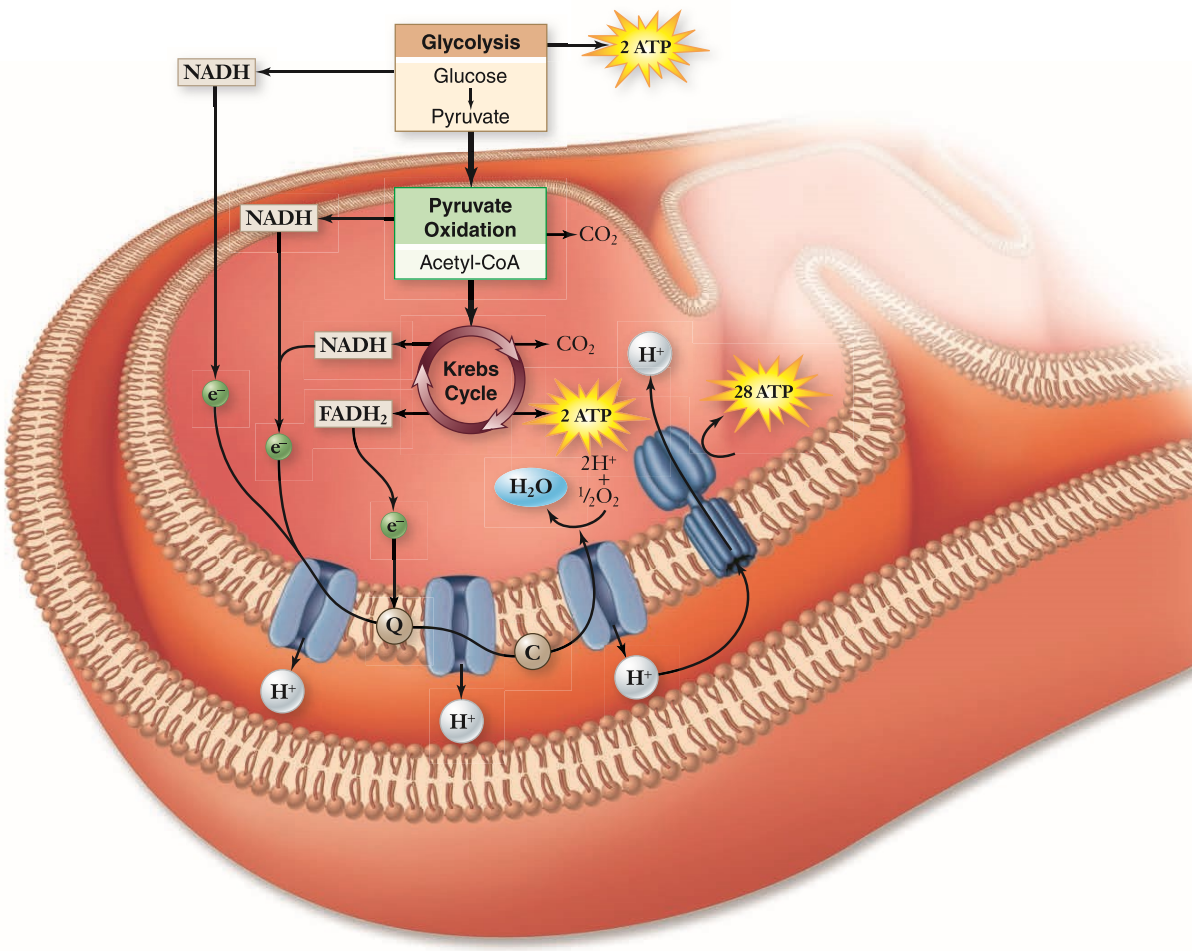

Figure 7.14 Aerobic respiration in the mitochondria. The entire process of aerobic respiration is shown in cellular context. Glycolysis occurs in the cytoplasm with the pyruvate and NADH produced entering the mitochondria. Here, pyruvate is oxidized and fed into the Krebs cycle to complete the oxidation process. All the energetic electrons harvested by oxidations in the overall process are transferred by NADH and $FADH_2$ to the electron transport chain. The electron transport chain uses the energy released during electron transport to pump protons across the inner membrane. This creates an electrochemical gradient that contains potential energy. The enzyme ATP synthase uses this gradient to phosphorylate ADP to form ATP.

ATP synthase is a molecular rotary motor

ATP synthase uses a fascinating molecular mechanism to perform ATP synthesis (figure 7.15). Structurally, the enzyme has a membrane-bound portion and a narrow stalk that connects the membrane portion to a knoblike catalytic portion. This complex can be dissociated into two subportions: the F_0 membrane-bound complex, and the F_1 complex composed of the stalk and a knob, or head domain.

The F_1 complex has enzymatic activity. The F_0 complex contains a channel through which protons move across the membrane down their concentration gradient. As they do so, their movement causes part of the F_0 complex and the stalk to rotate relative to the knob. The mechanical energy of this rotation is used to change the conformation of the catalytic domain in the F_1 complex.

Thus, the synthesis of ATP is achieved by a tiny rotary motor, the rotation of which is driven directly by a gradient of protons. The flow of protons is like that of water in a hydroelectric power plant. Like the flow of water driven by gravity causes a turbine to rotate and generate electrical current, the proton gradient produces the energy that drives the rotation of the ATP synthase generator.

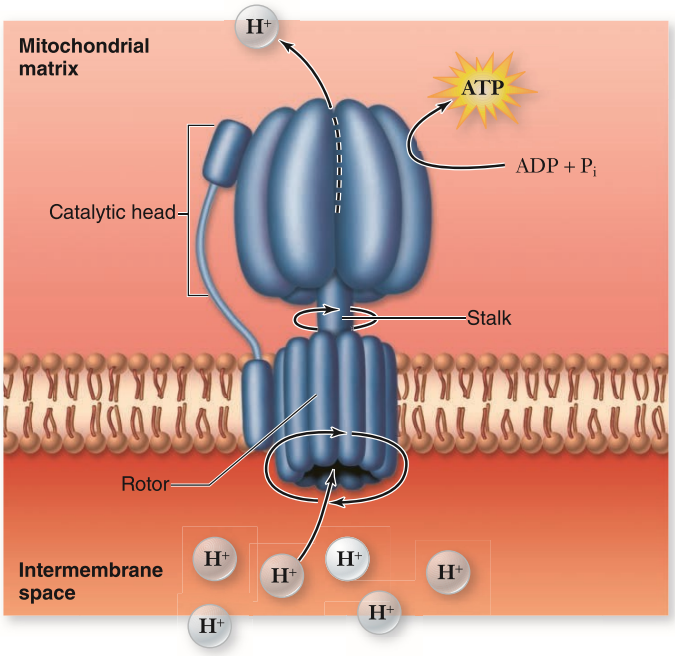

Figure 7.15 The ATP rotary engine. Protons move across the membrane down their concentration gradient. The energy released causes the rotor and stalk structures to rotate. This mechanical energy alters the conformation of the ATP synthase enzyme to catalyze the formation of ATP.

Learning Outcomes Review 7.5

The electron transport chain receives electrons from NADH and $FADH_2$ and passes them down the chain to oxygen. The protein complexes of the electron transport chain, in the inner membrane of mitochondria, use the energy from electron transfer to pump protons across the membrane, creating an electrochemical gradient. The enzyme ATP synthase uses this gradient to drive the endergonic reaction of phosphorylating ADP to ATP.

- *How would poking a small hole in the outer membrane affect ATP synthesis?*

7.6 Energy Yield of Aerobic Respiration

Learning Outcome

1. *Calculate the number of ATP molecules produced by aerobic respiration.*

How much metabolic energy (in the form of ATP) does a cell gain from aerobic breakdown of glucose? This simple question has actually been a source of some controversy in biochemistry.

The theoretical yield for eukaryotes is 30 molecules of ATP per glucose molecule

The number of molecules of ATP produced by ATP synthase per molecules of glucose depends on the number of protons transported across the inner membrane, and the number of protons needed per ATP synthesized. The number of protons transported per NADH and $FADH_2$ is 10 and 6 H^+, respectively. Each ATP synthesized requires 4 H^+, leading to 10/4 = 2.5 ATP/NADH, and 6/4 = 1.5 ATP/$FADH_2$.

To finish the bookkeeping: oxidizing glucose to pyruvate via glycolysis yields 2 ATP directly, and 2 × 2.5 = 5 ATP from NADH. The oxidation of pyruvate to acetyl-CoA yields another 2 × 2.5 = 5 ATP from NADH. Lastly, the Krebs cycle produces 2 ATP directly, 6 × 2.5 = 15 ATP from NADH, and 2 × 1.5 = 3 ATP from $FADH_2$. Summing all of these leads to 32 ATP for respiration (figure 7.16).

This number is accurate for bacteria, but it does not hold for eukaryotes because the NADH produced in the cytoplasm by glycolysis needs to be transported into the mitochondria by active transport, which costs one ATP per NADH transported. This reduces the predicted yield for eukaryotes to 30 ATP.

Calculation of P/O ratios has changed over time

The value for the amount of ATP synthesized per O_2 molecule reduced is called the phosphate-to-oxygen ratio (P/O ratio). Both theoretical calculations, and direct measurement of this value, have been contentious issues. When theoretical calculations were first made, we lacked detailed knowledge of the respiratory chain, and the mechanism for coupling electron transport to ATP synthesis. Since redox reactions occur at three sites for NADH and two sites for $FADH_2$, it was assumed that three molecules of ATP were produced per NADH and two per $FADH_2$. We now know that assumption was overly simplistic.

Understanding that a proton gradient is the link between electron transport and ATP synthesis changed the nature of the calculations. We need to know the number of protons pumped during electron transport: 10 H^+ per NADH, and 6 H^+ per $FADH_2$. Then we need to know the number of protons needed per ATP. Since ATP synthase is a rotary motor, this calculation depends on the number of binding sites for ATP, and the number of protons required for rotation. We know that ATP synthase has three binding sites for ATP. If 12 protons are used per rotation, you get the value of 4 H^+ per ATP

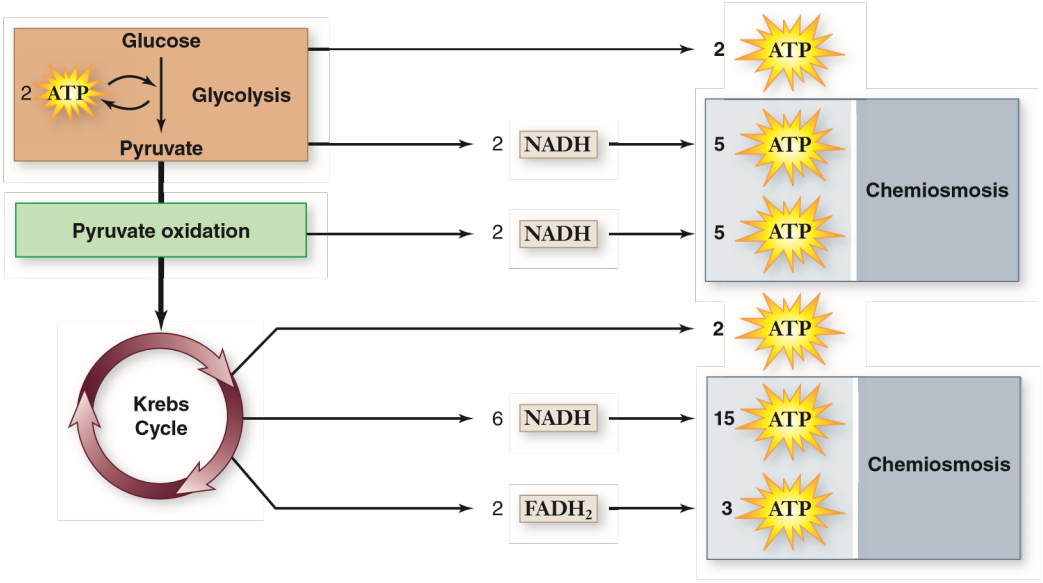

Figure 7.16 Theoretical ATP yield. The theoretical yield of ATP harvested from glucose by aerobic respiration totals 32 molecules. In eukaryotes this is reduced to 30 because it takes 1 ATP to transport each molecule of NADH that is generated by glycolysis in the cytoplasm into the mitochondria.

used in the previous calculation. Actual measurements of the P/O ratio have been problematic, but now appear to be at most 2.5.

We can also calculate how efficiently respiration captures the free energy released by the oxidation of glucose in the form of ATP. The amount of free energy released by the oxidation of glucose is 686 kcal/mol, and the free energy stored in each ATP is 7.3 kcal/mol. Therefore, a eukaryotic cell harvests about $(7.3 \times 30)/686 = 32\%$ of the energy available in glucose. (By comparison, a typical car converts only about 25% of the energy in gasoline into useful energy.)

The higher energy yield of aerobic respiration was one of the key factors that fostered the evolution of heterotrophs. As this mechanism for producing ATP evolved, nonphotosynthetic organisms became more effective at using respiration to extract energy from molecules derived from other organisms. As long as some organisms captured energy by photosynthesis, others could exist solely by feeding on them.

Learning Outcome Review 7.6

Passage of electrons down the electron transport chain produces roughly 2.5 molecules of ATP per molecule of NADH (1.5 ATP per $FADH_2$). This process plus the ATP from substrate-level phosphorylation can yield a maximum of 32 ATP for the complete oxidation of glucose. NADH generated in the cytoplasm of eukaryotes yields only two ATP/NADH due to the cost of transport into the mitochondria, lowering the yield to 30 ATP.

- *How does chemiosmosis allow for noninteger numbers of ATP/NADH?*

7.7 Regulation of Aerobic Respiration

Learning Outcome

1. *Understand the control points for cellular respiration.*

When cells possess plentiful amounts of ATP, the key reactions of glycolysis, the Krebs cycle, and fatty acid breakdown are inhibited, slowing ATP production. The regulation of these biochemical pathways by the level of ATP is an example of feedback inhibition. Conversely, when ATP levels in the cell are low, ADP levels are high, and ADP activates enzymes in the pathways of carbohydrate catabolism to stimulate the production of more ATP.

Control of glucose catabolism occurs at two key points in the catabolic pathway, namely at a point in glycolysis and at the beginning of the Krebs cycle (figure 7.17). The control point in glycolysis is the enzyme phosphofructokinase, which catalyzes the conversion of fructose phosphate to fructose bisphosphate. This is the first reaction of glycolysis that is not readily reversible, committing the substrate to the glycolytic sequence. ATP itself is an allosteric inhibitor (see chapter 6) of phosphofructokinase, as is the Krebs cycle intermediate citrate. High levels of both ATP and citrate inhibit phosphofructokinase. Thus, under conditions when

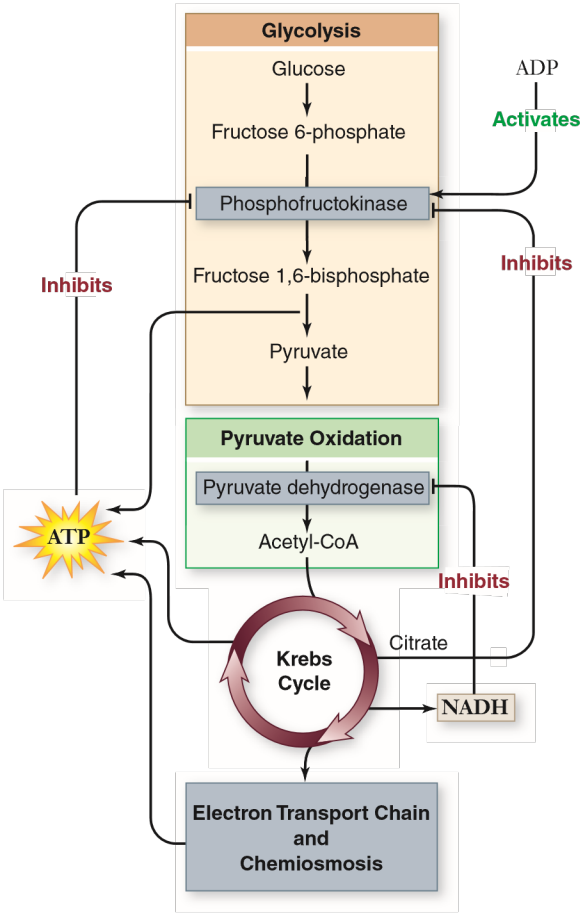

Figure 7.17 Control of glucose catabolism. The relative levels of ADP and ATP and key intermediates NADH and citrate control the catabolic pathway at two key points: the committing reactions of glycolysis and the Krebs cycle.

ATP is in excess, or when the Krebs cycle is producing citrate faster than it is being consumed, glycolysis is slowed.

The main control point in the oxidation of pyruvate occurs at the committing step in the Krebs cycle with the enzyme pyruvate dehydrogenase, which converts pyruvate to acetyl-CoA. This enzyme is inhibited by high levels of NADH, a key product of the Krebs cycle.

Another control point in the Krebs cycle is the enzyme citrate synthetase, which catalyzes the first reaction, the conversion of oxaloacetate and acetyl-CoA into citrate. High levels of ATP inhibit citrate synthetase (as well as phosphofructo-kinase, pyruvate dehydrogenase, and two other Krebs cycle enzymes), slowing down the entire catabolic pathway.

Learning Outcome Review 7.7

Respiration is controlled by levels of ATP in the cell and levels of key intermediates in the process. The control point for glycolysis is the enzyme phosphofructokinase, which is inhibited by ATP or citrate (or both). The main control point in oxidation of pyruvate is the enzyme pyruvate dehydrogenase, inhibited by NADH.

- *How does feedback inhibition ensure economic production of ATP?*

7.8 Oxidation Without O_2

Learning Outcomes

1. *Compare anaerobic and aerobic respiration.*
2. *Distinguish between fermentation and anaerobic respiration.*

In the presence of oxygen, cells can use oxygen to produce a large amount of ATP. But even when no oxygen is present to accept electrons, some organisms can still respire *anaerobically,* using inorganic molecules as final electron acceptors for an electron transport chain.

For example, many prokaryotes use sulfur, nitrate, carbon dioxide, or even inorganic metals as the final electron acceptor in place of oxygen (figure 7.18). The free energy released by using these other molecules as final electron acceptors is not as great as that using oxygen because they have a lower affinity for electrons. The amount of ATP produced is less, but the process is still respiration and not fermentation.

Methanogens use carbon dioxide

Among the heterotrophs that practice anaerobic respiration are Archaea such as thermophiles and methanogens. Methanogens use carbon dioxide (CO_2) as the electron acceptor, reducing CO_2 to CH_4 (methane). The hydrogens are derived from organic molecules produced by other organisms. Methanogens are found in diverse environments, including soil and the digestive systems of ruminants like cows.

Sulfur bacteria use sulfate

Evidence of a second anaerobic respiratory process among primitive bacteria is seen in a group of rocks about 2.7 BYA, known as the Woman River iron formation. Organic material in these rocks is enriched for the light isotope of sulfur, ^{32}S, relative to the heavier isotope, ^{34}S. No known geochemical process produces such enrichment, but biological sulfur reduction does, in a process still carried out today by certain prokaryotes.

In this sulfate respiration, the prokaryotes derive energy from the reduction of inorganic sulfates (SO_4) to hydrogen sulfide (H_2S). The hydrogen atoms are obtained from organic molecules other organisms produce. These prokaryotes thus are similar to methanogens, but they use SO_4 as the oxidizing (that is, electron-accepting) agent in place of CO_2.

The early sulfate reducers set the stage for the evolution of photosynthesis, creating an environment rich in H_2S. As discussed in chapter 8, the first form of photosynthesis obtained hydrogens from H_2S using the energy of sunlight.

Fermentation uses organic compounds as electron acceptors

In the absence of oxygen, cells that cannot utilize an alternative electron acceptor for respiration must rely exclusively on glycolysis to produce ATP. Under these conditions, the electrons generated

Figure 7.18 Sulfur-respiring prokaryote. ***a.*** The micrograph shows the archaeal species *Thermoproteus tenax*. This organism can use elemental sulfur as a final electron acceptor for anaerobic respiration. ***b.*** *Thermoproteus* is often found in sulfur-containing hot springs such as the Norris Geyser Basin in Yellowstone National Park, shown here.

by glycolysis are donated to organic molecules in a process called *fermentation*. This process recycles NAD^+, the electron acceptor that allows glycolysis to proceed.

Bacteria carry out more than a dozen kinds of fermentation reactions, often using pyruvate or a derivative of pyruvate to accept the electrons from NADH. Organic molecules other than pyruvate and its derivatives can be used as well; the important point is that the process regenerates NAD^+:

$$\text{organic molecule} + \text{NADH} \longrightarrow \text{reduced organic molecule} + \text{NAD}^+$$

Often the reduced organic compound is an organic acid—such as acetic acid, butyric acid, propionic acid, or lactic acid—or an alcohol.

Ethanol fermentation

Eukaryotic cells are capable of only a few types of fermentation. In one type, which occurs in yeast, the molecule that accepts electrons from NADH is derived from pyruvate, the end-product of glycolysis.

Yeast enzymes remove a terminal CO_2 group from pyruvate through decarboxylation, producing a 2-carbon molecule called acetaldehyde. The CO_2 released causes bread made with yeast to rise. The acetaldehyde accepts a pair of electrons from NADH, producing NAD^+ and ethanol (ethyl alcohol) (figure 7.19).

This particular type of fermentation is of great interest to humans, because it is the source of the ethanol in wine and beer. Ethanol is a by-product of fermentation that is actually toxic to yeast; as it approaches a concentration of about 12%, it begins to kill the yeast. That explains why naturally fermented wine contains only about 12% ethanol.

Lactic acid fermentation

Most animal cells regenerate NAD^+ without decarboxylation. Muscle cells, for example, use the enzyme lactate dehydrogenase to transfer electrons from NADH back to the pyruvate that is produced by glycolysis. This reaction converts pyruvate into lactic acid and regenerates NAD^+ from NADH (figure 7.19). It therefore closes the metabolic circle, allowing glycolysis to continue as long as glucoseis available.

Circulating blood removes excess lactate, the ionized form of lactic acid, from muscles, but when removal cannot keep pace with production, the accumulating lactic acid interferes with muscle function and contributes to muscle fatigue.

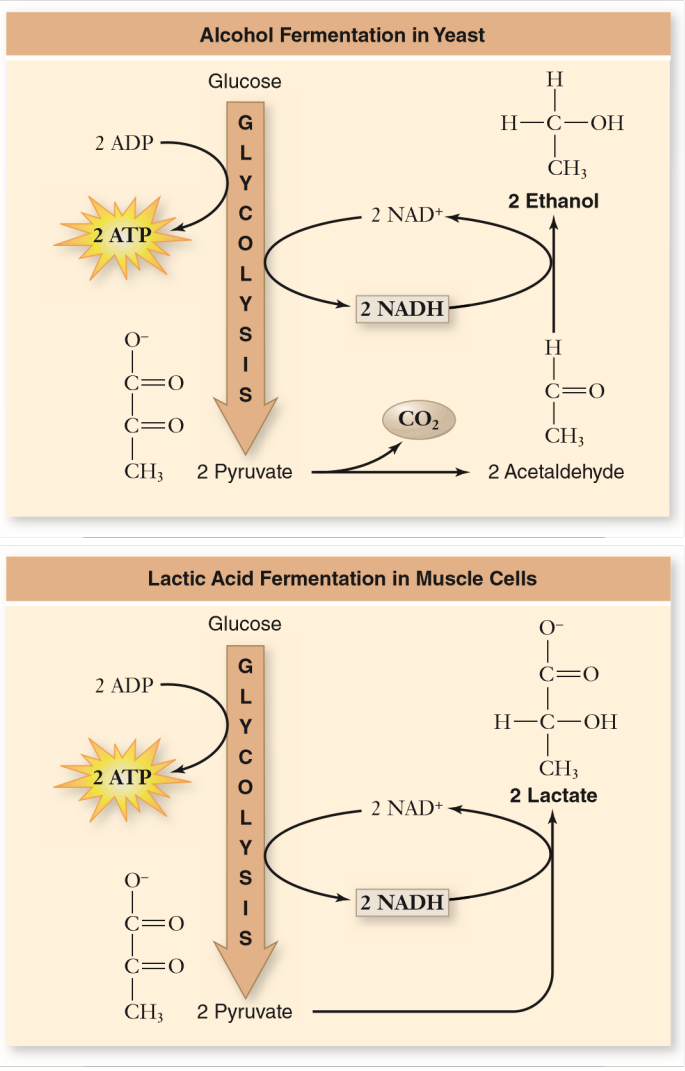

Figure 7.19 Fermentation. Yeasts carry out the conversion of pyruvate to ethanol. Muscle cells convert pyruvate into lactate, which is less toxic than ethanol. In each case, the reduction of a metabolite of glucose has oxidized NADH back to NAD^+ to allow glycolysis to continue under anaerobic conditions.

7.9 Catabolism of Proteins and Fats

Learning Outcomes

1. *Identify the entry points for proteins and fats in energy metabolism.*
2. *Recognize the importance of key intermediates in metabolism.*

Thus far we have focused on the aerobic respiration of glucose, which organisms obtain from the digestion of carbohydrates or from photosynthesis. Organic molecules other than glucose,

Learning Outcomes Review 7.8

Nitrate, sulfur, and CO_2 are all used as terminal electron acceptors in anaerobic respiration of different organisms.
Organic molecules can also accept electrons in fermentation reactions that regenerate NAD^+. Fermentation reactions produce a variety of compounds, including ethanol in yeast and lactic acid in humans.

- *In what kinds of ecosystems would you expect to find anaerobic respiration?*