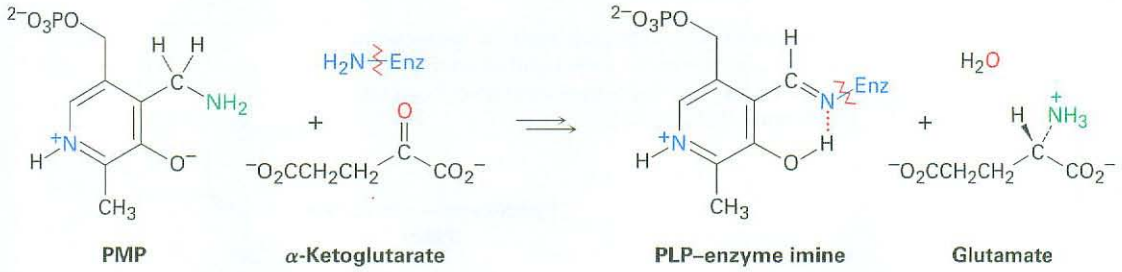



**Figure 29.15** Mechanism of steps 2–4 of amino acid transamination, the conversion of a PLP–amino acid imine to PMP and an α-keto acid.

imine formation and occurs by nucleophilic addition of water to the imine, followed by proton transfer and expulsion of PMP as leaving group.

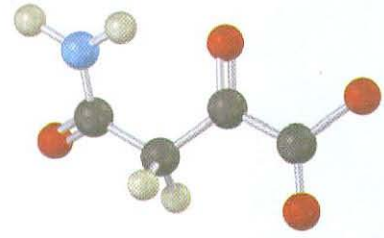
With PLP plus the α-amino acid now converted into PMP plus an α-keto acid, PMP must be transformed back into PLP to complete the catalytic cycle. The conversion occurs by another transamination reaction, this one between PMP and an α-keto acid, usually α-ketoglutarate. PLP plus glutamate are the products, and the mechanism of the process is the reverse of that shown in Figure 29.14. That is, PMP and α-ketoglutarate give an imine; the PMP–ketoglutarate imine undergoes tautomerization of the C=N bond to give a PLP–glutamate imine; and the PLP–glutamate imine reacts with a lysine residue on the enzyme in a transimination process to yield PLP–enzyme imine plus glutamate.




**Problem 29.14** Write all the steps in the transamination reaction of PMP with α-ketoglutarate plus a lysine residue in the enzyme to give the PLP–enzyme imine plus glutamate.

**Problem 29.15** What α-keto acid is formed on transamination of leucine?

**Problem 29.16** From what amino acid is the following α-keto acid derived?



## 29.10 Some Conclusions about Biological Chemistry

As promised in the chapter introduction, the past few sections have been a fast-paced tour of a large number of reactions. Following it all undoubtedly required a lot of work and a lot of page turning to look at earlier sections.

After examining the various metabolic pathways, perhaps the main conclusion about biological chemistry is the remarkable similarity between the mechanisms of biological reactions and the mechanisms of laboratory reactions. In all the pathways described in this chapter, terms like *imine formation, aldol reaction, nucleophilic acyl substitution reaction, E1cB reaction,* and *Claisen reaction* appear constantly. Biological reactions are not mysterious—the vitalistic theory described on page 1 died long ago. There are clear, understandable reasons for the reactions carried out within living organisms. Biological chemistry *is* organic chemistry.

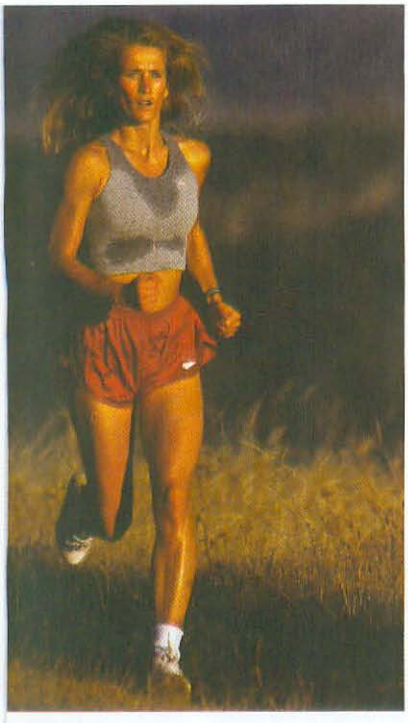
*Focus On . . .*

## Basal Metabolism

The minimum amount of energy per unit time an organism must expend to stay alive is called the organism's *basal metabolic rate (BMR)*. This rate is measured by monitoring respiration and finding the rate of oxygen consumption, which is proportional to the amount of energy used. Assuming an average dietary mix of fats, carbohydrates, and proteins, approximately 4.82 kcal are required for each liter of oxygen consumed.

The average basal metabolic rate for humans is about 65 kcal/h, or 1600 kcal/day. Obviously, the rate varies for different people depending on sex, age, weight, and physical condition. As a rule, the BMR is lower for older people than for younger people, is lower for females than for males, and is lower for people in good physical condition than for those who are out of shape and overweight. A BMR substantially above the expected value indicates an unusually rapid metabolism, perhaps caused by a fever or some biochemical abnormality.

*(continued)*



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Endurance trail runners can use up to 10,000 kcal to fuel their prodigious energy needs in runs of over 100 miles.

The total number of calories a person needs each day is the sum of the basal requirement plus the energy used for physical activities, as shown in Table 29.1. A relatively inactive person needs about 30% above basal requirements per day, a lightly active person needs about 50% above basal, and a very active person such as an athlete or construction worker may need 100% above basal requirements. Some endurance athletes in ultradistance events can use as many as 10,000 kcal/day above the basal level. Each day that your caloric intake is above what you use, fat is stored in your body and your weight rises. Each day that your caloric intake is below what you use, fat in your body is metabolized and your weight drops.

**Table 29.1 Energy Cost of Various Activities[a]**

| Activity | Kcal/min |
|---|---|
| Sleeping | 1.2 |
| Sitting, reading | 1.6 |
| Standing still | 1.8 |
| Walking | 3–6 |
| Tennis | 7–9 |
| Basketball | 9–10 |
| Walking up stairs | 10–18 |
| Running | 9–22 |

[a]For a 70 kg man.

## SUMMARY AND KEY WORDS

anabolism, 1126
β-oxidation pathway, 1133
catabolism, 1126
citric acid cycle, 1154
gluconeogenesis, 1161
glycolysis, 1143
metabolism, 1126
Schiff base, 1147
transamination, 1165

**Metabolism** is the sum of all chemical reactions in the body. Reactions that break down large molecules into smaller fragments are called **catabolism**; reactions that build up large molecules from small pieces are called **anabolism**. Although the details of specific biochemical pathways are sometimes complex, all the reactions that occur follow the normal rules of organic chemical reactivity.

The catabolism of fats begins with digestion, in which ester bonds are hydrolyzed to give glycerol and fatty acids. The fatty acids are degraded in the four-step **β-oxidation pathway** by removal of two carbons at a time, yielding acetyl CoA. Catabolism of carbohydrates begins with the hydrolysis of glycoside bonds to give glucose, which is degraded in the ten-step **glycolysis** pathway. Pyruvate, the initial product of glycolysis, is then converted into acetyl CoA. Acetyl CoA next enters the eight-step **citric acid cycle**, where it is further degraded into $CO_2$. The cycle is a closed loop of reactions in which the product of the final step (oxaloacetate) is a reactant in the first step. The intermediates are constantly regenerated and flow continuously through the cycle, which operates as long as the oxidizing coenzymes $NAD^+$ and FAD are available.

Catabolism of proteins is more complex than that of fats or carbohydrates because each of the 20 different amino acids is degraded by its own unique pathway. In general, though, the amino nitrogen atoms are removed and the substances that remain are converted into compounds that enter the citric acid cycle. Most amino acids lose their nitrogen atom by **transamination**, a reaction in which the $-NH_2$ group of the amino acid changes places with the keto group of an $\alpha$-keto acid such as $\alpha$-ketoglutarate. The products are a new $\alpha$-keto acid and glutamate.

The energy released in catabolic pathways is used in the *electron-transport chain* to make molecules of adenosine triphosphate, ATP. ATP, the final result of food catabolism, couples to and drives many otherwise unfavorable reactions.

Biomolecules are synthesized as well as degraded, but the pathways for anabolism and catabolism are not the exact reverse of one another. Fatty acids are biosynthesized from acetate by an 8-step pathway, and carbohydrates are made from pyruvate by the 11-step **gluconeogenesis** pathway.

## EXERCISES

### *Organic* KNOWLEDGE TOOLS

ThomsonNOW Sign in at **www.thomsonedu.com** to assess your knowledge of this chapter's topics by taking a pre-test. The pre-test will link you to interactive organic chemistry resources based on your score in each concept area.

Online homework for this chapter may be assigned in Organic OWL.

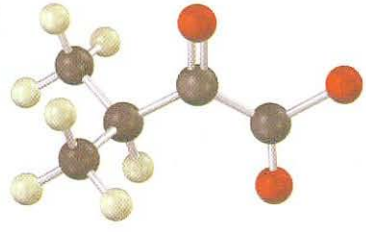
■ indicates problems assignable in Organic OWL.

### VISUALIZING CHEMISTRY

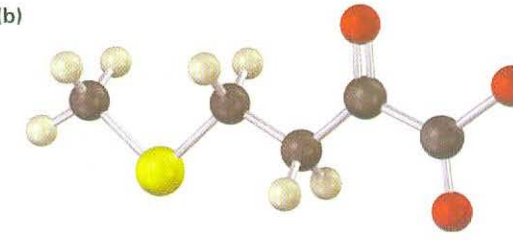
(Problems 29.1–29.16 appear within the chapter.)

**29.17** ■ Identify the amino acid that is a catabolic precursor of each of the following $\alpha$-keto acids:

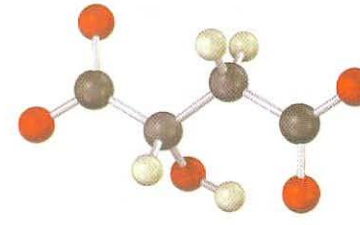
(a)



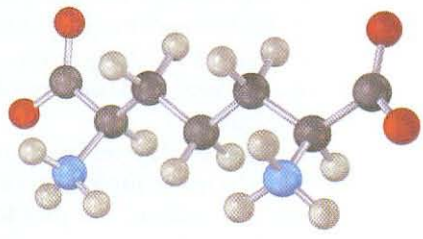
(b)



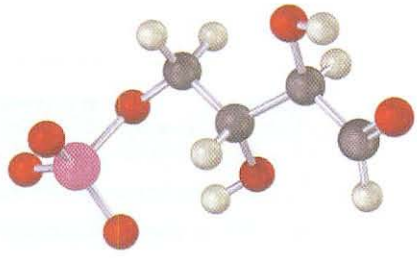
**29.18** ■ Identify the following intermediate in the citric acid cycle, and tell whether it has *R* or *S* stereochemistry:



**29.19** The following compound is an intermediate in the biosynthesis of one of the twenty common α-amino acids. Which one is it likely to be, and what kind of chemical change must take place to complete the biosynthesis?



**29.20** The following compound is an intermediate in the pentose phosphate pathway, an alternative route for glucose metabolism. Identify the sugar it is derived from.



## ADDITIONAL PROBLEMS

**29.21** What chemical events occur during the digestion of food?

**29.22** What is the difference between digestion and metabolism?

**29.23** What is the difference between anabolism and catabolism?

**29.24** ■ Draw the structure of adenosine 5′-monophosphate (AMP), an intermediate in some biochemical pathways.

**29.25** ■ Cyclic adenosine monophosphate (cyclic AMP), a modulator of hormone action, is related to AMP (Problem 29.24) but has its phosphate group linked to *two* hydroxyl groups at C3′ and C5′ of the sugar. Draw the structure of cyclic AMP.

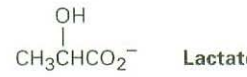
**29.26** ■ What general kind of reaction does ATP carry out?

**29.27** ■ What general kind of reaction does $NAD^+$ carry out?

**29.28** ■ What general kind of reaction does FAD carry out?

**29.29** Why aren't the glycolysis and gluconeogenesis pathways the exact reverse of one another?

**29.30** ■ Lactate, a product of glucose catabolism in oxygen-starved muscles, can be converted into pyruvate by oxidation. What coenzyme do you think is needed? Write the equation in the normal biochemical format using a curved arrow.



**Lactate**

**29.31** ■ How many moles of acetyl CoA are produced by catabolism of the following substances?

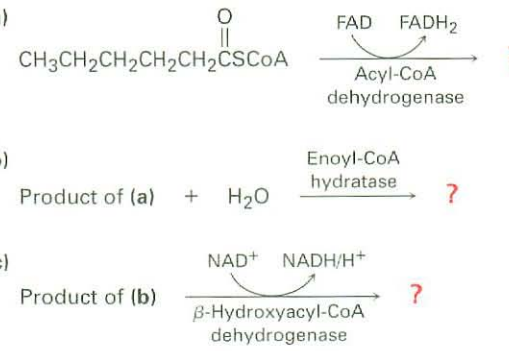
(a) 1.0 mol glucose (b) 1.0 mol palmitic acid (c) 1.0 mol maltose

**29.32** ■ How many grams of acetyl CoA (MW = 809.6 amu) are produced by catabolism of the following substances? Which substances is the most efficient precursor of acetyl CoA on a weight basis?

(a) 100.0 g glucose (b) 100.0 g palmitic acid (c) 100.0 g maltose

**29.33** Write the equation for the final step in the $\beta$-oxidation pathway of any fatty acid with an even number of carbon atoms.

**29.34** ■ Show the products of each of the following reactions:




**29.35** ■ What is the structure of the $\alpha$-keto acid formed by transamination of each of the following amino acids?

(a) Threonine (b) Phenylalanine (c) Asparagine

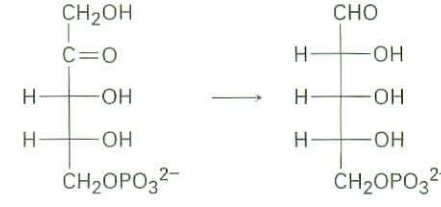
**29.36** ■ What enzyme cofactor is associated with each of the following kinds of reactions?

(a) Transamination (b) Carboxylation of a ketone

(c) Decarboxylation of an $\alpha$-keto acid

**29.37** The glycolysis pathway shown in Figure 29.7 has a number of intermediates that contain phosphate groups. Why can 3-phosphoglyceryl phosphate and phosphoenolpyruvate transfer a phosphate group to ADP while glucose 6-phosphate cannot?

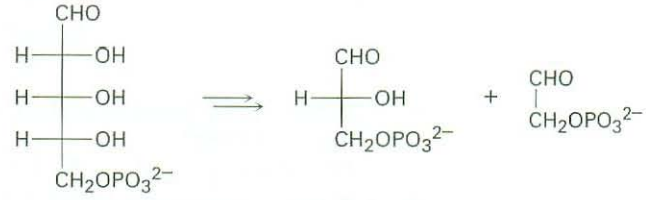
**29.38** ■ In the *pentose phosphate* pathway for degrading sugars, ribulose 5-phosphate is converted to ribose 5-phosphate. Propose a mechanism for the isomerization.




**Ribulose 5-phosphate** **Ribose 5-phosphate**

■ Assignable in OWL

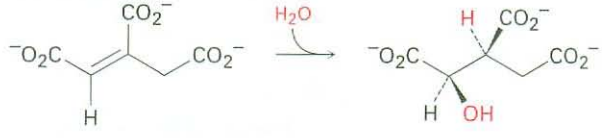
**29.39** Another step in the pentose phosphate pathway for degrading sugars (see Problem 29.38) is the conversion of ribose 5-phosphate to glyceraldehyde 3-phosphate. What kind of organic process is occurring? Propose a mechanism for the conversion.




**Ribose 5-phosphate** **Glyceraldehyde 3-phosphate**

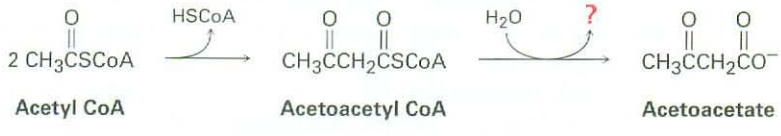
**29.40** Write a mechanism for the conversion of α-ketoglutarate to succinyl CoA in step 4 of the citric acid cycle (Figure 29.12).

**29.41** ■ In step 2 of the citric acid cycle (Figure 29.12), *cis*-aconitate reacts with water to give (2*R*,3*S*)-isocitrate. Does —OH add from the *Re* face of the double bond or from the *Si* face? What about —H? Does the addition of water occur with syn or anti geometry?

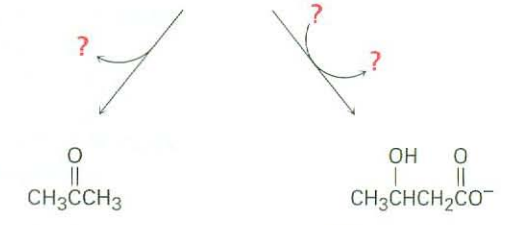



***cis*-Aconitate** **(2*R*,3*S*)-Isocitrate**

**29.42** ■ The primary fate of acetyl CoA under normal metabolic conditions is degradation in the citric acid cycle to yield $CO_2$. When the body is stressed by prolonged starvation, however, acetyl CoA is converted into compounds called *ketone bodies*, which can be used by the brain as a temporary fuel. Fill in the missing information indicated by the four question marks in the following biochemical pathway for the synthesis of ketone bodies from acetyl CoA:




**Acetyl CoA** **Acetoacetyl CoA** **Acetoacetate**

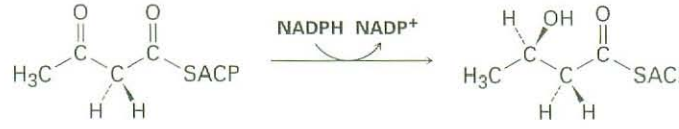



**Acetone** **3-Hydroxybutyrate**

**Ketone bodies**

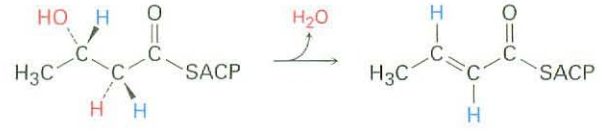
**29.43** ■ The initial reaction in Problem 29.42, conversion of two molecules of acetyl CoA to one molecule of acetoacetyl CoA, is a Claisen reaction. Assuming that there is a base present, show the mechanism of the reaction.

**29.44** In step 6 of fatty-acid biosynthesis (Figure 29.5), acetoacetyl ACP is reduced stereospecifically by NADPH to yield an alcohol. Does hydride ion add to the *Si* face or the *Re* face of acetoacetyl ACP?




**Acetoacetyl ACP** **β-Hydroxybutyryl ACP**

**29.45** In step 7 of fatty-acid biosynthesis (Figure 29.5), dehydration of a β-hydroxy thioester occurs to give *trans*-crotonyl ACP. Is the dehydration a syn elimination or an anti elimination?

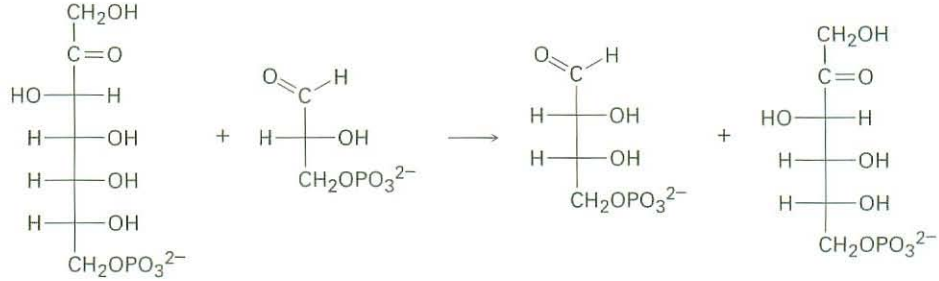



***trans*-Crotonyl ACP**

**29.46** ■ In step 8 of fatty-acid biosynthesis (Figure 29.5), reduction of *trans*-crotonyl ACP gives butyryl ACP. A hydride from NADPH adds to C3 of the crotonyl group from the *Re* face, and protonation on C2 occurs on the *Si* face. Is the reduction a syn addition or an anti addition?


**Crotonyl ACP** **Butyryl ACP**

**29.47** One of the steps in the pentose phosphate pathway for glucose catabolism is the reaction of sedoheptulose 7-phosphate with glyceraldehyde 3-phosphate in the presence of a transaldolase to yield erythrose 4-phosphate and fructose 6-phosphate.

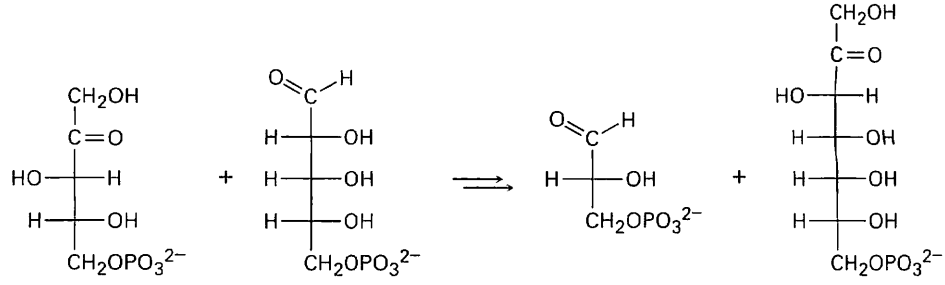


**Sedoheptulose 7-phosphate** **Glyceraldehyde 3-phosphate** **Erythrose 4-phosphate** **Fructose 6-phosphate**

(a) The first part of the reaction is formation of a protonated Schiff base of sedoheptulose 7-phosphate with a lysine residue in the enzyme followed by a retro-aldol cleavage to give an enamine plus erythrose 4-phosphate. Show the structure of the enamine and the mechanism by which it is formed.

(b) The second part of the reaction is nucleophilic addition of the enamine to glyceraldehyde 3-phosphate followed by hydrolysis of the Schiff base to give fructose 6-phosphate. Show the mechanism.

**29.48** One of the steps in the pentose phosphate pathway for glucose catabolism is the reaction of xylulose 5-phosphate with ribose 5-phosphate in the presence of a transketolase to give glyceraldehyde 3-phosphate and sedoheptulose 7-phosphate.

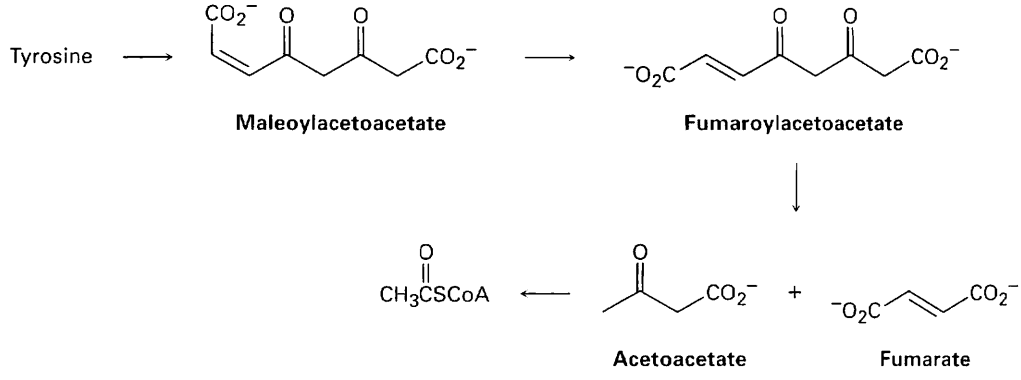


**Xylulose 5-phosphate** **Ribose 5-phosphate** **Glyceraldehyde 3-phosphate** **Sedoheptulose 7-phosphate**

(a) The first part of the reaction is nucleophilic addition of thiamin diphosphate (TPP) ylide to xylulose 5-phosphate, followed by a retro-aldol cleavage to give glyceraldehyde 3-phosphate and a TPP-containing enamine. Show the structure of the enamine and the mechanism by which it is formed.

(b) The second part of the reaction is addition of the enamine to ribose 5-phosphate followed by loss of TPP ylide to give sedoheptulose 7-phosphate. Show the mechanism.

**29.49** The amino acid tyrosine is biologically degraded by a series of steps that include the following transformations:



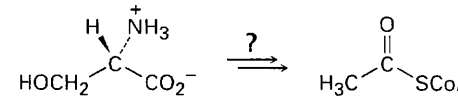
**Maleoylacetoacetate** **Fumaroylacetoacetate** **Acetoacetate** **Fumarate**

The double-bond isomerization of maleoylacetoacetate to fumaroyl acetoacetate is catalyzed by practically any nucleophile, $:Nu^-$. Propose a mechanism.

**29.50** Propose a mechanism for the conversion of fumaroylacetoacetate to fumarate plus acetoacetate (Problem 29.49).

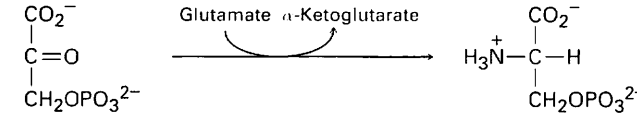
**29.51** Propose a mechanism for the conversion of acetoacetate to acetyl CoA (Problem 29.49).

**29.52** Design your own degradative pathway. You know the rules (organic mechanisms), and you've seen the kinds of reactions that occur in the biological degradation of fats and carbohydrates into acetyl CoA. If you were Mother Nature, what series of steps would you use to degrade the amino acid serine into acetyl CoA?



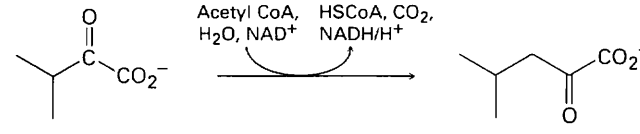

**Serine** **Acetyl CoA**

**29.53** The amino acid serine is biosynthesized by a route that involves reaction of 3-phosphohydroxypyruvate with glutamate. Propose a mechanism.



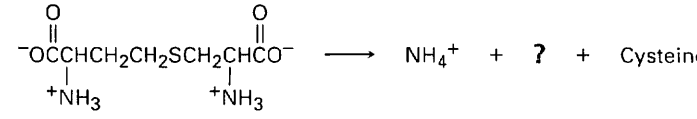

**3-Phosphohydroxypyruvate** **3-Phosphoserine**

**29.54** The amino acid leucine is biosynthesized from α-ketoisocaproate, which is itself prepared from α-ketoisovalerate by a multistep route that involves (1) reaction with acetyl CoA, (2) hydrolysis, (3) dehydration, (4) hydration, (5) oxidation, and (6) decarboxylation. Show the steps in the transformation, and propose a mechanism for each.




**α-Ketoisovalerate** **α-Ketoisocaproate**

**29.55** The amino acid cysteine, $C_3H_7NO_2S$, is biosynthesized from a substance called cystathionine by a multistep pathway.




**Cystathionine**

(a) The first step is a transamination. What is the product?
(b) The second step is an E1cB reaction. Show the products and the mechanism of the reaction.
(c) The final step is a double-bond reduction. What organic cofactor is required for this reaction, and what is the product represented by the question mark in the equation?