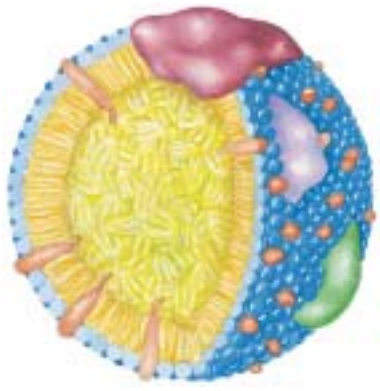


chapter 17

FATTY ACID CATABOLISM

Jack Sprat could eat no fat,
His wife could eat no lean,
And so between them both you see,
They licked the platter clean.

—*John Clarke,* Paroemiologia Anglo-Latina (Proverbs English and Latin), *1639*

The oxidation of long-chain fatty acids to acetyl-CoA is a central energy-yielding pathway in many organisms and tissues. In mammalian heart and liver, for example, it provides as much as 80% of the energetic needs under all physiological circumstances. The electrons removed from fatty acids during oxidation pass through the respiratory chain, driving ATP synthesis; the acetyl-CoA produced from the fatty acids may be completely oxidized to CO_2 in the citric acid cycle, resulting in further energy conservation. In some species and in some tissues, the acetyl-CoA has alternative fates. In liver, acetyl-CoA may be converted to ketone bodies—water-soluble fuels exported to the brain and other tissues when glucose is not available. In higher plants, acetyl-CoA serves primarily as a biosynthetic precursor, only secondarily as fuel. Although the biological role of fatty acid oxidation differs from organism to organism, the mechanism is essentially the same. The repetitive four-step process, called ***β* oxidation,** by which fatty acids are converted into acetyl-CoA is the main topic of this chapter.

In Chapter 10 we described the properties of triacylglycerols (also called triglycerides or neutral fats) that make them especially suitable as storage fuels. The long alkyl chains of their constituent fatty acids are essentially hydrocarbons, highly reduced structures with an energy of complete oxidation (~38 kJ/g) more than twice that for the same weight of carbohydrate or protein. This advantage is compounded by the extreme insolubility of lipids in water; cellular triacylglycerols aggregate in lipid droplets, which do not raise the osmolarity of the cytosol, and they are unsolvated. (In storage polysaccharides, by contrast, water of solvation can account for two-thirds of the overall weight of the stored molecules.) And because of their relative chemical inertness, triacylglycerols can be stored in large quantity in cells without the risk of undesired chemical reactions with other cellular constituents.

The properties that make triacylglycerols good storage compounds, however, present problems in their role as fuels. Because they are insoluble in water, ingested triacylglycerols must be emulsified before they can be digested by water-soluble enzymes in the intestine, and triacylglycerols absorbed in the intestine or mobilized from storage tissues must be carried in the blood bound to proteins that counteract their insolubility. To overcome the relative stability of the C—C bonds in a fatty acid, the carboxyl group at C-1 is activated by attachment to coenzyme A, which allows stepwise oxidation of the fatty acyl group at the C-3, or *β*, position—hence the name *β* oxidation.

We begin this chapter with a brief discussion of the sources of fatty acids and the routes by which they travel to the site of their oxidation, with special emphasis on the process in vertebrates. We then describe the chemical steps of fatty acid oxidation in mitochondria. The complete oxidation of fatty acids to CO_2 and H_2O takes place in three stages: the oxidation of long-chain fatty acids to two-carbon fragments, in the form of acetyl-CoA (*β* oxidation); the oxidation of acetyl-CoA to CO_2 in the citric acid cycle (Chapter 16); and the transfer of

electrons from reduced electron carriers to the mitochondrial respiratory chain (Chapter 19). In this chapter we focus on the first of these stages. We begin our discussion of β oxidation with the simple case in which a fully saturated fatty acid with an even number of carbon atoms is degraded to acetyl-CoA. We then look briefly at the extra transformations necessary for the degradation of unsaturated fatty acids and fatty acids with an odd number of carbons. Finally, we discuss variations on the β-oxidation theme in specialized organelles—peroxisomes and glyoxysomes—and two less common pathways of fatty acid catabolism, ω and α oxidation. The chapter concludes with a description of an alternative fate for the acetyl-CoA formed by β oxidation in vertebrates: the production of ketone bodies in the liver.

17.1 Digestion, Mobilization, and Transport of Fats

Cells can obtain fatty acid fuels from three sources: fats consumed in the diet, fats stored in cells as lipid droplets, and fats synthesized in one organ for export to another. Some species use all three sources under various circumstances, others use one or two. Vertebrates, for example, obtain fats in the diet, mobilize fats stored in specialized tissue (adipose tissue, consisting of cells called adipocytes), and, in the liver, convert excess dietary carbohydrates to fats for export to other tissues. On average, 40% or more of the daily energy requirement of humans in highly industrialized countries is supplied by dietary triacylglycerols (although most nutritional guidelines recommend no more than 30% of daily caloric intake from fats). Triacylglycerols provide more than half the energy requirements of some organs, particularly the liver, heart, and resting skeletal muscle. Stored triacylglycerols are virtually the sole source of energy in hibernating animals and migrating birds. Protists obtain fats by consuming organisms lower in the food chain, and some also store fats as cytosolic lipid droplets. Vascular plants mobilize fats stored in seeds during germination, but do not otherwise depend on fats for energy.

Dietary Fats Are Absorbed in the Small Intestine

In vertebrates, before ingested triacylglycerols can be absorbed through the intestinal wall they must be converted from insoluble macroscopic fat particles to finely dispersed microscopic micelles. This solubilization is carried out by bile salts, such as taurocholic acid (p. 355), which are synthesized from cholesterol in the liver, stored in the gallbladder, and released into the small intestine after ingestion of a fatty meal. Bile salts are amphipathic compounds that act as biological detergents, converting dietary fats into mixed micelles of bile salts and triacylglycerols (Fig. 17–1, step ①). Micelle formation enormously increases the fraction of lipid molecules accessible to the action of water-soluble lipases in the intestine, and lipase action converts triacylglycerols to monoacylglycerols (monoglycerides) and diacylglycerols (diglycerides), free fatty acids, and glycerol (step ②). These products of lipase action diffuse into the epithelial cells lining the intestinal surface (the intestinal mucosa) (step ③), where they are reconverted to triacylglycerols and packaged with dietary cholesterol and specific proteins into lipoprotein aggregates called **chylomicrons** (Fig. 17–2; see also Fig. 17–1, step ④).

Apolipoproteins are lipid-binding proteins in the blood, responsible for the transport of triacylglycerols, phospholipids, cholesterol, and cholesteryl esters between organs. Apolipoproteins ("apo" means "detached" or "separate," designating the protein in its lipid-free form) combine with lipids to form several classes of lipoprotein particles, spherical aggregates with hydrophobic lipids at the core and hydrophilic protein side chains and lipid head groups at the surface. Various combinations of lipid and protein produce particles of different densities, ranging from chylomicrons and very-low-density lipoproteins (VLDL) to very-high-density lipoproteins (VHDL), which can be separated by ultracentrifugation. The structures of these lipoprotein particles and their roles in lipid transport are detailed in Chapter 21.

The protein moieties of lipoproteins are recognized by receptors on cell surfaces. In lipid uptake from the intestine, chylomicrons, which contain apolipoprotein C-II (apoC-II), move from the intestinal mucosa into the lymphatic system, and then enter the blood, which carries them to muscle and adipose tissue (Fig. 17–1, step ⑤). In the capillaries of these tissues, the extracellular enzyme **lipoprotein lipase,** activated by apoC-II, hydrolyzes triacylglycerols to fatty acids and glycerol (step ⑥), which are taken up by cells in the target tissues (step ⑦). In muscle, the fatty acids are oxidized for energy; in adipose tissue, they are reesterified for storage as triacylglycerols (step ⑧).

The remnants of chylomicrons, depleted of most of their triacylglycerols but still containing cholesterol and apolipoproteins, travel in the blood to the liver, where they are taken up by endocytosis, mediated by receptors for their apolipoproteins. Triacylglycerols that enter the liver by this route may be oxidized to provide energy or to provide precursors for the synthesis of ketone bodies, as described in Section 17.3. When the diet contains more fatty acids than are needed immediately for fuel or as precursors, the liver converts them to triacylglycerols, which are packaged with specific apolipoproteins into VLDLs. The VLDLs are transported in the blood to adipose tissues, where the triacylglycerols are removed and stored in lipid droplets within adipocytes.

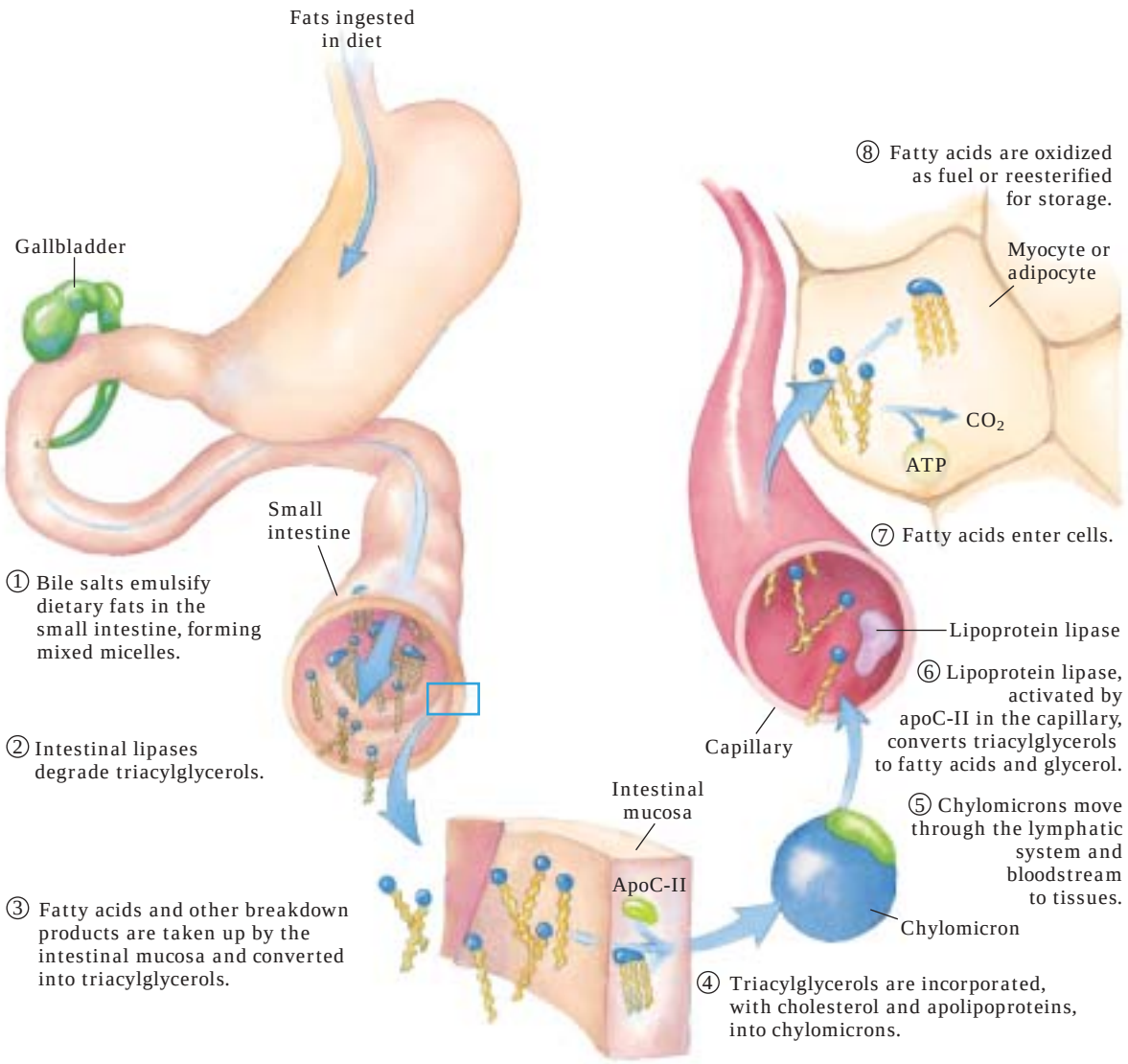


FIGURE 17–1 Processing of dietary lipids in vertebrates. Digestion and absorption of dietary lipids occur in the small intestine, and the fatty acids released from triacylglycerols are packaged and delivered to muscle and adipose tissues. The eight steps are discussed in the text.

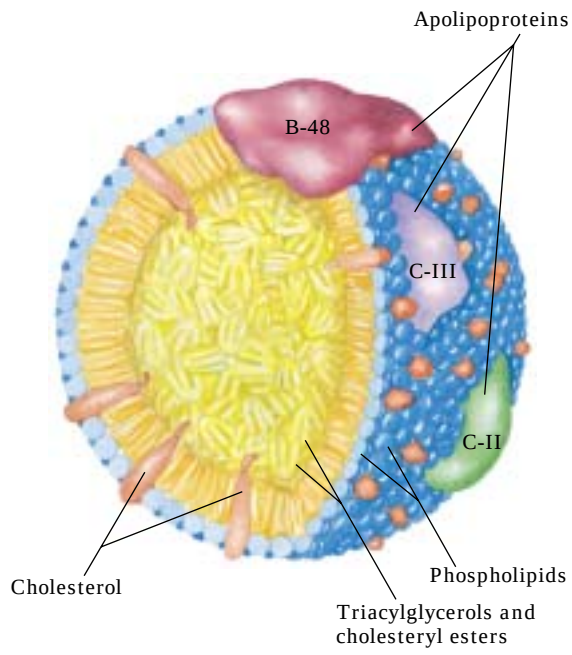


FIGURE 17–2 Molecular structure of a chylomicron. The surface is a layer of phospholipids, with head groups facing the aqueous phase. Triacylglycerols sequestered in the interior (yellow) make up more than 80% of the mass. Several apolipoproteins that protrude from the surface (B-48, C-III, C-II) act as signals in the uptake and metabolism of chylomicron contents. The diameter of chylomicrons ranges from about 100 to 500 nm.

Hormones Trigger Mobilization of Stored Triacylglycerols

Neutral lipids are stored in adipocytes (and in steroid-synthesizing cells of the adrenal cortex, ovary, and testes) in the form of lipid droplets, with a core of sterol esters and triacylglycerols surrounded by a monolayer of phospholipids. The surface of these droplets is coated with **perilipins,** a family of proteins that restrict access to lipid droplets, preventing untimely lipid mobilization. When hormones signal the need for metabolic energy, triacylglycerols stored in adipose tissue are mobilized (brought out of storage) and transported to tissues (skeletal muscle, heart, and renal cortex) in which fatty acids can be oxidized for energy production. The hormones epinephrine and glucagon, secreted in response to low blood glucose levels, activate the enzyme adenylyl cyclase in the adipocyte plasma membrane (Fig. 17–3), which produces the intracellular second messenger cyclic AMP (cAMP; see Fig. 12–13). Cyclic AMP–dependent protein kinase (PKA) phosphorylates perilipin A, and the phosphorylated perilipin causes **hormone-sensitive lipase** in the cytosol to move to the lipid droplet surface, where it can begin hydrolyzing triacylglycerols to free fatty acids and glycerol. PKA also phosphorylates hormone-sensitive lipase, doubling or tripling its activity, but the more than 50-fold increase in fat mobilization triggered by epinephrine is due primarily to perilipin phosphorylation. Cells with defective perilipin genes have almost no response to increases in cAMP concentration; their hormone-sensitive lipase does not associate with lipid droplets.

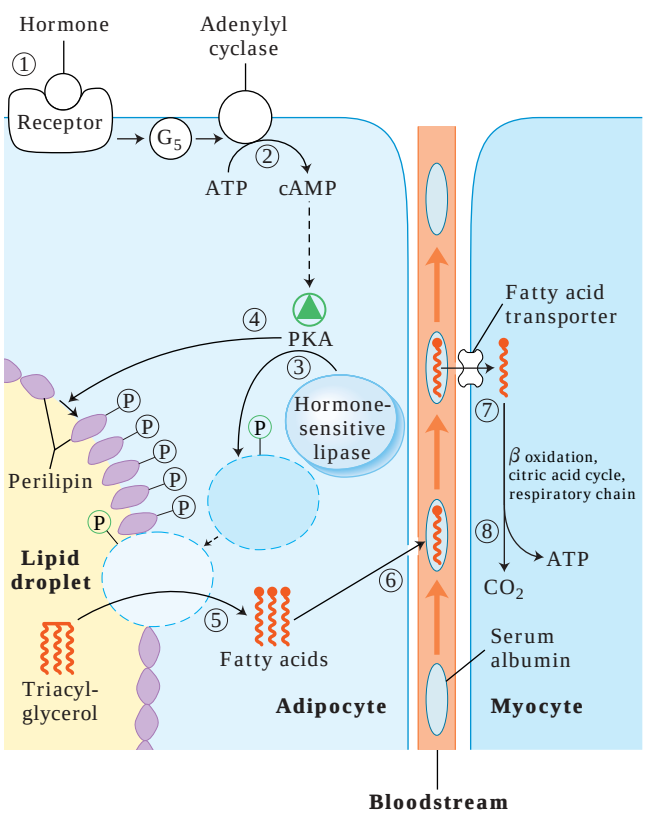


FIGURE 17–3 Mobilization of triacylglycerols stored in adipose tissue. When low levels of glucose in the blood trigger the release of glucagon, ① the hormone binds its receptor in the adipocyte membrane and thus ② stimulates adenylyl cyclase, via a G protein, to produce cAMP. This activates PKA, which phosphorylates ③ the hormone-sensitive lipase and ④ perilipin molecules on the surface of the lipid droplet. Phosphorylation of perilipin permits hormone-sensitive lipase access to the surface of the lipid droplet, where ⑤ it hydrolyzes triacylglycerols to free fatty acids. ⑥ Fatty acids leave the adipocyte, bind serum albumin in the blood, and are carried in the blood; they are released from the albumin and ⑦ enter a myocyte via a specific fatty acid transporter. ⑧ In the myocyte, fatty acids are oxidized to CO_2, and the energy of oxidation is conserved in ATP, which fuels muscle contraction and other energy requiring metabolism in the myocyte.

As hormone-sensitive lipase hydrolyzes triacylglycerol in adipocytes, the fatty acids thus released **(free fatty acids, FFA)** pass from the adipocyte into the blood, where they bind to the blood protein **serum albumin.** This protein (M_r 66,000), which makes up about half of the total serum protein, noncovalently binds as many as 10 fatty acids per protein monomer. Bound to this soluble protein, the otherwise insoluble fatty acids are carried to tissues such as skeletal muscle, heart, and renal cortex. In these target tissues, fatty acids dissociate from albumin and are moved by plasma membrane transporters into cells to serve as fuel.

About 95% of the biologically available energy of triacylglycerols resides in their three long-chain fatty acids; only 5% is contributed by the glycerol moiety. The glycerol released by lipase action is phosphorylated by **glycerol kinase** (Fig. 17–4), and the resulting glycerol 3-phosphate is oxidized to dihydroxyacetone phosphate. The glycolytic enzyme triose phosphate isomerase converts this compound to glyceraldehyde 3-phosphate, which is oxidized via glycolysis.

Fatty Acids Are Activated and Transported into Mitochondria

The enzymes of fatty acid oxidation in animal cells are located in the mitochondrial matrix, as demonstrated in 1948 by Eugene P. Kennedy and Albert Lehninger. The fatty acids with chain lengths of 12 or fewer carbons enter mitochondria without the help of membrane transporters. Those with 14 or more carbons, which constitute the majority of the FFA obtained in the diet or released from adipose tissue, cannot pass directly through the mitochondrial membranes—they must first undergo the three enzymatic reactions of the **carnitine shuttle.** The first reaction is catalyzed by a family of isozymes (different isozymes specific for fatty acids having short, intermediate, or long carbon chains) present

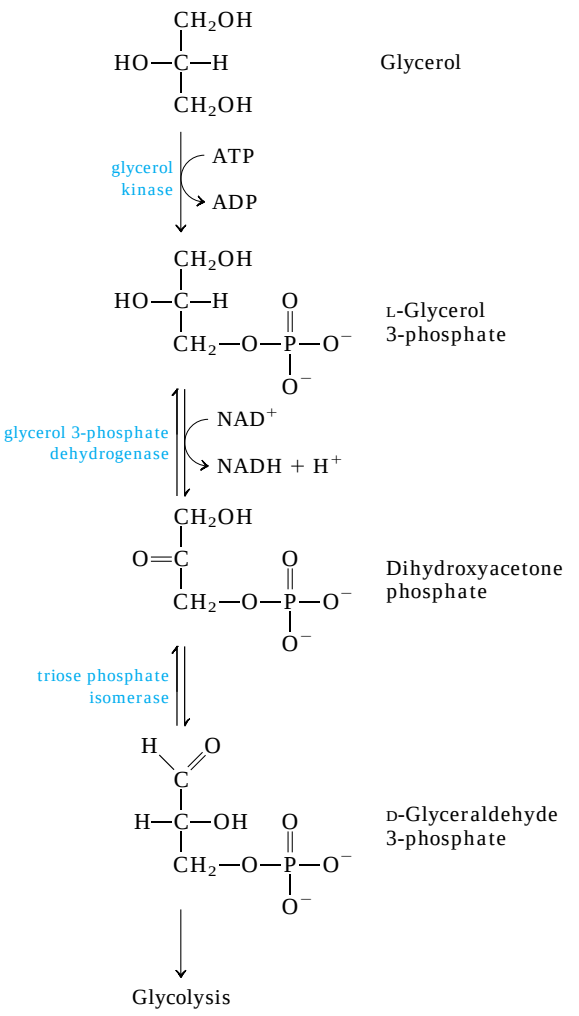


FIGURE 17–4 **Entry of glycerol into the glycolytic pathway.**

in the outer mitochondrial membrane, the **acyl-CoA synthetases,** which promote the general reaction

$$\text{Fatty acid} + \text{CoA} + \text{ATP} \rightleftharpoons \text{fatty acyl–CoA} + \text{AMP} + \text{PP}_i$$

Thus, acyl-CoA synthetases catalyze the formation of a thioester linkage between the fatty acid carboxyl group and the thiol group of coenzyme A to yield a **fatty acyl–CoA,** coupled to the cleavage of ATP to AMP and PP_i. (Recall the description of this reaction in Chapter 13, to illustrate how the free energy released by cleavage of phosphoanhydride bonds in ATP could be coupled to the formation of a high-energy compound; p. XXX.) The reaction occurs in two steps and involves a fatty acyl–adenylate intermediate (Fig. 17–5).

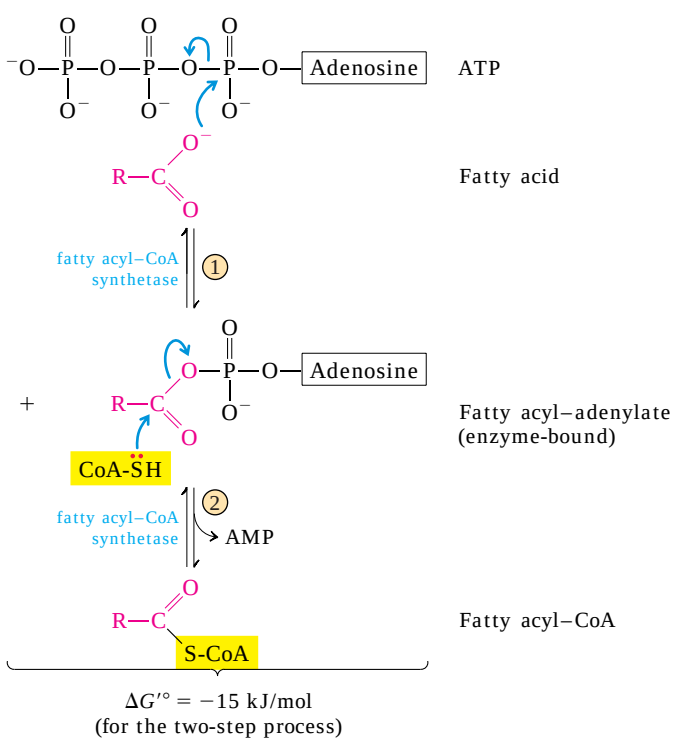
Fatty acyl–CoAs, like acetyl-CoA, are high-energy compounds; their hydrolysis to FFA and CoA has a large, negative standard free-energy change ($\Delta G'^\circ \approx -31$ kJ/mol). The formation of a fatty acyl–CoA is made more favorable by the hydrolysis of *two* high-energy bonds in ATP; the pyrophosphate formed in the activation reaction is immediately hydrolyzed by inorganic pyrophosphatase (left side of Fig. 17–5), which pulls the preceding activation reaction in the direction of fatty acyl–CoA formation. The overall reaction is

$$\text{Fatty acid} + \text{CoA} + \text{ATP} \longrightarrow \text{fatty acyl–CoA} + \text{AMP} + 2\text{P}_i \qquad (17\text{–}1)$$
$$\Delta G'^\circ = -34 \text{ kJ/mol}$$

Fatty acyl–CoA esters formed at the cytosolic side of the outer mitochondrial membrane can be transported into the mitochondrion and oxidized to produce ATP, or they can be used in the cytosol to synthesize

MECHANISM FIGURE 17–5

Conversion of a fatty acid to a fatty acyl–CoA. The conversion is catalyzed by fatty acyl–CoA synthetase and inorganic pyrophosphatase. Fatty acid activation by formation of the fatty acyl–CoA derivative occurs in two steps. In step (1), the carboxylate ion displaces the outer two (β and γ) phosphates of ATP to form a fatty acyl–adenylate, the mixed anhydride of a carboxylic acid and a phosphoric acid. The other product is PP_i, an excellent leaving group that is immediately hydrolyzed to two P_i, pulling the reaction in the forward direction. In step (2), the thiol group of coenzyme A carries out nucleophilic attack on the enzyme-bound mixed anhydride, displacing AMP and forming the thioester fatty acyl–CoA. The overall reaction is highly exergonic.



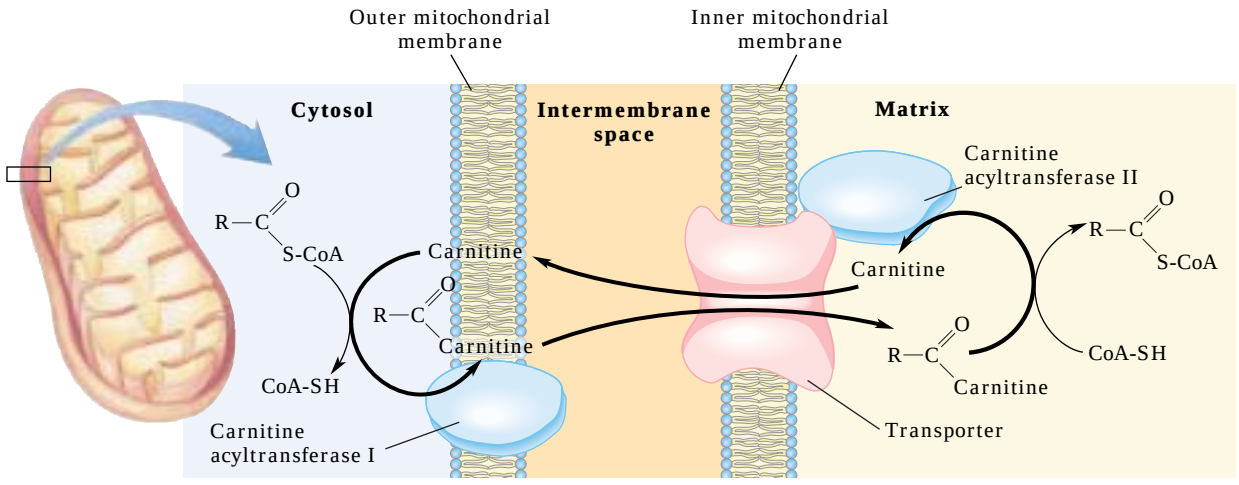
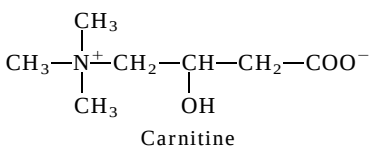


FIGURE 17–6 Fatty acid entry into mitochondria via the acyl-carnitine/carnitine transporter. After fatty acyl–carnitine is formed at the outer membrane or in the intermembrane space, it moves into the matrix by facilitated diffusion through the transporter in the inner membrane. In the matrix, the acyl group is transferred to mitochondrial coenzyme A, freeing carnitine to return to the intermembrane space through the same transporter. Acyltransferase I is inhibited by malonyl-CoA, the first intermediate in fatty acid synthesis (see Fig. 21–1). This inhibition prevents the simultaneous synthesis and degradation of fatty acids.

membrane lipids. Fatty acids destined for mitochondrial oxidation are transiently attached to the hydroxyl group of **carnitine** to form fatty acyl–carnitine—the second reaction of the shuttle. This transesterification is catalyzed by **carnitine acyltransferase I** (M_r 88,000), in the outer membrane. Either the acyl-CoA passes through the outer membrane and is converted to the carnitine ester in the intermembrane space (Fig. 17–6), or the carnitine ester is formed on the cytosolic face of the outer membrane, then moved across the outer membrane to the intermembrane space—the current evidence does not reveal which. In either case, passage into the intermembrane space (the space between the outer and inner membranes) occurs through large pores (formed by the protein porin) in the outer membrane. The fatty acyl–carnitine ester then enters the matrix by facilitated diffusion through the **acyl-carnitine/carnitine transporter** of the inner mitochondrial membrane (Fig. 17–6).



Carnitine

In the third and final step of the carnitine shuttle, the fatty acyl group is enzymatically transferred from carnitine to intramitochondrial coenzyme A by **carnitine acyltransferase II.** This isozyme, located on the inner face of the inner mitochondrial membrane, regenerates fatty acyl–CoA and releases it, along with free carnitine, into the matrix (Fig. 17–6). Carnitine reenters the intermembrane space via the acyl-carnitine/carnitine transporter.

This three-step process for transferring fatty acids into the mitochondrion—esterification to CoA, transesterification to carnitine followed by transport, and transesterification back to CoA—links two separate pools of coenzyme A and of fatty acyl–CoA, one in the cytosol, the other in mitochondria. These pools have different functions. Coenzyme A in the mitochondrial matrix is largely used in oxidative degradation of pyruvate, fatty acids, and some amino acids, whereas cytosolic coenzyme A is used in the biosynthesis of fatty acids (see Fig. 21–10). Fatty acyl–CoA in the cytosolic pool can be used for membrane lipid synthesis or can be moved into the mitochondrial matrix for oxidation and ATP production. Conversion to the carnitine ester commits the fatty acyl moiety to the oxidative fate.

The carnitine-mediated entry process is the rate-limiting step for oxidation of fatty acids in mitochondria and, as discussed later, is a regulation point. Once inside the mitochondrion, the fatty acyl–CoA is acted upon by a set of enzymes in the matrix.

SUMMARY 17.1 Digestion, Mobilization, and Transport of Fats

- The fatty acids of triacylglycerols furnish a large fraction of the oxidative energy in animals. Dietary triacylglycerols are emulsified in the small intestine by bile salts, hydrolyzed by intestinal lipases, absorbed by intestinal epithelial cells, reconverted into triacylglycerols, then formed into chylomicrons by combination with specific apolipoproteins.

- Chylomicrons deliver triacylglycerols to tissues, where lipoprotein lipase releases free fatty acids for entry into cells. Triacylglycerols stored in adipose tissue are mobilized by a hormone-sensitive triacylglycerol lipase. The released fatty acids bind to serum albumin and are carried in the blood to the heart, skeletal muscle, and other tissues that use fatty acids for fuel.
- Once inside cells, fatty acids are activated at the outer mitochondrial membrane by conversion to fatty acyl–CoA thioesters. Fatty acyl–CoA to be oxidized enters mitochondria in three steps, via the carnitine shuttle.

17.2 Oxidation of Fatty Acids

As noted earlier, mitochondrial oxidation of fatty acids takes place in three stages (Fig. 17–7). In the first stage—β oxidation—fatty acids undergo oxidative removal of successive two-carbon units in the form of acetyl-CoA, starting from the carboxyl end of the fatty acyl chain. For example, the 16-carbon palmitic acid (palmitate at pH 7) undergoes seven passes through the oxidative sequence, in each pass losing two carbons as acetyl-CoA. At the end of seven cycles the last two carbons of palmitate (originally C-15 and C-16) remain as acetyl-CoA. The overall result is the conversion of the 16-carbon chain of palmitate to eight two-carbon acetyl groups of acetyl-CoA molecules. Formation of each acetyl-CoA requires removal of four hydrogen atoms (two pairs of electrons and four H^+) from the fatty acyl moiety by dehydrogenases.

In the second stage of fatty acid oxidation, the acetyl groups of acetyl-CoA are oxidized to CO_2 in the citric acid cycle, which also takes place in the mitochondrial matrix. Acetyl-CoA derived from fatty acids thus enters a final common pathway of oxidation with the acetyl-CoA derived from glucose via glycolysis and pyruvate oxidation (see Fig. 16–1). The first two stages of fatty acid oxidation produce the reduced electron carriers NADH and $FADH_2$, which in the third stage donate electrons to the mitochondrial respiratory chain, through which the electrons pass to oxygen with the concomitant phosphorylation of ADP to ATP (Fig. 17–7). The energy released by fatty acid oxidation is thus conserved as ATP.

We now take a closer look at the first stage of fatty acid oxidation, beginning with the simple case of a saturated fatty acyl chain with an even number of carbons, then turning to the slightly more complicated cases of unsaturated and odd-number chains. We also consider the regulation of fatty acid oxidation, the β-oxidative processes as they occur in organelles other than mitochondria, and, finally, two less-general modes of fatty acid catabolism, α oxidation and ω oxidation.

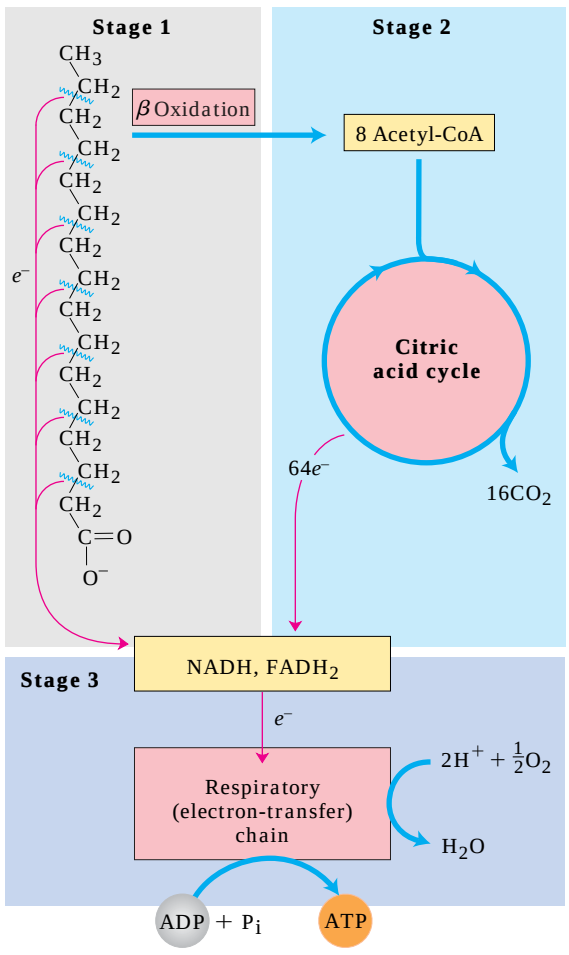


FIGURE 17–7 Stages of fatty acid oxidation. Stage 1: A long-chain fatty acid is oxidized to yield acetyl residues in the form of acetyl-CoA. This process is called β oxidation. Stage 2: The acetyl groups are oxidized to CO_2 via the citric acid cycle. Stage 3: Electrons derived from the oxidations of stages 1 and 2 pass to O_2 via the mitochondrial respiratory chain, providing the energy for ATP synthesis by oxidative phosphorylation.

The β Oxidation of Saturated Fatty Acids Has Four Basic Steps

Four enzyme-catalyzed reactions make up the first stage of fatty acid oxidation (Fig. 17–8a). First, dehydrogenation of fatty acyl–CoA produces a double bond between the α and β carbon atoms (C-2 and C-3), yielding a ***trans*-Δ^2-enoyl-CoA** (the symbol Δ^2 designates the position of the double bond; you may want to review fatty acid nomenclature, p. 343.) Note that the new double bond has the trans configuration, whereas the double bonds in naturally occurring unsaturated fatty acids are normally in the cis configuration. We consider the significance of this difference later.

This first step is catalyzed by three isozymes of **acyl-CoA dehydrogenase,** each specific for a range of fatty-acyl chain lengths: very-long-chain acyl-CoA dehydrogenase (VLCAD), acting on fatty acids of 12 to 18

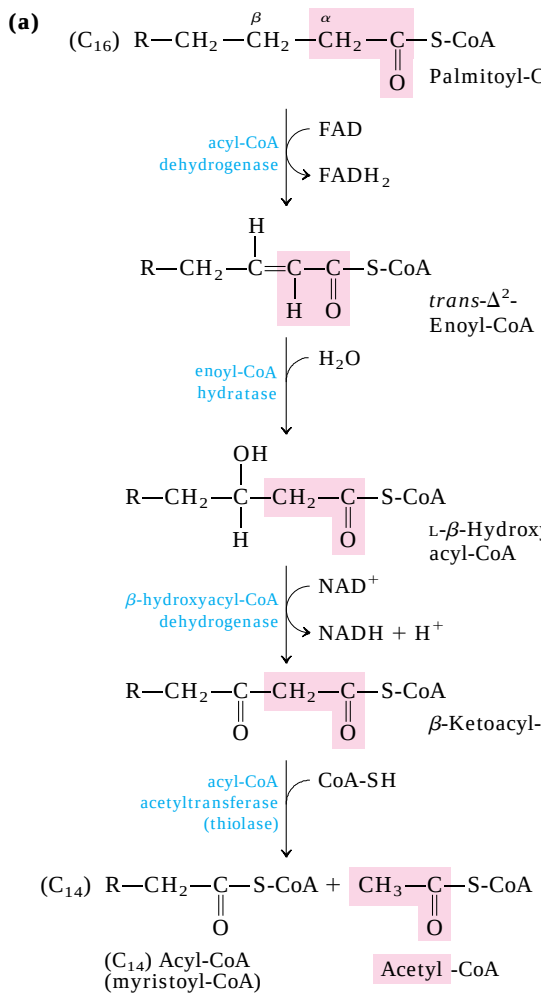
carbons; medium-chain (MCAD), acting on fatty acids of 4 to 14 carbons; and short-chain (SCAD), acting on fatty acids of 4 to 8 carbons. All three isozymes are flavoproteins with FAD (see Fig. 13–18) as a prosthetic group. The electrons removed from the fatty acyl–CoA are transferred to FAD, and the reduced form of the dehydrogenase immediately donates its electrons to an electron carrier of the mitochondrial respiratory chain, the **electron-transferring flavoprotein (ETF)** (see Fig. 19–8). The oxidation catalyzed by an acyl-CoA dehydrogenase is analogous to succinate dehydrogenation in the citric acid cycle (p. XXX); in both reactions the enzyme is bound to the inner membrane, a double bond is introduced into a carboxylic acid between the α and β carbons, FAD is the electron acceptor, and electrons from the reaction ultimately enter the respiratory chain and pass to O_2, with the concomitant synthesis of about 1.5 ATP molecules per electron pair.

In the second step of the β-oxidation cycle (Fig. 17–8a), water is added to the double bond of the *trans*-Δ^2-enoyl-CoA to form the L stereoisomer of ***β*-hydroxyacyl-CoA (3-hydroxyacyl-CoA).** This reaction, catalyzed by **enoyl-CoA hydratase,** is formally analogous to the fumarase reaction in the citric acid cycle, in which H_2O adds across an α–β double bond (p. XXX).

In the third step, L-β-hydroxyacyl-CoA is dehydrogenated to form ***β*-ketoacyl-CoA,** by the action of ***β*-hydroxyacyl-CoA dehydrogenase;** NAD^+ is the electron acceptor. This enzyme is absolutely specific for the L stereoisomer of hydroxyacyl-CoA. The NADH formed in the reaction donates its electrons to **NADH dehydrogenase,** an electron carrier of the respiratory chain, and ATP is formed from ADP as the electrons pass to O_2. The reaction catalyzed by β-hydroxyacyl-CoA dehydrogenase is closely analogous to the malate dehydrogenase reaction of the citric acid cycle (p. XXX).

The fourth and last step of the β-oxidation cycle is catalyzed by **acyl-CoA acetyltransferase,** more commonly called **thiolase,** which promotes reaction of β-ketoacyl-CoA with a molecule of free coenzyme A to split off the carboxyl-terminal two-carbon fragment of the original fatty acid as acetyl-CoA. The other product is the coenzyme A thioester of the fatty acid, now shortened by two carbon atoms (Fig. 17–8a). This reaction is called thiolysis, by analogy with the process of hydrolysis, because the β-ketoacyl-CoA is cleaved by reaction with the thiol group of coenzyme A.

The last three steps of this four-step sequence are catalyzed by either of two sets of enzymes, with the enzymes employed depending on the length of the fatty acyl chain. For fatty acyl chains of 12 or more carbons, the reactions are catalyzed by a multienzyme complex associated with the inner mitochondrial membrane, the **trifunctional protein (TFP).** TFP is a heterooctamer of $\alpha_4\beta_4$ subunits. Each α subunit contains two activities, the enoyl-CoA hydratase and the β-hydroxyacyl-CoA dehydrogenase; the β subunits contain the thiolase activity. This tight association of three enzymes may allow efficient substrate channeling from one active site to the



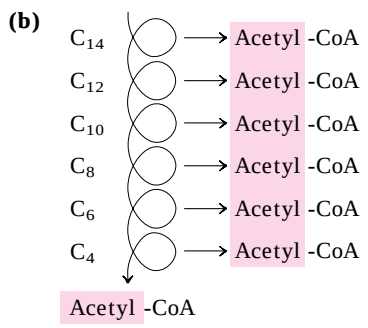


FIGURE 17–8 **The β-oxidation pathway. (a)** In each pass through this four-step sequence, one acetyl residue (shaded in pink) is removed in the form of acetyl-CoA from the carboxyl end of the fatty acyl chain—in this example palmitate (C_{16}), which enters as palmitoyl-CoA. **(b)** Six more passes through the pathway yield seven more molecules of acetyl-CoA, the seventh arising from the last two carbon atoms of the 16-carbon chain. Eight molecules of acetyl-CoA are formed in all.

next, without diffusion of the intermediates away from the enzyme surface. When TFP has shortened the fatty acyl chain to 12 or fewer carbons, further oxidations are catalyzed by a set of four soluble enzymes in the matrix.

As noted earlier, the single bond between methylene (—CH_2—) groups in fatty acids is relatively stable. The β-oxidation sequence is an elegant mechanism for destabilizing and breaking these bonds. The first three reactions of β oxidation create a much less stable C—C bond, in which the α carbon (C-2) is bonded to *two* carbonyl carbons (the β-ketoacyl-CoA intermediate). The ketone function on the β carbon (C-3) makes it a good target for nucleophilic attack by the —SH of coenzyme A, catalyzed by thiolase. The acidity of the α hydrogen and the resonance stabilization of the carbanion generated by the departure of this hydrogen make the terminal —CH_2—CO—S-CoA a good leaving group, facilitating breakage of the α–β bond.

The Four β-Oxidation Steps Are Repeated to Yield Acetyl-CoA and ATP

In one pass through the β-oxidation sequence, one molecule of acetyl-CoA, two pairs of electrons, and four protons (H^+) are removed from the long-chain fatty acyl–CoA, shortening it by two carbon atoms. The equation for one pass, beginning with the coenzyme A ester of our example, palmitate, is

$$\text{Palmitoyl-CoA} + \text{CoA} + \text{FAD} + \text{NAD}^+ + \text{H}_2\text{O} \longrightarrow \text{myristoyl-CoA} + \text{acetyl-CoA} + \text{FADH}_2 + \text{NADH} + \text{H}^+ \qquad (17\text{–}2)$$

Following removal of one acetyl-CoA unit from palmitoyl-CoA, the coenzyme A thioester of the shortened fatty acid (now the 14-carbon myristate) remains. The myristoyl-CoA can now go through another set of four β-oxidation reactions, exactly analogous to the first, to yield a second molecule of acetyl-CoA and lauroyl-CoA, the coenzyme A thioester of the 12-carbon laurate. Altogether, seven passes through the β-oxidation sequence are required to oxidize one molecule of palmitoyl-CoA to eight molecules of acetyl-CoA (Fig. 17–8b). The overall equation is

$$\text{Palmitoyl-CoA} + 7\text{CoA} + 7\text{FAD} + 7\text{NAD}^+ + 7\text{H}_2\text{O} \longrightarrow 8\ \text{acetyl-CoA} + 7\text{FADH}_2 + 7\text{NADH} + 7\text{H}^+ \qquad (17\text{–}3)$$

Each molecule of $FADH_2$ formed during oxidation of the fatty acid donates a pair of electrons to ETF of the respiratory chain, and about 1.5 molecules of ATP are generated during the ensuing transfer of each electron pair to O_2. Similarly, each molecule of NADH formed delivers a pair of electrons to the mitochondrial NADH dehydrogenase, and the subsequent transfer of each pair of electrons to O_2 results in formation of about 2.5 molecules of ATP. Thus four molecules of ATP are formed for each two-carbon unit removed in one pass through the sequence. Note that water is also produced in this process. Transfer of electrons from NADH or $FADH_2$ to O_2 yields one H_2O per electron pair. Reduction of O_2 by NADH also consumes one H^+ per NADH molecule: $\text{NADH} + \text{H}^+ + \frac{1}{2}\text{O}_2 \longrightarrow \text{NAD}^+ + \text{H}_2\text{O}$. In hibernating animals, fatty acid oxidation provides metabolic energy, heat, and water—all essential for survival of an animal that neither eats nor drinks for long periods (Box 17–1). Camels obtain water to supplement the meager supply available in their natural environment by oxidation of fats stored in their hump.

The overall equation for the oxidation of palmitoyl-CoA to eight molecules of acetyl-CoA, including the electron transfers and oxidative phosphorylations, is

$$\text{Palmitoyl-CoA} + 7\text{CoA} + 7\text{O}_2 + 28\text{P}_i + 28\text{ADP} \longrightarrow 8\ \text{acetyl-CoA} + 28\text{ATP} + 7\text{H}_2\text{O} \qquad (17\text{–}4)$$

Acetyl-CoA Can Be Further Oxidized in the Citric Acid Cycle

The acetyl-CoA produced from the oxidation of fatty acids can be oxidized to CO_2 and H_2O by the citric acid cycle. The following equation represents the balance sheet for the second stage in the oxidation of palmitoyl-CoA, together with the coupled phosphorylations of the third stage:

$$8\ \text{Acetyl-CoA} + 16\text{O}_2 + 80\text{P}_i + 80\text{ADP} \longrightarrow 8\text{CoA} + 80\text{ATP} + 16\text{CO}_2 + 16\text{H}_2\text{O} \qquad (17\text{–}5)$$

Combining Equations 17–4 and 17–5, we obtain the overall equation for the complete oxidation of palmitoyl-CoA to carbon dioxide and water:

$$\text{Palmitoyl-CoA} + 23\text{O}_2 + 108\text{P}_i + 108\text{ADP} \longrightarrow \text{CoA} + 108\text{ATP} + 16\text{CO}_2 + 23\text{H}_2\text{O} \qquad (17\text{–}6)$$

Table 17–1 summarizes the yields of NADH, $FADH_2$, and ATP in the successive steps of palmitoyl-CoA oxidation. Note that because the activation of palmitate to palmitoyl-CoA breaks both phosphoanhydride bonds in ATP (Fig. 17–5), the energetic cost of activating a fatty acid is equivalent to two ATP, and the net gain per molecule of palmitate is 106 ATP. The standard free-energy change for the oxidation of palmitate to CO_2 and H_2O is about 9,800 kJ/mol. Under standard conditions, the energy recovered as the phosphate bond energy of ATP is 106×30.5 kJ/mol = 3,230 kJ/mol, about 33% of the theoretical maximum. However, when the free-energy changes are calculated from actual concentrations of reactants and products under intracellular conditions (see Box 13–1), the free-energy recovery is more than 60%; the energy conservation is remarkably efficient.

Oxidation of Unsaturated Fatty Acids Requires Two Additional Reactions

The fatty acid oxidation sequence just described is typical when the incoming fatty acid is saturated (that is, has only single bonds in its carbon chain). However,

BOX 17–1 THE WORLD OF BIOCHEMISTRY

Fat Bears Carry Out β Oxidation in Their Sleep

Many animals depend on fat stores for energy during hibernation, during migratory periods, and in other situations involving radical metabolic adjustments. One of the most pronounced adjustments of fat metabolism occurs in hibernating grizzly bears. These animals remain in a continuous state of dormancy for periods as long as seven months. Unlike most hibernating species, the bear maintains a body temperature of between 32 and 35 °C, close to the normal (nonhibernating) level. Although expending about 25,000 kJ/day (6,000 kcal/day), the bear does not eat, drink, urinate, or defecate for months at a time.

A grizzly bear prepares its hibernation nest, near the McNeil River in Canada.

Experimental studies have shown that hibernating grizzly bears use body fat as their sole fuel. Fat oxidation yields sufficient energy for maintenance of body temperature, active synthesis of amino acids and proteins, and other energy-requiring activities, such as membrane transport. Fat oxidation also releases large amounts of water, as described in the text, which replenishes water lost in breathing. The glycerol released by degradation of triacylglycerols is converted into blood glucose by gluconeogenesis. Urea formed during breakdown of amino acids is reabsorbed in the kidneys and recycled, the amino groups reused to make new amino acids for maintaining body proteins.

Bears store an enormous amount of body fat in preparation for their long sleep. An adult grizzly consumes about 38,000 kJ/day during the late spring and summer, but as winter approaches it feeds 20 hours a day, consuming up to 84,000 kJ daily. This change in feeding is a response to a seasonal change in hormone secretion. Large amounts of triacylglycerols are formed from the huge intake of carbohydrates during the fattening-up period. Other hibernating species, including the tiny dormouse, also accumulate large amounts of body fat.

most of the fatty acids in the triacylglycerols and phospholipids of animals and plants are unsaturated, having one or more double bonds. These bonds are in the cis configuration and cannot be acted upon by enoyl-CoA hydratase, the enzyme catalyzing the addition of H_2O to the trans double bond of the Δ^2-enoyl-CoA generated during β oxidation. Two auxiliary enzymes are needed for β oxidation of the common unsaturated fatty acids: an isomerase and a reductase. We illustrate these auxiliary reactions with two examples.

TABLE 17–1 Yield of ATP during Oxidation of One Molecule of Palmitoyl-CoA to CO_2 and H_2O

Enzyme catalyzing the oxidation step	*Number of NADH or $FADH_2$ formed*	*Number of ATP ultimately formed**
Acyl-CoA dehydrogenase	7 $FADH_2$	10.5
β-Hydroxyacyl-CoA dehydrogenase	7 NADH	17.5
Isocitrate dehydrogenase	8 NADH	20
α-Ketoglutarate dehydrogenase	8 NADH	20
Succinyl-CoA synthetase		8†
Succinate dehydrogenase	8 $FADH_2$	12
Malate dehydrogenase	8 NADH	20
Total		108

*These calculations assume that mitochondrial oxidative phosphorylation produces 1.5 ATP per $FADH_2$ oxidized and 2.5 ATP per NADH oxidized.

†GTP produced directly in this step yields ATP in the reaction catalyzed by nucleoside diphosphate kinase (p. XXX).

Oleate is an abundant 18-carbon monounsaturated fatty acid with a cis double bond between C-9 and C-10 (denoted Δ^9). In the first step of oxidation, oleate is converted to oleoyl-CoA and, like the saturated fatty acids, enters the mitochondrial matrix via the carnitine shuttle (Fig. 17–6). Oleoyl-CoA then undergoes three passes through the fatty acid oxidation cycle to yield three molecules of acetyl-CoA and the coenzyme A ester of a Δ^3, 12-carbon unsaturated fatty acid, *cis*-Δ^3-dodecenoyl-CoA (Fig. 17–9). This product cannot serve as a substrate for enoyl-CoA hydratase, which acts only on trans double bonds. The auxiliary enzyme **Δ^3,Δ^2-enoyl-CoA isomerase** isomerizes the *cis*-Δ^3-enoyl-CoA to the *trans*-Δ^2-enoyl-CoA, which is converted by enoyl-CoA hydratase into the corresponding L-β-hydroxyacyl-CoA (*trans*-Δ^2-dodecenoyl-CoA). This intermediate is now acted upon by the remaining enzymes of β oxidation to yield acetyl-CoA and the coenzyme A ester of a 10-carbon saturated fatty acid, decanoyl-CoA. The latter undergoes four more passes through the pathway to yield five more molecules of acetyl-CoA. Altogether, nine acetyl-CoAs are produced from one molecule of the 18-carbon oleate.

The other auxiliary enzyme (a reductase) is required for oxidation of polyunsaturated fatty acids—for example, the 18-carbon linoleate, which has a *cis*-Δ^9,*cis*-Δ^{12} configuration (Fig. 17–10). Linoleoyl-CoA undergoes three passes through the β-oxidation sequence to yield three molecules of acetyl-CoA and the coenzyme A ester of a 12-carbon unsaturated fatty acid with a *cis*-Δ^3,*cis*-Δ6 configuration. This intermediate cannot be used by the enzymes of the β-oxidation pathway; its double bonds are in the wrong position and have the wrong configuration (cis, not trans). However, the combined action of enoyl-CoA isomerase and **2,4-dienoyl-CoA reductase,** as shown in Figure 17–10, allows reentry of this intermediate into the β-oxidation pathway

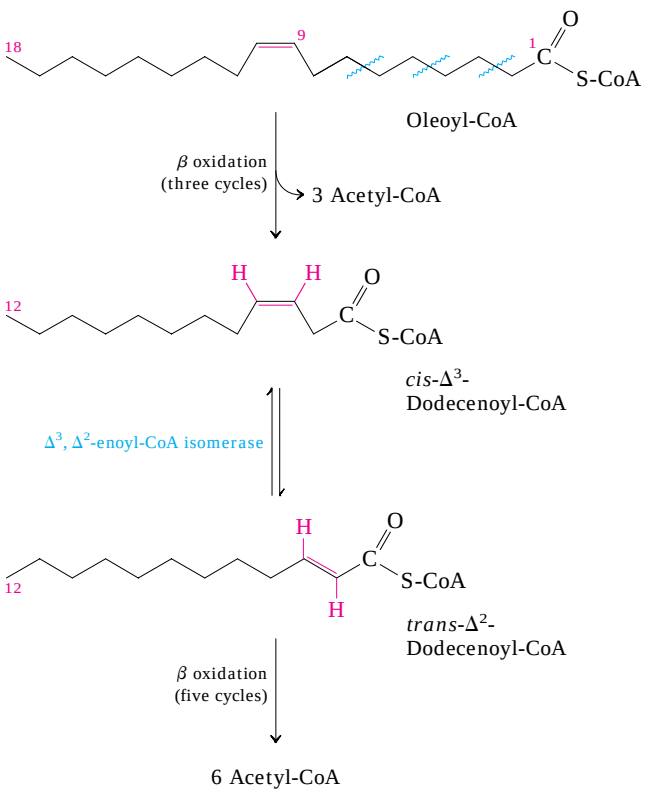


FIGURE 17–9 **Oxidation of a monounsaturated fatty acid.** Oleic acid, as oleoyl-CoA (Δ^9), is the example used here. Oxidation requires an additional enzyme, enoyl-CoA isomerase, to reposition the double bond, converting the cis isomer to a trans isomer, a normal intermediate in β oxidation.

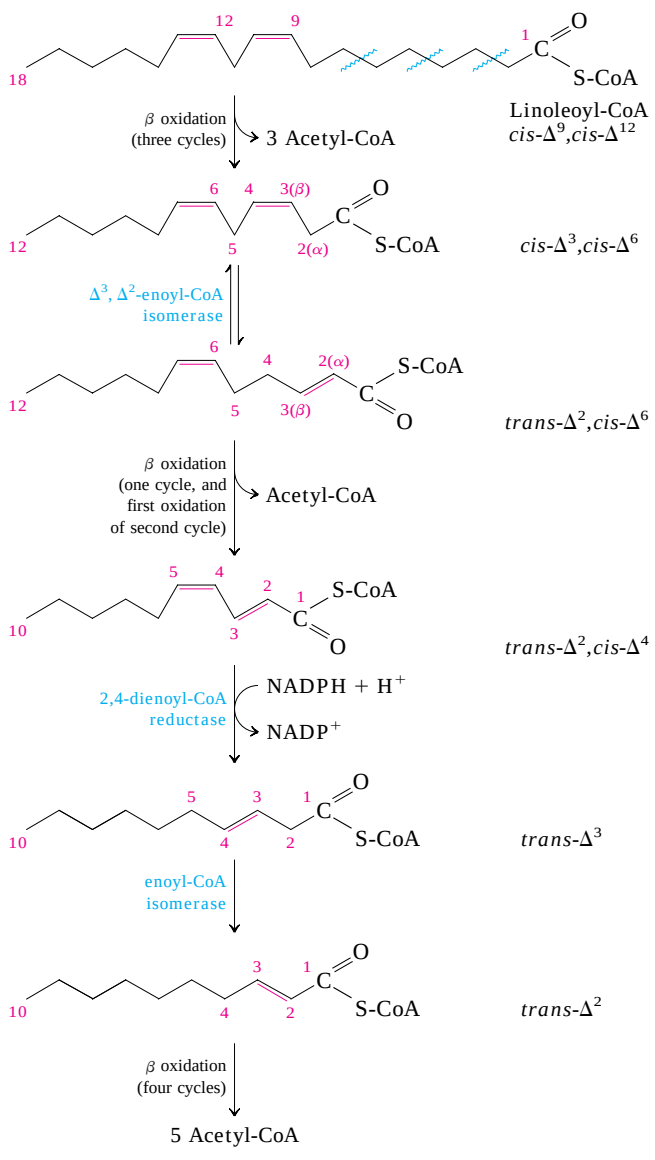


FIGURE 17–10 **Oxidation of a polyunsaturated fatty acid.** The example here is linoleic acid, as linoleoyl-CoA ($\Delta^{9,12}$). Oxidation requires a second auxiliary enzyme in addition to enoyl-CoA isomerase: NADPH-dependent 2,4-dienoyl-CoA reductase. The combined action of these two enzymes converts a *trans*-Δ^2,*cis*-Δ^4-dienoyl-CoA intermediate to the *trans*-Δ^2-enoyl-CoA substrate necessary for β oxidation.

and its degradation to six acetyl-CoAs. The overall result is conversion of linoleate to nine molecules of acetyl-CoA.

Complete Oxidation of Odd-Number Fatty Acids Requires Three Extra Reactions

Although most naturally occurring lipids contain fatty acids with an even number of carbon atoms, fatty acids with an odd number of carbons are common in the lipids of many plants and some marine organisms. Cattle and other ruminant animals form large amounts of the three-carbon **propionate** ($CH_3—CH_2—COO^-$) during fermentation of carbohydrates in the rumen. The propionate is absorbed into the blood and oxidized by the liver and other tissues. And small quantities of propionate are added as a mold inhibitor to some breads and cereals, thus entering the human diet.

Long-chain odd-number fatty acids are oxidized in the same pathway as the even-number acids, beginning at the carboxyl end of the chain. However, the substrate for the last pass through the β-oxidation sequence is a fatty acyl–CoA with a five-carbon fatty acid. When this is oxidized and cleaved, the products are acetyl-CoA and **propionyl-CoA.** The acetyl-CoA can be oxidized in the citric acid cycle, of course, but propionyl-CoA enters a different pathway involving three enzymes.

Propionyl-CoA is first carboxylated to form the D stereoisomer of **methylmalonyl-CoA** (Fig. 17–11) by **propionyl-CoA carboxylase,** which contains the cofactor biotin. In this enzymatic reaction, as in the pyruvate carboxylase reaction (see Fig. 16–16), CO_2 (or its hydrated ion, HCO_3^-) is activated by attachment to biotin before its transfer to the substrate, in this case the propionate moiety. Formation of the carboxybiotin intermediate requires energy, which is provided by the cleavage of ATP to ADP and P_i. The D-methylmalonyl-CoA thus formed is enzymatically epimerized to its L stereoisomer by **methylmalonyl-CoA epimerase** (Fig. 17–11). The L-methylmalonyl-CoA then undergoes an intramolecular rearrangement to form succinyl-CoA, which can enter the citric acid cycle. This rearrangement is catalyzed by **methylmalonyl-CoA mutase,** which requires as its coenzyme **5′-deoxyadenosylcobalamin,** or **coenzyme B_{12},** which is derived from vitamin B_{12} (cobalamin). Box 17–2 describes the role of coenzyme B_{12} in this remarkable exchange reaction.

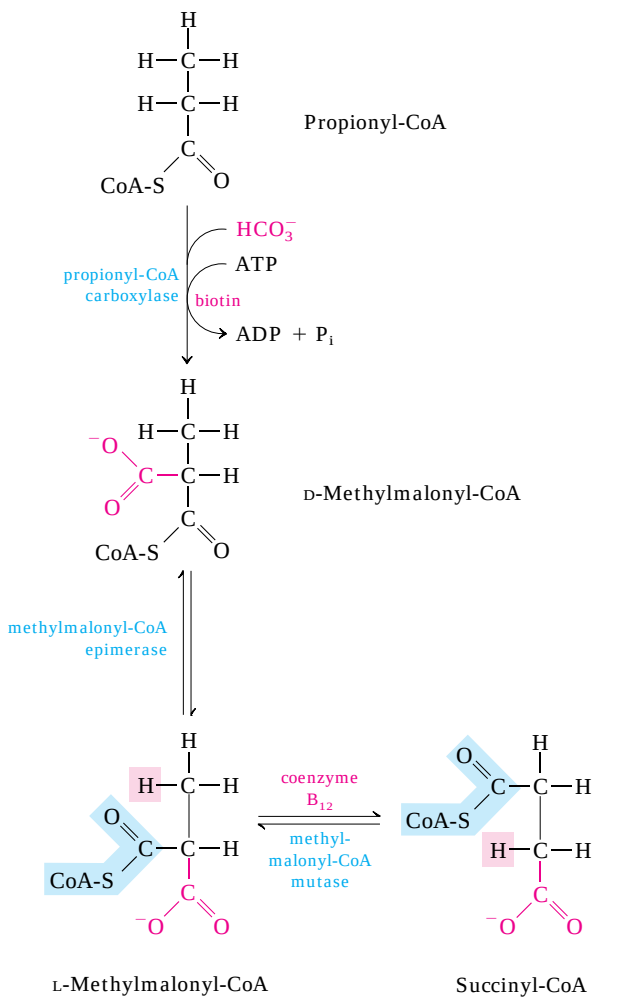


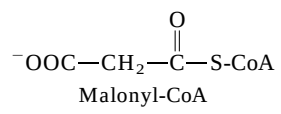
FIGURE 17–11 Oxidation of propionyl-CoA produced by β oxidation of odd-number fatty acids. The sequence involves the carboxylation of propionyl-CoA to D-methylmalonyl-CoA and conversion of the latter to succinyl-CoA. This conversion requires epimerization of D- to L-methylmalonyl-CoA, followed by a remarkable reaction in which substituents on adjacent carbon atoms exchange positions (see Box 17–2).

Fatty Acid Oxidation Is Tightly Regulated

Oxidation of fatty acids consumes a precious fuel, and it is regulated so as to occur only when the need for energy requires it. In the liver, fatty acyl–CoA formed in the cytosol has two major pathways open to it: (1) β oxidation by enzymes in mitochondria or (2) conversion into triacylglycerols and phospholipids by enzymes in the cytosol. The pathway taken depends on the rate of transfer of long-chain fatty acyl–CoA into mitochondria. The three-step process (carnitine shuttle) by which fatty acyl groups are carried from cytosolic fatty acyl–CoA into the mitochondrial matrix (Fig. 17–6) is rate-limiting for fatty acid oxidation and is an important point of regulation. Once fatty acyl groups have entered the mitochondrion, they are committed to oxidation to acetyl-CoA.

Malonyl-CoA, the first intermediate in the cytosolic biosynthesis of long-chain fatty acids from acetyl-CoA (see Fig. 21–1), increases in concentration whenever the animal is well supplied with carbohydrate; excess glucose that cannot be oxidized or stored as glycogen is converted in the cytosol into fatty acids for storage as triacylglycerol. The inhibition of carnitine acyltransferase I by malonyl-CoA ensures that the oxidation of

fatty acids is inhibited whenever the liver is amply supplied with glucose as fuel and is actively making triacylglycerols from excess glucose.



Malonyl-CoA

Two of the enzymes of β oxidation are also regulated by metabolites that signal energy sufficiency. When the [NADH]/[NAD^+] ratio is high, β-hydroxyacyl-CoA dehydrogenase is inhibited; in addition, high concentrations of acetyl-CoA inhibit thiolase (Fig. 17–12).

Genetic Defects in Fatty Acyl–CoA Dehydrogenases Cause Serious Disease

Stored triacylglycerols are typically the chief source of energy for muscle contraction, and an inability to oxidize fatty acids from triacylglycerols has serious consequences for health. The most common genetic defect in fatty acid catabolism in U.S. and northern European populations is due to a mutation in the gene encoding the **medium-chain acyl-CoA dehydrogenase (MCAD).** Among northern Europeans, the frequency of carriers (individuals with this recessive mutation on one of the two homologous chromosomes) is about 1 in 40, and about 1 individual in 10,000 has the disease—that is, has two copies of the mutant MCAD allele and is unable to oxidize fatty acids of 6 to 12 carbons. The disease is characterized by recurring episodes of a syndrome that includes fat accumulation in the liver, high blood levels of octanoic acid, low blood glucose (hypoglycemia), sleepiness, vomiting, and coma. The pattern of organic acids in the urine helps in the diagnosis of this disease: the urine commonly contains high levels of 6-carbon to 10-carbon dicarboxylic acids (produced by ω oxidation) and low levels of urinary ketone bodies (we discuss ω oxidation below and ketone bodies in Section 17.3). Although individuals may have no symptoms between episodes, the episodes are very serious; mortality from this disease is 25% to 60% in early childhood. If the genetic defect is detected shortly after birth, the infant can be started on a low-fat, high-carbohydrate diet. With early detection and careful management of the diet—including avoiding long intervals between meals, to prevent the body from turning to its fat reserves for energy—the prognosis for these individuals is good.

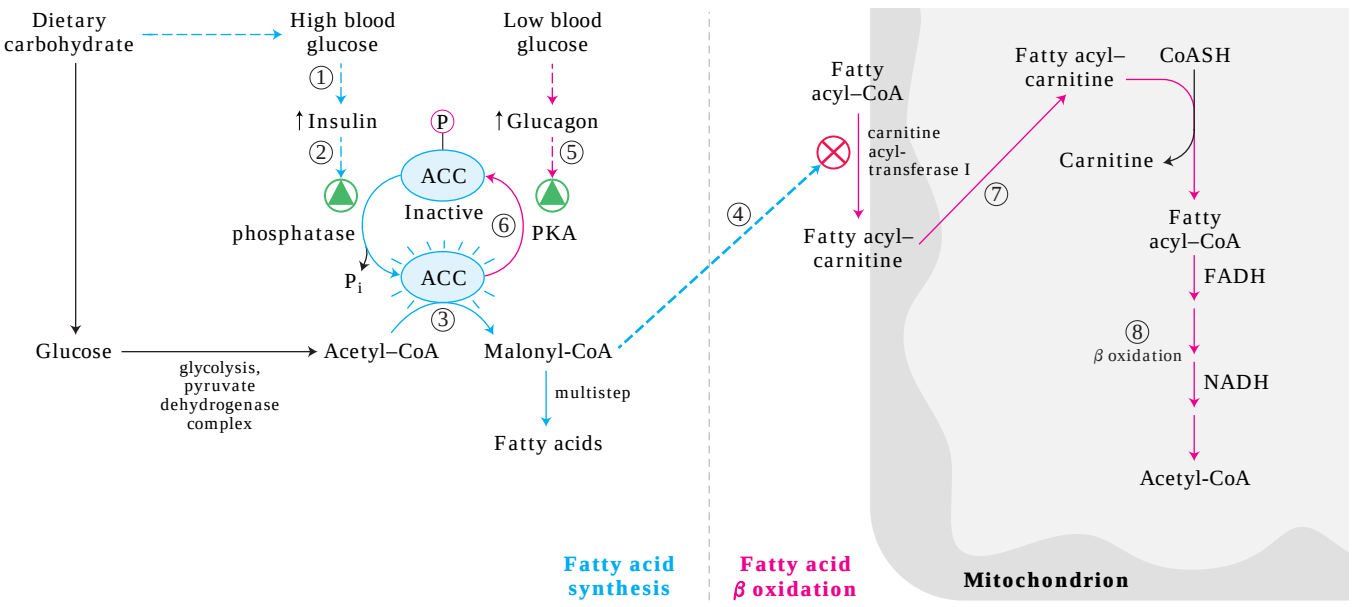


FIGURE 17–12 Coordinated regulation of fatty acid synthesis and breakdown. When the diet provides a ready source of carbohydrate as fuel, β oxidation of fatty acids is unnecessary and is therefore down-regulated. Two enzymes are key to the coordination of fatty acid metabolism: acetyl-CoA carboxylase (ACC), the first enzyme in the synthesis of fatty acids (see Fig. 21–1), and carnitine acyl transferase I, which limits the transport of fatty acids into the mitochondrial matrix for β oxidation (see Fig. 17–6). Ingestion of a high-carbohydrate meal raises the blood glucose level and thus ① triggers the release of insulin. ② Insulin-dependent protein phosphatase dephosphorylates ACC, activating it. ③ ACC catalyzes the formation of malonyl-CoA (the first intermediate of fatty acid synthesis), and ④ malonyl-CoA inhibits carnitine acyltransferase I, thereby preventing fatty acid entry into the mitochondrial matrix.

When blood glucose levels drop between meals, ⑤ glucagon release activates cAMP-dependent protein kinase (PKA), which ⑥ phosphorylates and inactivates ACC. The concentration of malonyl-CoA falls, the inhibition of fatty acid entry into mitochondria is relieved, and ⑦ fatty acids enter the mitochondrial matrix and ⑧ become the major fuel. Because glucagon also triggers the mobilization of fatty acids in adipose tissue, a supply of fatty acids begins arriving in the blood.

BOX 17–2 WORKING IN BIOCHEMISTRY

Coenzyme B_{12}: A Radical Solution to a Perplexing Problem

In the methylmalonyl-CoA mutase reaction (see Fig. 17–11), the group —CO—S-CoA at C-2 of the original propionate exchanges position with a hydrogen atom at C-3 of the original propionate (Fig. 1a). Coenzyme B_{12} is the cofactor for this reaction, as it is for almost all enzymes that catalyze reactions of this general type (Fig. 1b). These coenzyme B_{12}–dependent processes are among the very few enzymatic reactions in biology in which there is an exchange of an alkyl or substituted alkyl group (X) with a hydrogen atom on an adjacent carbon, *with no mixing of the transferred hydrogen atom with the hydrogen of the solvent,* H_2O. How can the hydrogen atom move between two carbons without mixing with the enormous excess of hydrogen atoms in the solvent?

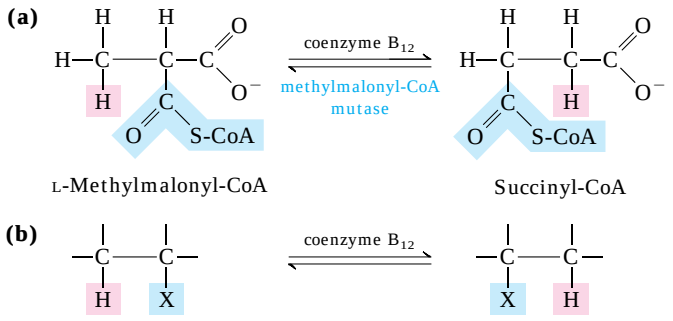


FIGURE 1

Coenzyme B_{12} is the cofactor form of vitamin B_{12}, which is unique among all the vitamins in that it contains not only a complex organic molecule but an essential trace element, cobalt. The complex **corrin ring system** of vitamin B_{12} (colored blue in Fig. 2), to which cobalt (as Co^{3+}) is coordinated, is chemically related to the porphyrin ring system of heme and heme proteins (see Fig. 5–1). A fifth coordination position of cobalt is filled by dimethylbenzimidazole ribonucleotide (shaded yellow), bound covalently by its 3′-phosphate group to a side chain of the corrin ring, through aminoisopropanol. The formation of this complex cofactor occurs in one of only two known reactions in which triphosphate is cleaved from ATP (Fig. 3); the other reaction is the formation of *S*-adenosylmethionine from ATP and methionine (see Fig. 18–18).

Vitamin B_{12} as usually isolated is called **cyanocobalamin,** because it contains a cyano group (picked up during purification) attached to cobalt in the sixth coordination position. In **5′-deoxyadenosylcobalamin,** the cofactor for methylmalonyl-CoA mutase, the cyano group is replaced by the **5′-deoxyadenosyl** group (red in Fig. 2), covalently bound through C-5′ to the cobalt. The three-dimensional structure of the cofactor was determined by Dorothy Crowfoot Hodgkin in 1956, using x-ray crystallography.

The key to understanding how coenzyme B_{12} catalyzes hydrogen exchange lies in the properties of the covalent bond between cobalt and C-5′ of the deoxyadenosyl group (Fig. 2). This is a relatively weak bond; its bond dissociation energy is about 110 kJ/mol, compared with 348 kJ/mol for a typical C—C bond or 414 kJ/mol for a C—H bond. Merely illuminating the compound with visible light is enough to break this Co—C bond. (This extreme photolability probably accounts for the absence of vitamin B_{12} in plants.) Dissociation produces a 5′-deoxyadenosyl radical and the Co^{2+}

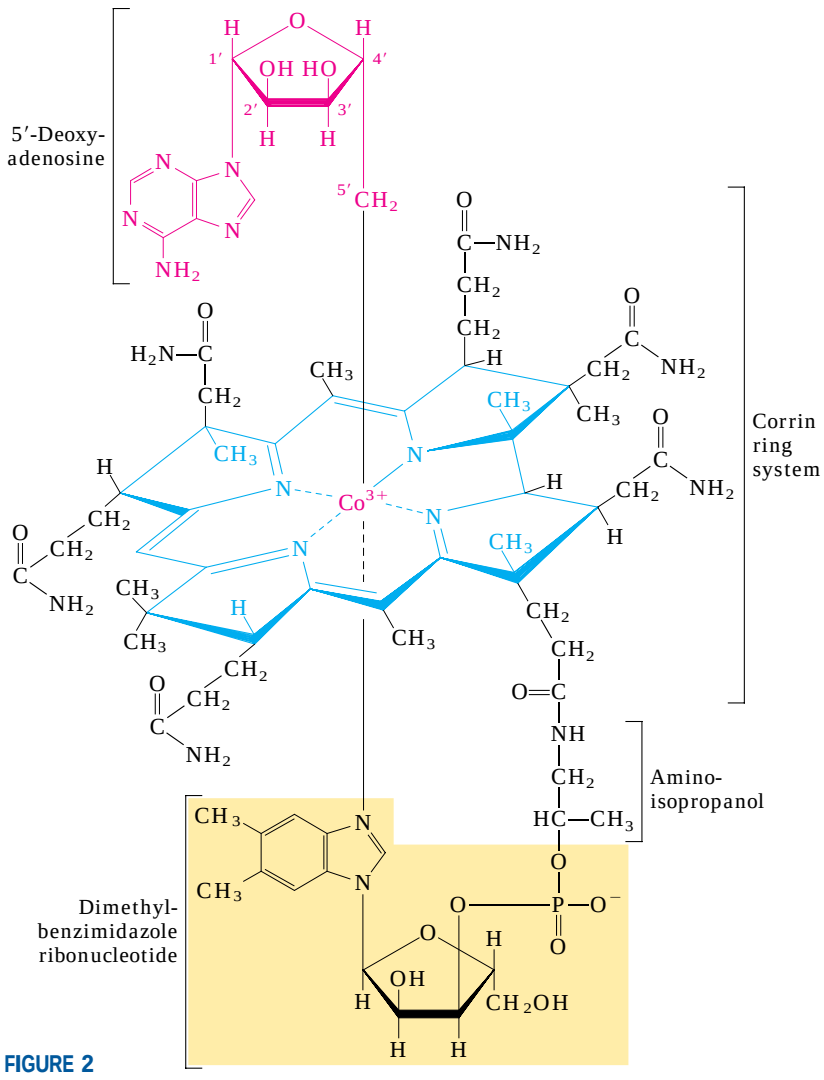


FIGURE 2

Dorothy Crowfoot Hodgkin, 1910–1994

form of the vitamin. The chemical function of 5′-deoxyadenosylcobalamin is to generate free radicals in this way, thus initiating a series of transformations such as that illustrated in Figure 4—a postulated mechanism for the reaction catalyzed by methylmalonyl-CoA mutase and a number of other coenzyme B_{12}–dependent transformations.

① The enzyme first breaks the Co—C bond in the cofactor, leaving the coenzyme in its Co^{2+} form and producing the 5′-deoxyadenosyl free radical. ② This radical now abstracts a hydrogen atom from the substrate, converting the substrate to a radical and producing 5′-deoxyadenosine. ③ Rearrangement of the substrate radical yields another radical, in which the migrating group X (—CO—S-CoA for methylmalonyl-CoA mutase) has moved to the adjacent carbon to form a radical that has the carbon skeleton of the eventual product (a four-carbon straight chain). The hydrogen atom initially abstracted from the substrate is now part of the $—CH_3$ group of 5′-deoxyadenosine. ④ One of the hydrogens from this same $—CH_3$ group (it can be the same one originally abstracted) is now returned to the productlike radical, generating the product and regenerating the deoxyadenosyl free radical. ⑤ The bond re-forms between cobalt and the $—CH_2$ group of the deoxyadenosyl radical, destroying the free radical and regenerating the cofactor in its Co^{3+} form, ready to undergo another catalytic cycle. In this postulated mechanism, the migrating hydrogen atom never exists as a free species and is thus never free to exchange with the hydrogen of surrounding water molecules.

Vitamin B_{12} deficiency results in serious disease. This vitamin is not made by plants or animals and can be synthesized only by a few species of microorganisms. It is required by healthy people in only minute amounts, about 3 μg/day. The severe disease **pernicious anemia** results from failure to absorb vitamin B_{12} efficiently from the intestine, where it is synthesized by intestinal bacteria or obtained from digestion of meat. Individuals with this disease do not produce sufficient amounts of **intrinsic factor,** a glycoprotein essential to vitamin B_{12} absorption. The pathology in pernicious anemia includes reduced production of erythrocytes, reduced levels of hemoglobin, and severe, progressive impairment of the central nervous system. Administration of large doses of vitamin B_{12} alleviates these symptoms in at least some cases. ■

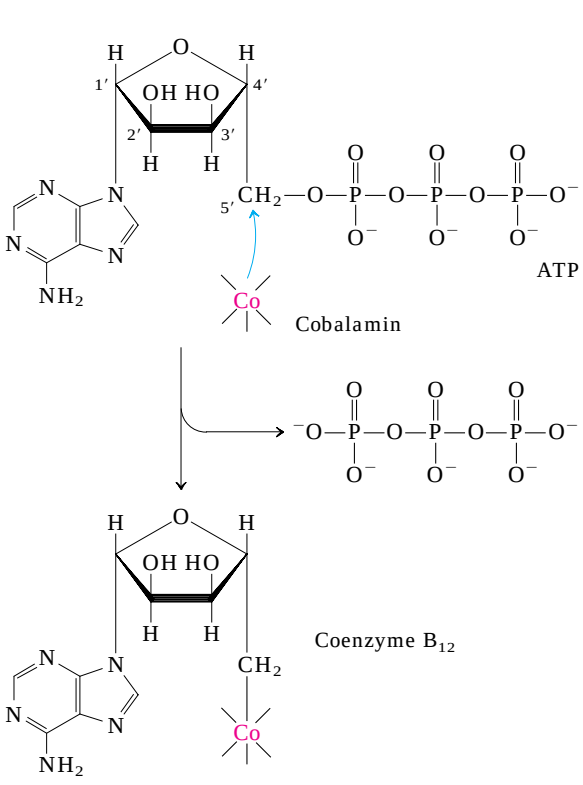
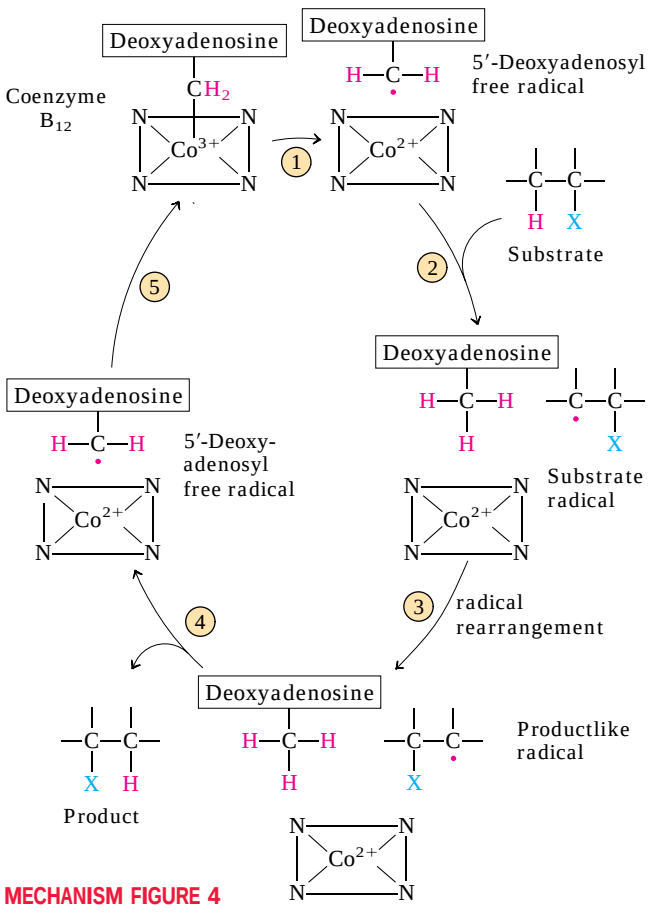


FIGURE 3



MECHANISM FIGURE 4

More than 20 other human genetic defects in fatty acid transport or oxidation have been documented, most much less common than the defect in MCAD. One of the most severe disorders results from loss of the long-chain β-hydroxyacyl-CoA dehydrogenase activity of the trifunctional protein, TFP. Other disorders include defects in the α or β subunits that affect all three activities of TFP and cause serious heart disease and abnormal skeletal muscle. ■

Peroxisomes Also Carry Out β Oxidation

The mitochondrial matrix is the major site of fatty acid oxidation in animal cells, but in certain cells other compartments also contain enzymes capable of oxidizing fatty acids to acetyl-CoA, by a pathway similar to, but not identical with, that in mitochondria. In plant cells, the major site of β oxidation is not mitochondria but peroxisomes.

In **peroxisomes,** membrane-enclosed organelles of animal and plant cells, the intermediates for β oxidation of fatty acids are coenzyme A derivatives, and the process consists of four steps, as in mitochondrial β oxidation (Fig. 17–13): (1) dehydrogenation, (2) addition of water to the resulting double bond, (3) oxidation of the β-hydroxyacyl-CoA to a ketone, and (4) thiolytic cleavage by coenzyme A. (The identical reactions also occur in glyoxysomes, as discussed below.)

One difference between the peroxisomal and mitochondrial pathways is in the chemistry of the first step. In peroxisomes, the flavoprotein acyl-CoA oxidase that introduces the double bond passes electrons directly to O_2, producing H_2O_2 (Fig. 17–13). This strong and potentially damaging oxidant is immediately cleaved to H_2O and O_2 by **catalase.** Recall that in mitochondria, the electrons removed in the first oxidation step pass through the respiratory chain to O_2 to produce H_2O, and this process is accompanied by ATP synthesis. In peroxisomes, the energy released in the first oxidative step of fatty acid breakdown is not conserved as ATP, but is dissipated as heat.

A second important difference between mitochondrial and peroxisomal β oxidation in mammals is in the specificity for fatty acyl–CoAs; the peroxisomal system is much more active on very-long-chain fatty acids such as hexacosanoic acid (26:0) and on branched-chain fatty acids such as phytanic acid and pristanic acid (see Fig. 17–17). These less-common fatty acids are obtained in the diet from dairy products, the fat of ruminant animals, meat, and fish. Their catabolism in the peroxisome involves several auxiliary enzymes unique to this organelle. The inability to oxidize these compounds is responsible for several serious human diseases. Individuals with **Zellweger syndrome** are unable to make peroxisomes and therefore lack all the metabolism unique to that organelle. In **X-linked adrenoleukodystrophy (XALD),** peroxisomes fail to oxidize very-long-chain fatty acids, apparently for lack of a functional transporter for these fatty acids in the peroxisomal membrane. Both defects lead to accumulation in the blood of very-long-chain fatty acids, especially 26:0. XALD affects young boys before the age of 10 years, causing loss of vision, behavioral disturbances, and death within a few years. ■

In mammals, high concentrations of fats in the diet result in increased synthesis of the enzymes of peroxisomal β oxidation in the liver. Liver peroxisomes do not contain the enzymes of the citric acid cycle and cannot catalyze the oxidation of acetyl-CoA to CO_2. Instead,

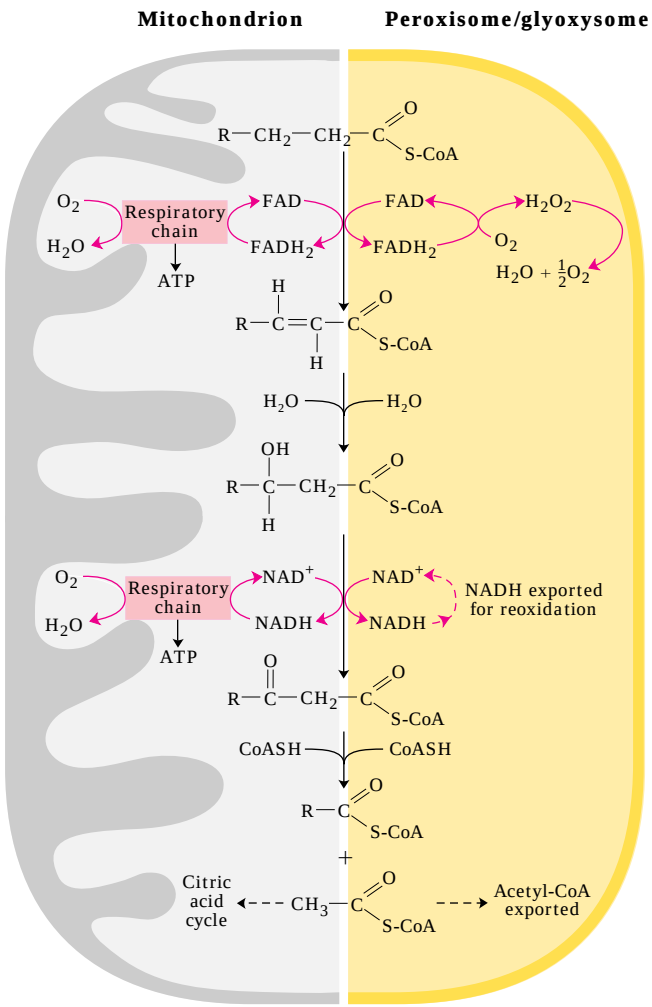


FIGURE 17–13 Comparison of β oxidation in mitochondria and in peroxisomes and glyoxysomes. The peroxisomal/glyoxysomal system differs from the mitochondrial system in two respects: (1) in the first oxidative step electrons pass directly to O_2, generating H_2O_2, and (2) the NADH formed in the second oxidative step cannot be reoxidized in the peroxisome or glyoxysome, so reducing equivalents are exported to the cytosol, eventually entering mitochondria. The acetyl-CoA produced by peroxisomes and glyoxysomes is also exported; the acetate from glyoxysomes (organelles found only in germinating seeds) serves as a biosynthetic precursor (see Fig. 17–14). Acetyl-CoA produced in mitochondria is further oxidized in the citric acid cycle.

long-chain or branched fatty acids are catabolized to shorter-chain products, such as hexanoyl-CoA, which are exported to mitochondria and completely oxidized.

Plant Peroxisomes and Glyoxysomes Use Acetyl-CoA from β Oxidation as a Biosynthetic Precursor

In plants, fatty acid oxidation does not occur primarily in mitochondria (as noted earlier) but in the peroxisomes of leaf tissue and in the glyoxysomes of germinating seeds. Plant peroxisomes and glyoxysomes are similar in structure and function; glyoxysomes, which occur only in germinating seeds, may be considered specialized peroxisomes. The biological role of β oxidation in these organelles is to use stored lipids primarily to provide but biosynthetic precursors, not energy.

During seed germination, stored triacylglycerols are converted into glucose, sucrose, and a wide variety of essential metabolites (Fig. 17–14). Fatty acids released from the triacylglycerols are first activated to their coenzyme A derivatives and oxidized in glyoxysomes by the same four-step process that takes place in peroxisomes (Fig. 17–13). The acetyl-CoA produced is converted via the glyoxylate cycle (see Fig. 16–20) to four-carbon precursors for gluconeogenesis (see Fig. 14–18). Glyoxysomes, like peroxisomes, contain high concentrations of catalase, which converts the H_2O_2 produced by β oxidation to H_2O and O_2.

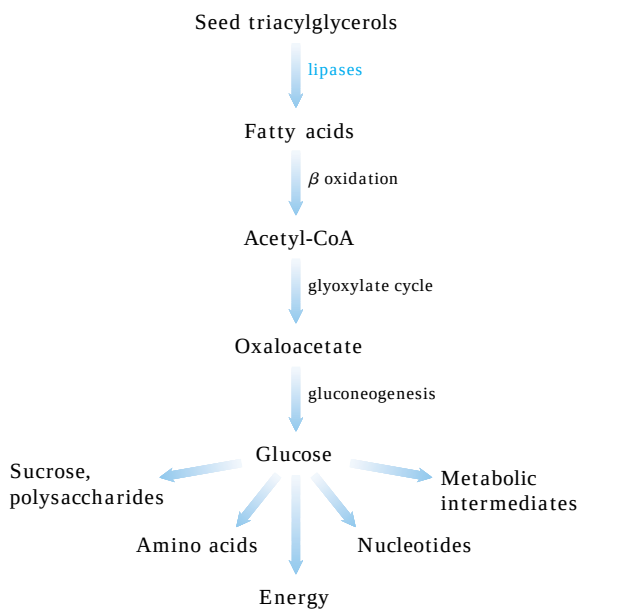


FIGURE 17–14 Triacylglycerols as glucose source in seeds. β Oxidation is one stage in a pathway that converts stored triacylglycerols to glucose in germinating seeds. For more detail, see Figure 16–22.

The β-Oxidation Enzymes of Different Organelles Have Diverged during Evolution

Although the β-oxidation reactions in mitochondria are essentially the same as those in peroxisomes and glyoxysomes, the enzymes (isozymes) differ significantly between the two types of organelles. The differences apparently reflect an evolutionary divergence that occurred very early, with the separation of gram-positive and gram-negative bacteria (see Fig. 1–6).

In mitochondria, the four β-oxidation enzymes that act on short-chain fatty acyl–CoAs are separate, soluble proteins (as noted earlier), similar in structure to the analogous enzymes of gram-positive bacteria (Fig. 17–15a). The gram-negative bacteria have four activities in three soluable subunits (Fig. 17–15b), and the eukaryotic enzyme system that acts on long-chain fatty acids—the trifunctional protein, TFP—has three enzyme activities in two subunits that are membrane-associated (Fig. 17–15c). The β-oxidation enzymes of plant peroxisomes and glyoxysomes, however, form a complex of proteins, one of which contains four enzymatic activities in a single polypeptide chain (Fig. 17–15d). The first enzyme, acyl-CoA oxidase, is a single polypeptide chain; the **multifunctional protein (MFP)** contains the second and third enzyme activities (enoyl-CoA hydratase and hydroxyacyl-CoA dehydrogenase) as well as two auxiliary activities needed for the oxidation of unsaturated fatty acids (D-3-hydroxyacyl-CoA epimerase and Δ^3,Δ^2-enoyl-CoA isomerase); the fourth enzyme, thiolase, is a separate, soluble polypeptide.

It is interesting that the enzymes that catalyze essentially the reversal of β oxidation in the synthesis of fatty acids are also organized differently in prokaryotes and eukaryotes; in bacteria, the seven enzymes needed for fatty acid synthesis are separate polypeptides, but in mammals, all seven activities are part of a single, huge polypeptide chain (see Fig. 21–7). One advantage to the cell in having several enzymes of the same pathway encoded in a single polypeptide chain is that this solves the problem of regulating the synthesis of enzymes that must interact functionally; regulation of the expression of *one* gene ensures production of the same number of active sites for all enzymes in the path. When each enzyme activity is on a separate polypeptide, some mechanism is required to coordinate the synthesis of all the gene products. The *disadvantage* of having several activities on the same polypeptide is that the longer the polypeptide chain, the greater is the probability of a mistake in its synthesis: a single incorrect amino acid in the chain may make all the enzyme activities in that chain useless. Comparison of the gene structures for these proteins in many species may shed light on the reasons for the selection of one or the other strategy in evolution.

The ω Oxidation of Fatty Acids Occurs in the Endoplasmic Reticulum

Although mitochondrial β oxidation, in which enzymes act at the carboxyl end of a fatty acid, is by far the most

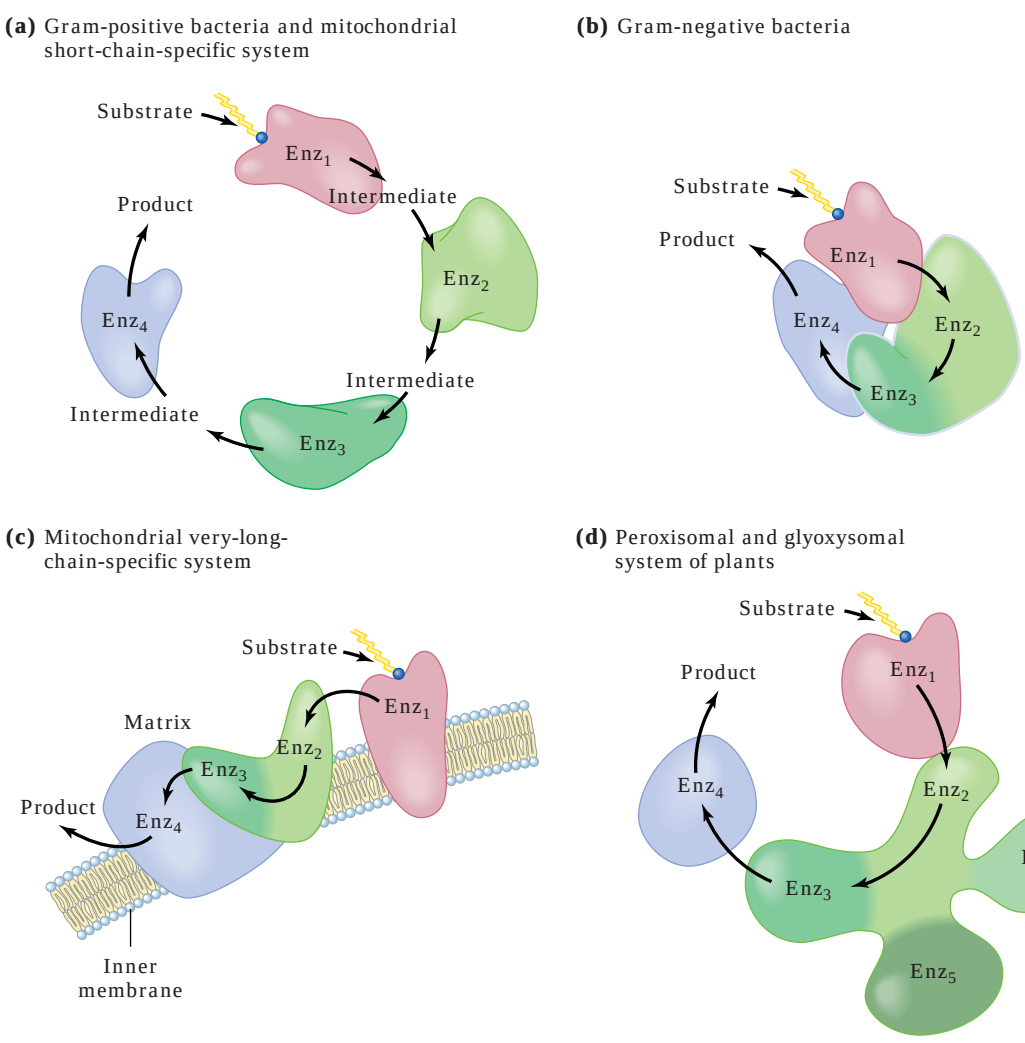


FIGURE 17–15 The enzymes of β oxidation. Shown here are the different subunit structures of the enzymes of β oxidation in gram-positive and gram-negative bacteria, mitochondria, and plant peroxisomes and glyoxysomes. Enz_1 is acyl-CoA dehydrogenase; Enz_2, enoyl-CoA hydratase; Enz_3, L-β-hydroxyacyl-CoA dehydrogenase; Enz_4, thiolase; Enz_5, D-3-hydroxyacyl-CoA epimerase, and Enz_6, Δ^3,Δ^2-enoyl-CoA isomerase. **(a)** The four enzymes of β oxidation in gram-positive bacteria are separate, soluble entities, as are those of the short-chain-specific system of mitochondria. **(b)** In gram-negative bacteria, the four enzyme activities reside in three polypeptides; enzymes 2 and 3 are parts of a single polypeptide chain. **(c)** The very-long-chain-specific system of mitochondria is also composed of three polypeptides, one of which includes the activities of enzymes 2 and 3; in this case, the system is bound to the inner mitochondrial membrane. **(d)** In the peroxisomal and glyoxysomal β-oxidation systems of plants, enzymes 1 and 4 are separate polypeptides, but enzymes 2 and 3, as well as two auxiliary enzymes, are part of a single polypeptide chain, the multifunctional protein, MFP.

important catabolic fate for fatty acids in animal cells, there is another pathway in some species, including vertebrates, that involves oxidation of the ω (omega) carbon—the carbon most distant from the carboxyl group. The enzymes unique to ***ω* oxidation** are located (in vertebrates) in the endoplasmic reticulum of liver and kidney, and the preferred substrates are fatty acids of 10 or 12 carbon atoms. In mammals ω oxidation is normally a minor pathway for fatty acid degradation, but when β oxidation is defective (because of mutation or a carnitine deficiency, for example) it becomes more important.

The first step introduces a hydroxyl group onto the ω carbon (Fig. 17–16). The oxygen for this group comes from molecular oxygen (O_2) in a complex reaction that involves cytochrome P450 and the electron donor NADPH. Reactions of this type are catalyzed by **mixed-function oxidases,** described in Box 21–1. Two more enzymes now act on the ω carbon: **alcohol dehydrogenase** oxidizes the hydroxyl group to an aldehyde, and **aldehyde dehydrogenase** oxidizes the aldehyde group to a carboxylic acid, producing a fatty acid with a carboxyl group at each end. At this point, either end can be attached to coenzyme A, and the molecule can en-

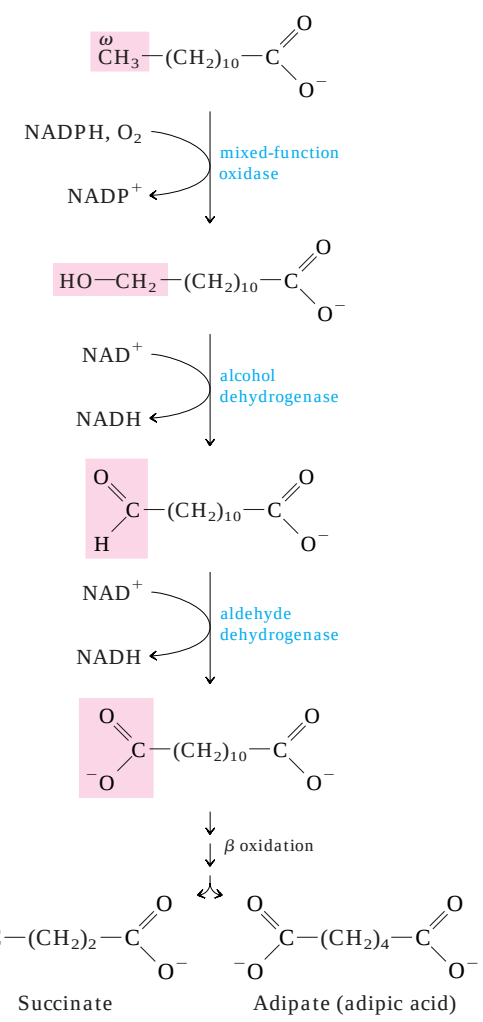


FIGURE 17–16 **The ω oxidation of fatty acids in the endoplasmic reticulum.** This alternative to β oxidation begins with oxidation of the carbon most distant from the α carbon—the ω (omega) carbon. The substrate is usually a medium-chain fatty acid; shown here is lauric acid (laurate). This pathway is generally not the major route for oxidative catabolism of fatty acids.

ter the mitochondrion and undergo β oxidation by the normal route. In each pass through the β-oxidation pathway, the "double-ended" fatty acid yields dicarboxylic acids such as succinic acid, which can enter the citric acid cycle, and adipic acid (Fig. 17–16).

Phytanic Acid Undergoes α Oxidation in Peroxisomes

The presence of a methyl group on the β carbon of a fatty acid makes β oxidation impossible, and these branched fatty acids are catabolized in peroxisomes of animal cells by ***α* oxidation.** In the oxidation of phytanic acid, for example (Fig. 17–17), phytanoyl-CoA is hydroxylated on its α carbon, in a reaction that involves molecular oxygen; decarboxylated to form an aldehyde one carbon shorter; and then oxidized to the

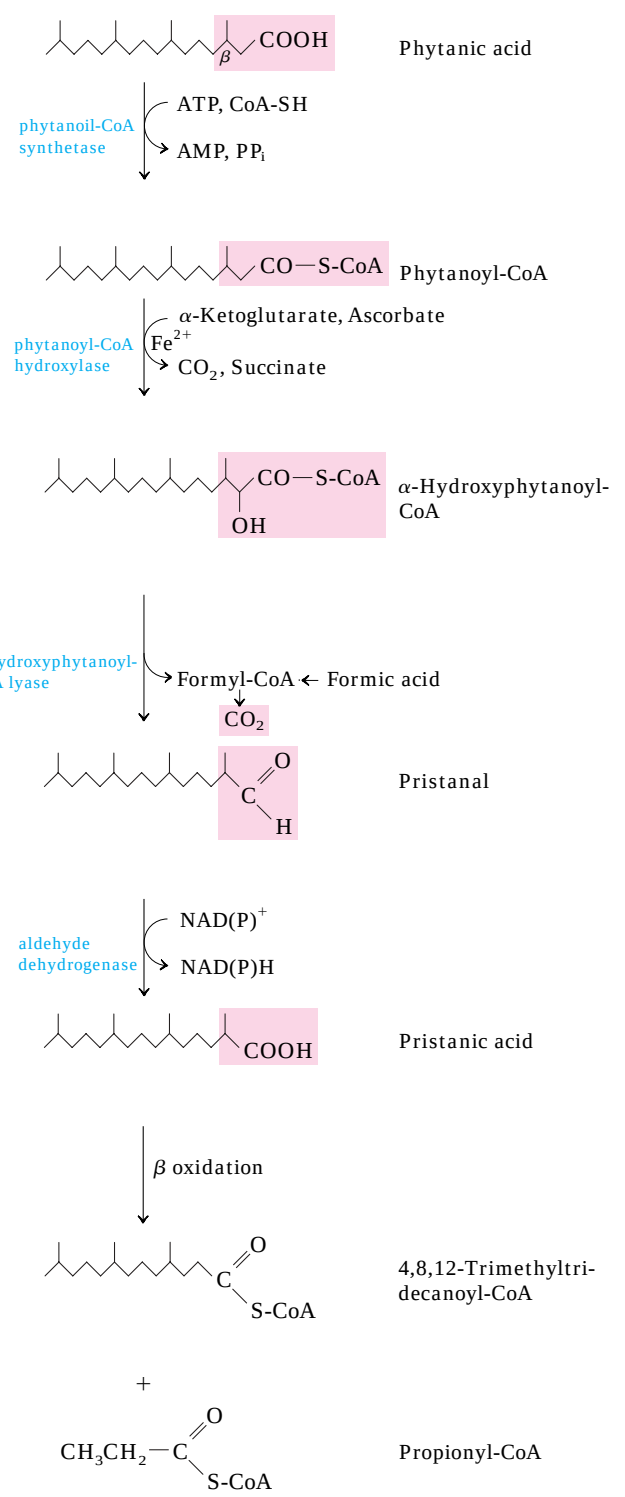


FIGURE 17–17 **The α oxidation of a branched-chain fatty acid (phytanic acid) in peroxisomes.** Phytanic acid has a methyl-substituted β carbon and therefore cannot undergo β oxidation. The combined action of the enzymes shown here removes the carboxyl carbon of phytanic acid, to produce pristanic acid, in which the β carbon is unsubstituted, allowing oxidation. Notice that β oxidation of pristanic acid releases propionyl-CoA, not acetyl-CoA. This is further catabolized as in Figure 17–11. (The details of the reaction that produces pristanal remain controversial.)

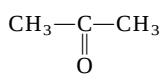
corresponding carboxylic acid, which now has no substituent on the β carbon and can be oxidized further by β oxidation. **Refsum's disease,** resulting from a genetic defect in phytanoyl-CoA hydroxylase, leads to very high blood levels of phytanic acid and severe neurological problems including blindness and deafness. ■

SUMMARY 17.2 Oxidation of Fatty Acids

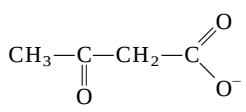
- In the first stage of β oxidation, four reactions remove each acetyl-CoA unit from the carboxyl end of a saturated fatty acyl–CoA: (1) dehydrogenation of the α and β carbons (C-2 and C-3) by FAD-linked acyl-CoA dehydrogenases, (2) hydration of the resulting *trans*-Δ^2 double bond by enoyl-CoA hydratase, (3) dehydrogenation of the resulting L-β-hydroxyacyl-CoA by NAD-linked β-hydroxyacyl-CoA dehydrogenase, and (4) CoA-requiring cleavage of the resulting β-ketoacyl-CoA by thiolase, to form acetyl-CoA and a fatty acyl–CoA shortened by two carbons. The shortened fatty acyl–CoA then reenters the sequence.
- In the second stage of fatty acid oxidation, the acetyl-CoA is oxidized to CO_2 in the citric acid cycle. A large fraction of the theoretical yield of free energy from fatty acid oxidation is recovered as ATP by oxidative phosphorylation, the final stage of the oxidative pathway.
- Malonyl-CoA, an early intermediate of fatty acid synthesis, inhibits carnitine acyltransferase I, preventing fatty acid entry into mitochondria. This blocks fatty acid breakdown while synthesis is occurring.
- Genetic defects in the medium-chain acyl-CoA dehydrogenase result in serious human disease, as do mutations in other components of the β-oxidation system.
- Oxidation of unsaturated fatty acids requires two additional enzymes: enoyl-CoA isomerase and 2,4-dienoyl-CoA reductase. Odd-number fatty acids are oxidized by the β-oxidation pathway to yield acetyl-CoA and a molecule of propionyl-CoA. This is carboxylated to methylmalonyl-CoA, which is isomerized to succinyl-CoA in a reaction catalyzed by methylmalonyl-CoA mutase, an enzyme requiring coenzyme B_{12}.
- Peroxisomes of plants and animals, and glyoxysomes of plants, carry out β oxidation in four steps similar to those of the mitochondrial pathway in animals. The first oxidation step, however, transfers electrons directly to O_2, generating H_2O_2. Peroxisomes of animal tissues specialize in the oxidation of very-long-chain fatty acids and branched fatty acids. In glyoxysomes, in germinating seeds, β oxidation is one step in the conversion of stored lipids into a variety of intermediates and products.
- The reactions of ω oxidation, occurring in the endoplasmic reticulum, produce dicarboxylic fatty acyl intermediates, which can undergo β oxidation at either end to yield short dicarboxylic acids such as succinate.

17.3 Ketone Bodies

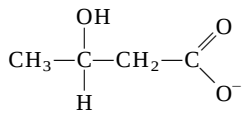
In humans and most other mammals, acetyl-CoA formed in the liver during oxidation of fatty acids can either enter the citric acid cycle (stage 2 of Fig. 17–7) or undergo conversion to the "ketone bodies," **acetone, acetoacetate,** and **D-β-hydroxybutyrate,** for export to other tissues. (The term "bodies" is a historical artifact; the term is occasionally applied to insoluble particles, but these compounds are quite soluble in blood and urine.) Acetone, produced in smaller quantities than the other ketone bodies, is exhaled. Acetoacetate and D-β-hydroxybutyrate are transported by the blood to tissues other than the liver (extrahepatic tissues), where they are converted to acetyl-CoA and oxidized in the citric acid cycle, providing much of the energy required by tissues such as skeletal and heart muscle and the renal cortex. The brain, which preferentially uses glucose as fuel, can adapt to the use of acetoacetate or D-β-hydroxybutyrate under starvation conditions, when glucose is unavailable. The production and export of ketone bodies from the liver to extrahepatic tissues allow continued oxidation of fatty acids in the liver when acetyl-CoA is not being oxidized in the citric acid cycle.



Acetone



Acetoacetate



D-β-Hydroxybutyrate

Ketone Bodies, Formed in the Liver, Are Exported to Other Organs as Fuel

The first step in the formation of acetoacetate, occurring in the liver (Fig. 17–18), is the enzymatic condensation of two molecules of acetyl-CoA, catalyzed by thiolase;

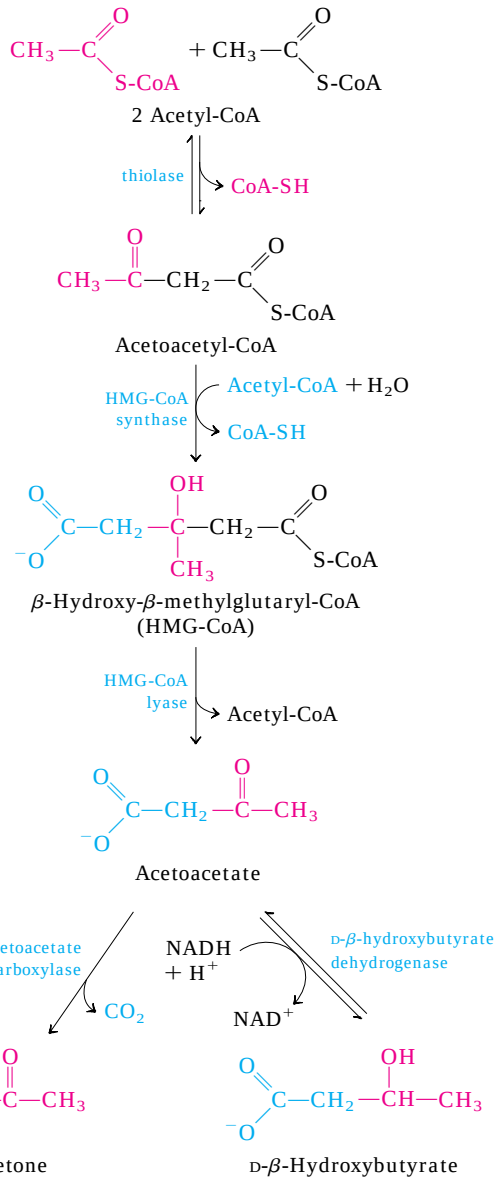


FIGURE 17–18 Formation of ketone bodies from acetyl-CoA. Healthy, well-nourished individuals produce ketone bodies at a relatively low rate. When acetyl-CoA accumulates (as in starvation or untreated diabetes, for example), thiolase catalyzes the condensation of two acetyl-CoA molecules to acetoacetyl-CoA, the parent compound of the three ketone bodies. The reactions of ketone body formation occur in the matrix of liver mitochondria. The six-carbon compound β-hydroxy-β-methylglutaryl-CoA (HMG-CoA) is also an intermediate of sterol biosynthesis, but the enzyme that forms HMG-CoA in that pathway is cytosolic. HMG-CoA lyase is present only in the mitochondrial matrix.

this is simply the reversal of the last step of β oxidation. The acetoacetyl-CoA then condenses with acetyl-CoA to form **β-hydroxy-β-methylglutaryl-CoA (HMG-CoA),** which is cleaved to free acetoacetate and acetyl-CoA. The acetoacetate is reversibly reduced by D-β-hydroxybutyrate dehydrogenase, a mitochondrial enzyme, to D-β-hydroxybutyrate. This enzyme is specific for the D stereoisomer; it does not act on L-β-hydroxyacyl-CoAs and is not to be confused with L-β-hydroxyacyl-CoA dehydrogenase of the β-oxidation pathway.

In healthy people, acetone is formed in very small amounts from acetoacetate, which is easily decarboxylated, either spontaneously or by the action of **acetoacetate decarboxylase** (Fig. 17–18). Because individuals with untreated diabetes produce large quantities of acetoacetate, their blood contains significant amounts of acetone, which is toxic. Acetone is volatile and imparts a characteristic odor to the breath, which is sometimes useful in diagnosing diabetes. ■

In extrahepatic tissues, D-β-hydroxybutyrate is oxidized to acetoacetate by D-β-hydroxybutyrate dehydrogenase (Fig. 17–19). The acetoacetate is activated to its coenzyme A ester by transfer of CoA from succinyl-CoA, an intermediate of the citric acid cycle (see Fig. 16–7), in a reaction catalyzed by ***β*-ketoacyl-CoA transferase.** The acetoacetyl-CoA is then cleaved by thiolase to yield two acetyl-CoAs, which enter the citric acid cycle. Thus the ketone bodies are used as fuels.

The production and export of ketone bodies by the liver allow continued oxidation of fatty acids with only minimal oxidation of acetyl-CoA. When intermediates of the citric acid cycle are being siphoned off for glucose

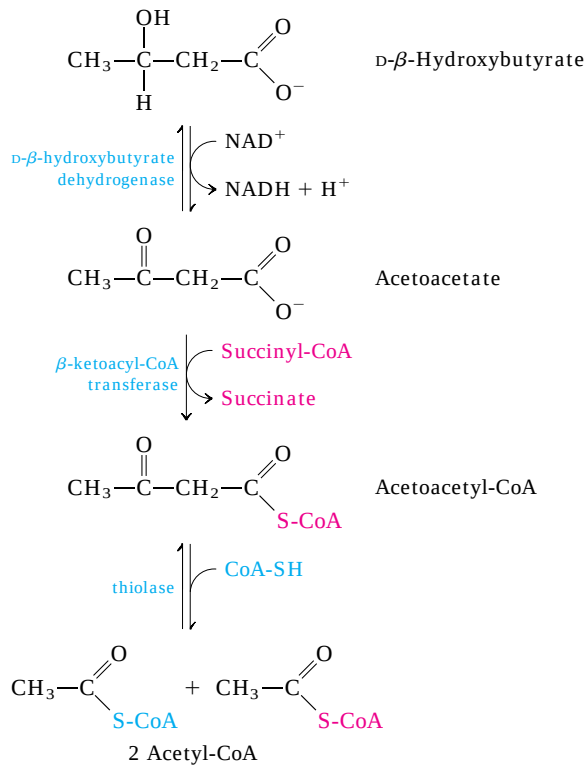


FIGURE 17–19 D-β-Hydroxybutyrate as a fuel. D-β-Hydroxybutyrate, synthesized in the liver, passes into the blood and thus to other tissues, where it is converted in three steps to acetyl-CoA. It is first oxidized to acetoacetate, which is activated with coenzyme A donated from succinyl-CoA, then split by thiolase. The acetyl-CoA thus formed is used for energy production.

synthesis by gluconeogenesis, for example, oxidation of cycle intermediates slows—and so does acetyl-CoA oxidation. Moreover, the liver contains only a limited amount of coenzyme A, and when most of it is tied up in acetyl-CoA, β oxidation slows for want of the free coenzyme. The production and export of ketone bodies free coenzyme A, allowing continued fatty acid oxidation.

Ketone Bodies Are Overproduced in Diabetes and during Starvation

Starvation and untreated diabetes mellitus lead to overproduction of ketone bodies, with several associated medical problems. During starvation, gluconeogenesis depletes citric acid cycle intermediates, diverting acetyl-CoA to ketone body production (Fig. 17–20). In untreated diabetes, when the insulin level is insufficient, extrahepatic tissues cannot take up glucose efficiently from the blood, either for fuel or for conversion to fat. Under these conditions, levels of malonyl-CoA (the starting material for fatty acid synthesis) fall, inhibition of carnitine acyltransferase I is relieved, and fatty acids enter mitochondria to be degraded to acetyl-CoA—which cannot pass through the citric acid cycle because cycle intermediates have been drawn off for use as substrates in gluconeogenesis. The resulting accumulation of acetyl-CoA accelerates the formation of ketone bodies beyond the capacity of extrahepatic tissues to oxidize them. The increased blood levels of acetoacetate and D-β-hydroxybutyrate lower the blood pH, causing the condition known as **acidosis.** Extreme acidosis can lead to coma and in some cases death. Ketone bodies in the blood and urine of untreated diabetics can reach extraordinary levels—a blood concentration of 90 mg/100 mL (compared with a normal level of $<$3 mg/100 mL) and urinary excretion of 5,000 mg/24 hr (compared with a normal rate of $\leq$125 mg/24 hr). This condition is called **ketosis.**

Individuals on very low-calorie diets, using the fats stored in adipose tissue as their major energy source, also have increased levels of ketone bodies in their blood and urine. These levels must be monitored to avoid the dangers of acidosis and ketosis (ketoacidosis). ■

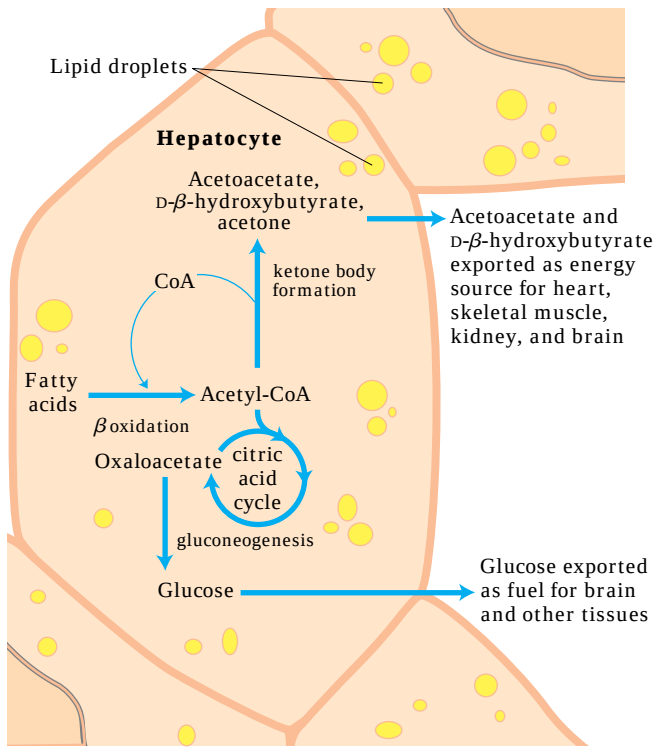


FIGURE 17–20 Ketone body formation and export from the liver. Conditions that promote gluconeogenesis (untreated diabetes, severely reduced food intake) slow the citric acid cycle (by drawing off oxaloacetate) and enhance the conversion of acetyl-CoA to acetoacetate. The released coenzyme A allows continued β oxidation of fatty acids.

SUMMARY 17.3 Ketone Bodies

- The ketone bodies—acetone, acetoacetate, and D-β-hydroxybutyrate—are formed in the liver. The latter two compounds serve as fuel molecules in extrahepatic tissues, through oxidation to acetyl-CoA and entry into the citric acid cycle.
- Overproduction of ketone bodies in uncontrolled diabetes or severely reduced calorie intake can lead to acidosis or ketosis.

Key Terms

Terms in bold are defined in the glossary.

β oxidation XXX
chylomicron XXX
apolipoprotein XXX
lipoprotein XXX
perilipins XXX
hormone-sensitive lipase XXX
free fatty acids XXX
serum albumin XXX
carnitine shuttle XXX
carnitine acyltransferase I XXX
acyl-carnitine/carnitine transporter XXX
carnitine acyltransferase II XXX
trifunctional protein (TFP) XXX
methylmalonyl-CoA mutase XXX
coenzyme B_{12} XXX
pernicious anemia XXX
intrinsic factor XXX
malonyl-CoA XXX
medium-chain acyl-CoA dehydrogenase (MCAD) XXX
multifunctional protein (MFP) XXX
ω oxidation XXX
mixed-function oxidases XXX
α oxidation XXX
acidosis XXX
ketosis XXX

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Problems

1. Energy in Triacylglycerols On a per-carbon basis, where does the largest amount of biologically available energy in triacylglycerols reside: in the fatty acid portions or the glycerol portion? Indicate how knowledge of the chemical structure of triacylglycerols provides the answer.

2. Fuel Reserves in Adipose Tissue Triacylglycerols, with their hydrocarbon-like fatty acids, have the highest energy content of the major nutrients.

(a) If 15% of the body mass of a 70.0 kg adult consists of triacylglycerols, what is the total available fuel reserve, in

both kilojoules and kilocalories, in the form of triacylglycerols? Recall that 1.00 kcal = 4.18 kJ.

(b) If the basal energy requirement is approximately 8,400 kJ/day (2,000 kcal/day), how long could this person survive if the oxidation of fatty acids stored as triacylglycerols were the only source of energy?

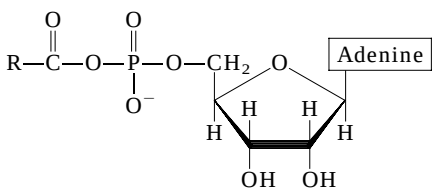
(c) What would be the weight loss in pounds per day under such starvation conditions (1 lb = 0.454 kg)?

3. Common Reaction Steps in the Fatty Acid Oxidation Cycle and Citric Acid Cycle Cells often use the same enzyme reaction pattern for analogous metabolic conversions. For example, the steps in the oxidation of pyruvate to acetyl-CoA and of α-ketoglutarate to succinyl-CoA, although catalyzed by different enzymes, are very similar. The first stage of fatty acid oxidation follows a reaction sequence closely resembling a sequence in the citric acid cycle. Use equations to show the analogous reaction sequences in the two pathways.

4. Chemistry of the Acyl-CoA Synthetase Reaction Fatty acids are converted to their coenzyme A esters in a reversible reaction catalyzed by acyl-CoA synthetase:

$$R{-}COO^- + ATP + CoA \rightleftharpoons R{-}\overset{\displaystyle O}{\overset{\|}{C}}{-}CoA + AMP + PP_i$$

(a) The enzyme-bound intermediate in this reaction has been identified as the mixed anhydride of the fatty acid and adenosine monophosphate (AMP), acyl-AMP:



Write two equations corresponding to the two steps of the reaction catalyzed by acyl-CoA synthetase.

(b) The acyl-CoA synthetase reaction is readily reversible, with an equilibrium constant near 1. How can this reaction be made to favor formation of fatty acyl–CoA?

5. Oxidation of Tritiated Palmitate Palmitate uniformly labeled with tritium (^3H) to a specific activity of 2.48×10^8 counts per minute (cpm) per micromole of palmitate is added to a mitochondrial preparation that oxidizes it to acetyl-CoA. The acetyl-CoA is isolated and hydrolyzed to acetate. The specific activity of the isolated acetate is 1.00×10^7 cpm/μmol. Is this result consistent with the β-oxidation pathway? Explain. What is the final fate of the removed tritium?

6. Compartmentation in β Oxidation Free palmitate is activated to its coenzyme A derivative (palmitoyl-CoA) in the cytosol before it can be oxidized in the mitochondrion. If palmitate and [^{14}C]coenzyme A are added to a liver homogenate, palmitoyl-CoA isolated from the cytosolic fraction is radioactive, but that isolated from the mitochondrial fraction is not. Explain.

7. Comparative Biochemistry: Energy-Generating Pathways in Birds One indication of the relative importance of various ATP-producing pathways is the V_{max} of certain enzymes of these pathways. The values of V_{max} of several enzymes from the pectoral muscles (chest muscles used for flying) of pigeon and pheasant are listed below.

	V_{max} (μmol substrate/min/g tissue)	
Enzyme	*Pigeon*	*Pheasant*
Hexokinase	3.0	2.3
Glycogen phosphorylase	18.0	120.0
Phosphofructokinase-1	24.0	143.0
Citrate synthase	100.0	15.0
Triacylglycerol lipase	0.07	0.01

(a) Discuss the relative importance of glycogen metabolism and fat metabolism in generating ATP in the pectoral muscles of these birds.

(b) Compare oxygen consumption in the two birds.

(c) Judging from the data in the table, which bird is the long-distance flyer? Justify your answer.

(d) Why were these particular enzymes selected for comparison? Would the activities of triose phosphate isomerase and malate dehydrogenase be equally good bases for comparison? Explain.

8. Effect of Carnitine Deficiency An individual developed a condition characterized by progressive muscular weakness and aching muscle cramps. The symptoms were aggravated by fasting, exercise, and a high-fat diet. The homogenate of a skeletal muscle specimen from the patient oxidized added oleate more slowly than did control homogenates, consisting of muscle specimens from healthy individuals. When carnitine was added to the patient's muscle homogenate, the rate of oleate oxidation equaled that in the control homogenates. The patient was diagnosed as having a carnitine deficiency.

(a) Why did added carnitine increase the rate of oleate oxidation in the patient's muscle homogenate?

(b) Why were the patient's symptoms aggravated by fasting, exercise, and a high-fat diet?

(c) Suggest two possible reasons for the deficiency of muscle carnitine in this individual.

9. Fatty Acids as a Source of Water Contrary to legend, camels do not store water in their humps, which actually consist of large fat deposits. How can these fat deposits serve as a source of water? Calculate the amount of water (in liters) that a camel can produce from 1.0 kg of fat. Assume for simplicity that the fat consists entirely of tripalmitoylglycerol.

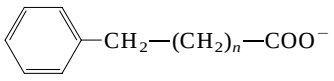
10. Petroleum as a Microbial Food Source Some microorganisms of the genera *Nocardia* and *Pseudomonas* can grow in an environment where hydrocarbons are the only food source. These bacteria oxidize straight-chain aliphatic hydrocarbons, such as octane, to their corresponding carboxylic acids:

$$CH_3(CH_2)_6CH_3 + NAD^+ + O_2 \rightleftharpoons CH_3(CH_2)_6COOH + NADH + H^+$$

How could these bacteria be used to clean up oil spills? What

would be some of the limiting factors to the efficiency of this process?

11. Metabolism of a Straight-Chain Phenylated Fatty Acid A crystalline metabolite was isolated from the urine of a rabbit that had been fed a straight-chain fatty acid containing a terminal phenyl group:



A 302 mg sample of the metabolite in aqueous solution was completely neutralized by 22.2 mL of 0.100 M NaOH.

(a) What is the probable molecular weight and structure of the metabolite?

(b) Did the straight-chain fatty acid contain an even or an odd number of methylene (—CH_2—) groups (i.e., is n even or odd)? Explain.

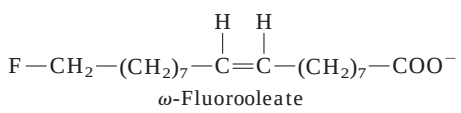
12. Fatty Acid Oxidation in Uncontrolled Diabetes When the acetyl-CoA produced during β oxidation in the liver exceeds the capacity of the citric acid cycle, the excess acetyl-CoA forms ketone bodies—acetone, acetoacetate, and D-β-hydroxybutyrate. This occurs in severe, uncontrolled diabetes: because the tissues cannot use glucose, they oxidize large amounts of fatty acids instead. Although acetyl-CoA is not toxic, the mitochondrion must divert the acetyl-CoA to ketone bodies. What problem would arise if acetyl-CoA were not converted to ketone bodies? How does the diversion to ketone bodies solve the problem?

13. Consequences of a High-Fat Diet with No Carbohydrates Suppose you had to subsist on a diet of whale blubber and seal blubber, with little or no carbohydrate.

(a) What would be the effect of carbohydrate deprivation on the utilization of fats for energy?

(b) If your diet were totally devoid of carbohydrate, would it be better to consume odd- or even-numbered fatty acids? Explain.

14. Metabolic Consequences of Ingesting ω-Fluorooleate The shrub *Dichapetalum toxicarium*, native to Sierra Leone, produces ω-fluorooleate, which is highly toxic to warm-blooded animals.



ω-Fluorooleate

This substance has been used as an arrow poison, and powdered fruit from the plant is sometimes used as a rat poison (hence the plant's common name, ratsbane). Why is this substance so toxic? (Hint: Review Chapter 16, Problem 13.)

15. Role of FAD as Electron Acceptor Acyl-CoA dehydrogenase uses enzyme-bound FAD as a prosthetic group to dehydrogenate the α and β carbons of fatty acyl–CoA. What is the advantage of using FAD as an electron acceptor rather than NAD^+? Explain in terms of the standard reduction potentials for the Enz-FAD/$FADH_2$ ($E'^\circ = -0.219$ V) and NAD^+/NADH ($E'^\circ = -0.320$ V) half-reactions.

16. β Oxidation of Arachidic Acid How many turns of the fatty acid oxidation cycle are required for complete oxidation of arachidic acid (see Table 10–1) to acetyl-CoA?

17. Fate of Labeled Propionate If [3-^{14}C]propionate (^{14}C in the methyl group) is added to a liver homogenate, ^{14}C-labeled oxaloacetate is rapidly produced. Draw a flow chart for the pathway by which propionate is transformed to oxaloacetate, and indicate the location of the ^{14}C in oxaloacetate.

18. Sources of H_2O Produced in β Oxidation The complete oxidation of palmitoyl-CoA to carbon dioxide and water is represented by the overall equation

$$\text{Palmitoyl-CoA} + 23O_2 + 108P_i + 108\text{ADP} \longrightarrow \text{CoA} + 16CO_2 + 108\text{ATP} + 23H_2O$$

Water is also produced in the reaction

$$\text{ADP} + P_i \longrightarrow \text{ATP} + H_2O$$

but is not included as a product in the overall equation. Why?

19. Biological Importance of Cobalt In cattle, deer, sheep, and other ruminant animals, large amounts of propionate are produced in the rumen through the bacterial fermentation of ingested plant matter. Propionate is the principal source of glucose for these animals, via the route propionate $\rightarrow$ oxaloacetate $\rightarrow$ glucose. In some areas of the world, notably Australia, ruminant animals sometimes show symptoms of anemia with concomitant loss of appetite and retarded growth, resulting from an inability to transform propionate to oxaloacetate. This condition is due to a cobalt deficiency caused by very low cobalt levels in the soil and thus in plant matter. Explain.

20. Fat Loss during Hibernation Bears expend about 25×10^6 J/day during periods of hibernation, which may last as long as seven months. The energy required to sustain life is obtained from fatty acid oxidation. How much weight loss (in kilograms) has occurred after seven months? How might ketosis be minimized during hibernation? (Assume the oxidation of fat yields 38 kJ/g.)