Chapter 9

Cellular Respiration and Fermentation

Key Concepts

9.1 **Catabolic pathways yield energy by oxidizing organic fuels**

9.2 **Glycolysis harvests chemical energy by oxidizing glucose to pyruvate**

9.3 **After pyruvate is oxidized, the citric acid cycle completes the energy-yielding oxidation of organic molecules**

9.4 **During oxidative phosphorylation, chemiosmosis couples electron transport to ATP synthesis**

9.5 **Fermentation and anaerobic respiration enable cells to produce ATP without the use of oxygen**

9.6 **Glycolysis and the citric acid cycle connect to many other metabolic pathways**

Framework

The catabolic pathways of glycolysis and cellular respiration release the chemical energy in glucose and other fuels and store it in ATP. Glycolysis, occurring in the cytosol, produces ATP, pyruvate, and NADH; the latter two may then enter the mitochondria for respiration. A mitochondrion consists of a matrix in which the enzymes of the citric acid cycle are localized and a highly folded inner membrane in which electron transport chains are embedded. The redox reactions of the electron transport chain pump H^+ into the intermembrane space. ATP is produced by oxidative phosphorylation, using a chemiosmotic mechanism in which a proton-motive force drives protons through ATP synthases located in the membrane. Anaerobic respiration uses a final electron acceptor other than oxygen. Fermentation breaks down glucose to pyruvate, producing 2 ATP and recycling NADH to NAD^+.

Chapter Review

9.1 Catabolic pathways yield energy by oxidizing organic fuels

Catabolic Pathways and Production of ATP The breaking down of complex organic molecules in catabolic pathways releases energy that cells can use to do work. **Fermentation** occurs without oxygen and partially degrades sugars or other fuels to release energy. **Aerobic respiration** uses oxygen in the breakdown of glucose (or other energy-rich organic compounds) to yield carbon dioxide and water and release energy as ATP and heat. The *anaerobic respiration* of some prokaryotes does not use oxygen as a reactant but is a similar process. Aerobic respiration is often referred to as **cellular respiration.** This exergonic process has a free energy change of –686 kcal/mol of glucose.

INTERACTIVE QUESTION 9.1

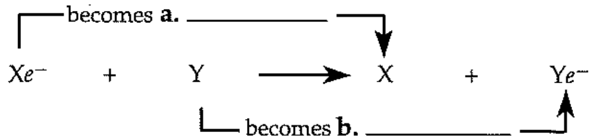
Fill in the following summary equation for cellular respiration, starting with the sugar glucose.

$$\_\_\_\_ + 6\,O_2 \rightarrow \_\_\_\_ + 6\,H_2O + \_\_\_\_$$

Redox Reactions: Oxidation and Reduction Oxidation–reduction reactions or **redox reactions** involve the partial or complete transfer of one or more electrons from one reactant to another. **Oxidation** is the loss of electrons from one substance; **reduction** is the addition of electrons to another substance. The substance that loses electrons becomes oxidized and acts as a **reducing agent** (electron donor). By gaining electrons, a substance acts as an **oxidizing agent** (electron acceptor) and becomes reduced.

INTERACTIVE QUESTION 9.2

Fill in the appropriate terms in the following equation.



Xe⁻ is the reducing agent; it **c.** ________ electrons.

Y is the **d.** ________; it **e.** ________ electrons.

Oxygen strongly attracts electrons and is one of the most powerful oxidizing agents. As electrons shift toward a more electronegative atom, they give up potential energy. Thus, chemical energy is released in a redox reaction that relocates electrons closer to oxygen.

Organic molecules with an abundance of hydrogen are rich in "hilltop" electrons, which release their potential energy when they "fall" closer to oxygen.

INTERACTIVE QUESTION 9.3

a. In the conversion of glucose and O_2 to CO_2 and H_2O, which molecule becomes reduced?

b. Which molecule becomes oxidized?

c. What happens to the energy that is released in this redox reaction?

At certain steps in the oxidation of glucose, two hydrogen atoms are removed by enzymes called dehydrogenases, and the two electrons and one proton are passed to a coenzyme, **NAD⁺** (nicotinamide adenine dinucleotide), reducing it to NADH.

Energy from respiration is slowly released in a series of redox reactions as electrons are passed from NADH down an **electron transport chain,** a group of carrier molecules located in the inner mitochondrial membrane (or in the plasma membrane of aerobic prokaryotes), to a stable location close to a highly electronegative oxygen atom, forming water.

INTERACTIVE QUESTION 9.4

a. NAD^+ is called a(n) ________________.

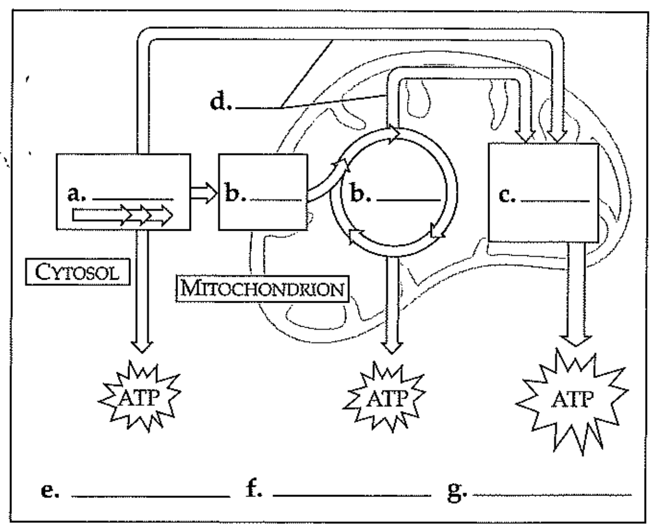
b. Its reduced form is ________.

The Stages of Cellular Respiration: A Preview
Glycolysis, which occurs in the cytosol, breaks glucose into two molecules of pyruvate. Within the mitochondrial matrix or in the cytosol of prokaryotes, pyruvate is oxidized to acetyl CoA. The **citric acid cycle** then oxidizes acetyl CoA to CO_2. In some steps, electrons are transferred to NAD^+. NADH passes electrons to the electron transport chain, at the bottom of which they combine with H^+ and oxygen to form water. The energy released in this chain of redox reactions is used to synthesize ATP by **oxidative phosphorylation,** a process that includes electron transport and chemiosmosis.

Up to 32 molecules of ATP may be generated for each glucose molecule oxidized to CO_2. About 10% of this ATP is produced by **substrate-level phosphorylation,** in which an enzyme transfers a phosphate group from a substrate molecule to ADP.

INTERACTIVE QUESTION 9.5

Fill in the three stages of cellular respiration (**a–c**). What do the arrows labeled **d.** represent? Indicate whether ATP is produced by substrate-level or oxidative phosphorylation (**e–g**).



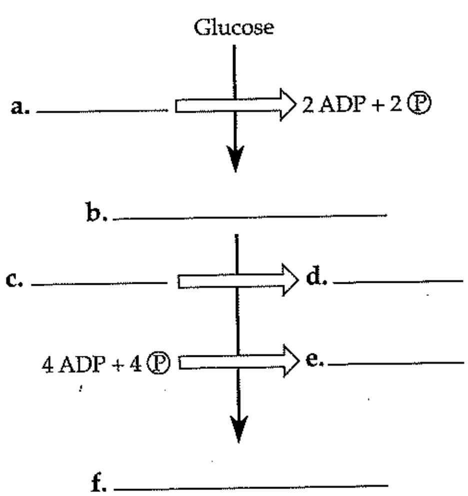
9.2 Glycolysis harvests chemical energy by oxidizing glucose to pyruvate

Glycolysis, a 10-step process occurring in the cytosol, has an energy-investment phase and an energy-payoff phase. Two molecules of ATP are consumed as glucose is split into two molecules of a three-carbon sugar (glyceraldehyde-3-phosphate). The oxidation of these molecules and the production of pyruvate yields 2 NADH and 4 ATP by substrate-level phosphorylation. For each molecule of glucose, glycolysis yields two molecules of pyruvate, 2 NADH, and a net gain of 2 ATP.

Enzymes catalyze each step in glycolysis. Kinases transfer phosphate groups; other enzymes cleave the six-carbon sugar and rearrange atoms in substrate molecules; and a dehydrogenase oxidizes glyceraldehyde-3-phosphate and reduces NAD^+.

INTERACTIVE QUESTION 9.6

Fill in the blanks in the following summary diagram of glycolysis.



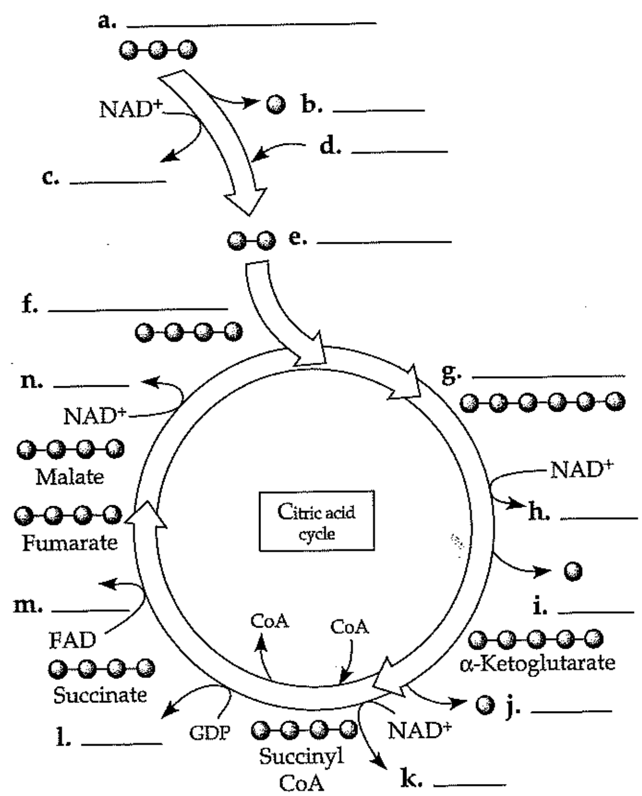
9.3 After pyruvate is oxidized, the citric acid cycle completes the energy-yielding oxidation of organic molecules

Oxidation of Pyruvate to Acetyl CoA The pyruvate produced in glycolysis is actively transported into the mitochondrion. In a series of steps within a multienzyme complex, a carboxyl group is removed from the three-carbon pyruvate and released as CO_2; the remaining two-carbon group is oxidized to form acetate, with the accompanying reduction of NAD^+ to NADH; and coenzyme A is attached by its sulfur atom to the acetate, forming **acetyl CoA.**

The Citric Acid Cycle In the citric acid cycle, the acetyl group of acetyl CoA is added to oxaloacetate to form citrate, which is progressively decomposed back to oxaloacetate. For each turn of the citric acid cycle, two carbons enter from acetyl CoA; two carbons exit completely oxidized as CO_2; three NADH and one $FADH_2$ are formed; and one ATP (GTP in most animal cells) is made by substrate-level phosphorylation. It takes two turns of the citric acid cycle to oxidize the two acetyl groups derived from a single glucose molecule.

INTERACTIVE QUESTION 9.7

Fill in the blanks in the following diagram of the citric acid cycle. Gray balls represent carbon atoms.



9.4 During oxidative phosphorylation, chemiosmosis couples electron transport to ATP synthesis

The first two stages of respiration have produced only 4 ATP molecules per glucose. What happens in the final stage to get the big ATP payout?

The Pathway of Electron Transport Thousands of electron transport chains are embedded in the cristae (infoldings) of the inner mitochondrial membrane (or in the plasma membrane of prokaryotes). The components of the electron transport chain are organized into four complexes, and most are proteins to which nonprotein *prosthetic groups* are tightly bound.

The electron carriers shift between reduced and oxidized states as they accept and donate electrons, with the free energy of electrons decreasing step by step. The transfer of electrons proceeds from NADH to a flavoprotein to an iron-sulfur protein (Complex I) to a mobile hydrophobic molecule called ubiquinone (CoQ or Coenzyme Q). Next, electrons are passed down a series of molecules called **cytochromes,** which are proteins with an iron-containing heme group. The last cytochrome, cyt a_3, passes two electrons to oxygen, which picks up two H^+, forming water.

$FADH_2$ adds its electrons to the chain at a lower energy level (within Complex II); thus, less energy is provided for ATP synthesis by $FADH_2$ as compared to NADH.

Chemiosmosis: The Energy-Coupling Mechanism **ATP synthase,** a protein complex embedded in the inner mitochondrial membrane or in the prokaryotic plasma membrane, uses the energy of a proton (H^+) gradient to make ATP, an example of the process called **chemiosmosis.**

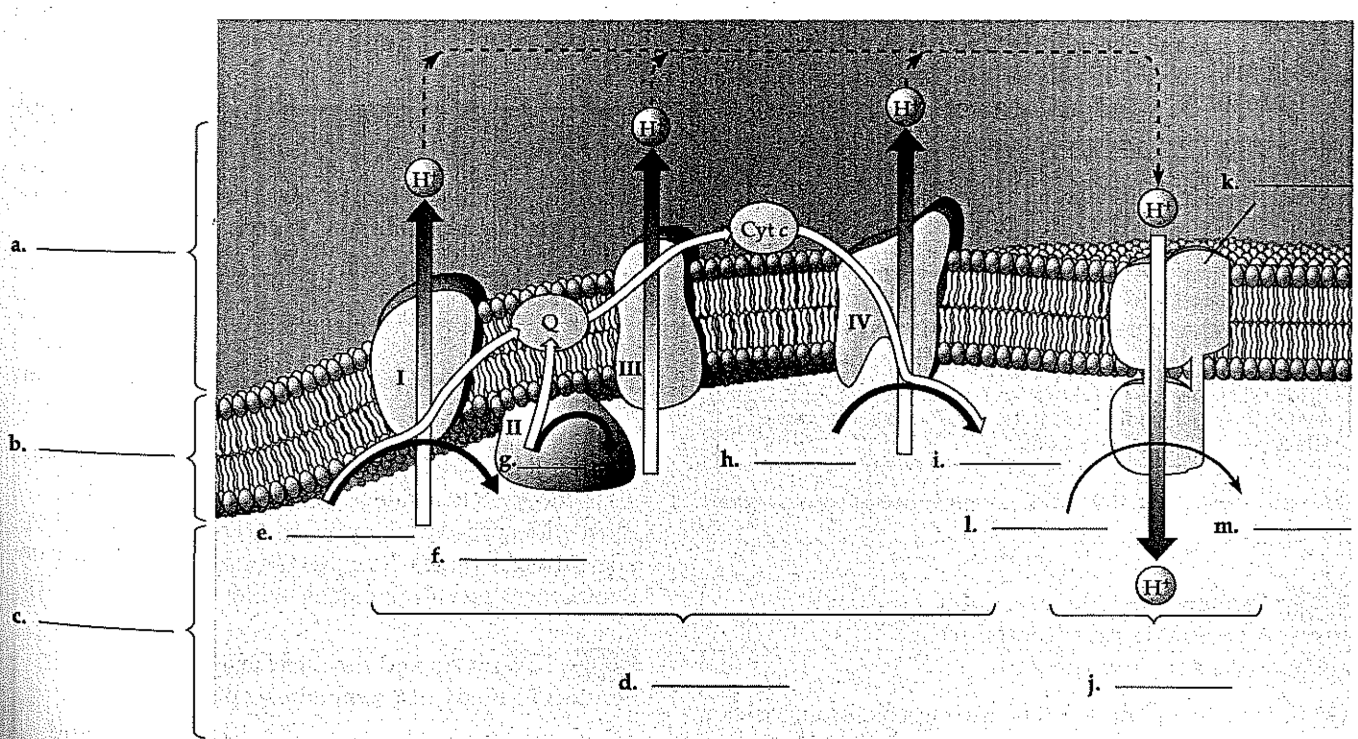
The flow of H^+ down their gradient through the stator and rotor part of the ATP synthase complex causes the rotor and attached rod to rotate, activating catalytic sites in the knob portion, where ADP and inorganic phosphate join to make ATP.

Where does the proton gradient that powers ATP synthase come from? When some members of the electron transport chain pass electrons, they also accept and release protons, which are deposited in the intermembrane space. The potential energy of the proton gradient is referred to as the **proton-motive force.**

In mitochondria, exergonic redox reactions produce the proton gradient that drives the production of ATP. Chloroplasts use light energy to create the proton-motive force used to make ATP. Prokaryotes use proton gradients generated across the plasma membrane to transport molecules, make ATP, and rotate flagella. These are all examples of chemiosmosis—the use of an H^+ gradient across a membrane to drive cellular work.

INTERACTIVE QUESTION 9.8

Label the following diagram of oxidative phosphorylation in a mitochondrial membrane.



An Accounting of ATP Production by Cellular Respiration About 30 to 32 ATPs may be produced per glucose molecule oxidized. These numbers are only estimates for three reasons: The ratio of NADH to ATP is not a whole number—experimental data indicate the production of 2.5 ATPs/NADH and of 1.5 ATPs/$FADH_2$; the electrons from NADH produced by glycolysis may be passed across the mitochondrial membrane to NAD^+ or FAD, depending on the type of shuttle used in the cell; and the proton-motive force generated by the electron transport chain is also used to power other work in the mitochondrion.

The efficiency of energy conversion in respiration is about 34%. The remaining 66% is lost as heat. In the brown fat cells of some mammals, an uncoupling protein allows protons to flow back across the inner mitochondrial membrane. Without the generation of ATP (which would inhibit cellular respiration), fats continue to be oxidized, and heat is generated during hibernation.

INTERACTIVE QUESTION 9.9

Fill in the following tally for the maximum ATP yield from the oxidation of one molecule of glucose to six molecules of carbon dioxide.

Process	# ATP
Initial phosphorylation of glucose:	a. ________
Substrate-level phosphorylation: in glycolysis	b. ________
In c. ________________	2
Oxidative phosphorylation:*	d. ________
Maximum Total	e. ________

*2.5 ATP for each of the f. ________ NADH from pyruvate → acetyl CoA and the g. ________ NADH from citric acid cycle; 1.5 ATP for each of the h. ________ $FADH_2$ from citric acid cycle; 2.5 or 1.5 ATP for each of the i. ________ NADH from glycolysis, depending on which shuttle passes electrons across the membrane.

9.5 Fermentation and anaerobic respiration enable cells to produce ATP without the use of oxygen

Organisms that generate ATP through anaerobic respiration have an electron transport chain that does not use oxygen as the final electron acceptor. Some bacteria use sulfate ions to accept electrons, generating H_2S instead of H_2O.

Is it possible for cells to generate ATP without using either oxygen or an electron transport chain? In fermentation, oxidation of glucose in glycolysis produces a net of 2 ATP by substrate-level phosphorylation, and NADH is recycled to NAD^+ by the transfer of electrons to pyruvate or derivatives of pyruvate.

Types of Fermentation In **alcohol fermentation,** pyruvate is converted to acetaldehyde, and CO_2 is released. Acetaldehyde is then reduced by NADH to form ethanol (ethyl alcohol), and NAD^+ is regenerated. In **lactic acid fermentation,** pyruvate is reduced directly by NADH to lactate, recycling NAD^+. Muscle cells make ATP by lactic acid fermentation when energy demand is high and O_2 supply is low.

Comparing Fermentation with Anaerobic and Aerobic Respiration Fermentation and aerobic and anaerobic respiration all use glycolysis, with NAD^+ as the oxidizing agent to convert glucose and other organic fuels to pyruvate. To oxidize NADH back to NAD^+, fermentation uses a molecule such as pyruvate or acetaldehyde as the final electron acceptor. Respiration uses oxygen or another electronegative molecule (in anaerobic respiration) as the final electron acceptor after electrons are passed down an electron transport chain.

Obligate anaerobes make ATP by fermentation or anaerobic respiration and are poisoned by oxygen. **Facultative anaerobes,** such as yeasts and many bacteria, can make ATP by fermentation or respiration, depending upon the availability of oxygen.

INTERACTIVE QUESTION 9.10

How much more ATP can be generated by aerobic respiration than by fermentation? Explain why.

The Evolutionary Significance of Glycolysis Glycolysis is common to fermentation and respiration. This most widespread of all processes probably evolved in ancient prokaryotes. Its cytosolic location and independence of oxygen also provide evidence of its antiquity.

9.6 Glycolysis and the citric acid cycle connect to many other metabolic pathways

The Versatility of Catabolism Fats, proteins, and carbohydrates can all be used in cellular respiration.

Proteins are digested into amino acids, which are then *deaminated* (the amino group is removed) and can enter glycolysis or the citric acid cycle at several points. The digestion of fats yields glycerol, which is fed into glycolysis, and fatty acids, which are broken down by **beta oxidation** to two-carbon fragments that enter the citric acid cycle as acetyl CoA.

Biosynthesis (Anabolic Pathways) The organic molecules of food also provide carbon skeletons for biosynthesis. Some monomers, such as amino acids, can be directly incorporated into the cell's macromolecules. Intermediate compounds of glycolysis and of the citric acid cycle serve as precursors for anabolic pathways. Carbohydrates, fats, and proteins can be interconverted to provide for a cell's needs.

Regulation of Cellular Respiration via Feedback Mechanisms Through feedback inhibition, the end product of an anabolic pathway inhibits an enzyme early in the pathway, thus preventing a cell from producing an excess of a particular substance.

The supply of ATP in the cell regulates respiration. The allosteric enzyme that catalyzes the third step of glycolysis, phosphofructokinase, is inhibited by ATP and activated by AMP (derived from ADP). Phosphofructokinase is also inhibited by citrate released from the mitochondria, thereby synchronizing the rates of glycolysis and the citric acid cycle. Other enzymes located at key intersections help to maintain metabolic balance.

Word Roots

aero- = air (*aerobic respiration:* a catabolic pathway for organic molecules using oxygen as the final electron acceptor in an electron transport chain and ultimately producing ATP)

an- = not (*facultative anaerobe:* an organism that makes ATP by aerobic respiration if oxygen is present but that switches to anaerobic respiration or fermentation if oxygen is not present)

chemi- = chemical (*chemiosmosis:* an energy-coupling mechanism that uses energy stored in the form of an H^+ gradient across a membrane to drive cellular work, such as the synthesis of ATP)

glyco- = sweet; **-lysis** = split (*glycolysis:* a series of reactions that splits glucose into pyruvate; glycolysis occurs in almost all living cells, serving as the starting point for fermentation or cellular respiration)

Structure Your Knowledge

1. This chapter describes how catabolic pathways release chemical energy and store it in ATP. One of the best ways to learn the three main components of cellular respiration is to teach them to someone. Find two study partners and have each person learn and explain the important concepts of glycolysis, pyruvate oxidation and the citric acid cycle, or oxidative phosphorylation. Use diagrams to illustrate the process you are explaining.
2. Create a concept map to organize your understanding of oxidative phosphorylation.
3. Fill in the following table to summarize glycolysis, the citric acid cycle, oxidative phosphorylation, and fermentation. Base the main inputs and outputs of these processes on one glucose molecule.

Process	Brief Description	Main Inputs	Main Outputs
Glycolysis			
Pyruvate to acetyl CoA and citric acid cycle			
Oxidative phosphorylation			
Fermentation			

Test Your Knowledge

MULTIPLE CHOICE: *Choose the one best answer.*

1. When electrons move closer to a more electronegative atom,
   a. energy is released.
   b. chemical energy is stored.
   c. a proton gradient is established.
   d. water is produced.

2. In the reaction $C_6H_{12}O_6 + 6\ O_2 \rightarrow 6\ CO_2 + 6\ H_2O$,
   a. glucose becomes reduced.
   b. oxygen becomes reduced.
   c. water is a reducing agent.
   d. oxygen is a reducing agent.

3. In which of the following conversions is the first molecule becoming reduced to the second molecule?
   a. pyruvate → acetyl CoA
   b. glucose → pyruvate
   c. $NADH + H^+ \rightarrow NAD^+ + 2\ H$
   d. pyruvate → lactate

4. Some prokaryotes use anaerobic respiration, a process that
   a. does not involve an electron transport chain.
   b. produces ATP solely by substrate-level phosphorylation.
   c. uses a substance other than oxygen as the final electron acceptor.
   d. Both **a** and **b** are correct.

5. Which of the following reactions is *incorrectly* paired with its location?
   a. ATP synthesis—inner membrane of the mitochondrion, mitochondrial matrix, and cytosol
   b. fermentation—cell cytosol
   c. glycolysis—cell cytosol
   d. citric acid cycle—cristae of mitochondrion

6. Which of the following enzymes uses NAD^+ as a coenzyme?
   a. phosphofructokinase
   b. phosphoglucoisomerase
   c. triose phosphate dehydrogenase
   d. hexokinase

7. Which of the following compounds produces the most ATP when completely oxidized to CO_2?
   a. acetyl CoA
   b. glucose
   c. pyruvate
   d. fructose-1,6-bisphosphate

8. When pyruvate is converted to acetyl CoA,
   a. CO_2 and ATP are released.
   b. a multienzyme complex removes CO_2, transfers electrons to NAD^+, and attaches a coenzyme.
   c. one turn of the citric acid cycle is completed.
   d. NAD^+ is regenerated so that glycolysis can continue to produce ATP by substrate-level phosphorylation.

9. Which of the following statements correctly describes the role of oxygen in cellular respiration?
   a. It is reduced in glycolysis as glucose is oxidized.
   b. It combines with H^+ diffusing through ATP synthase to produce H_2O.
   c. It is the final electron acceptor for the electron transport chain.
   d. It combines with the carbon removed during the citric acid cycle to form CO_2.

10. Which of the following statements is an accurate description of chemiosmosis?
    a. ATP production is linked to the proton gradient established by the electron transport chain.
    b. The difference in pH between the intermembrane space and the cytosol drives the formation of ATP.
    c. The flow of H^+ through ATP synthases rotates a rotor and rod, driving the hydrolysis of ADP.
    d. The production of water in the mitochondrial matrix by the reduction of oxygen leads to a net flow of water out of a mitochondrion.

11. When glucose is oxidized to CO_2 and water, approximately 66% of its energy is transformed to
    a. heat.
    b. ATP.
    c. a proton-motive force.
    d. potential energy.

12. A suspension of cells is ground up and then mixed with a chemical that dissolves fats. Which of the following stages of cellular respiration would be most disrupted by this chemical?
    a. glycolysis
    b. the citric acid cycle
    c. oxidative phosphorylation
    d. fermentation

13. Which of the following statements correctly describes a metabolic effect of cyanide, a poison that blocks the passage of electrons along the electron transport chain?
 a. The pH of the intermembrane space becomes much lower than normal.
 b. Alcohol would build up in the mitochondria.
 c. NADH supplies would be exhausted, and ATP synthesis would cease.
 d. No proton gradient would be produced, and ATP synthesis would cease.

14. Substrate-level phosphorylation
 a. involves the shifting of a phosphate group from ATP to a substrate.
 b. takes place only in the cytosol.
 c. accounts for 10% of the ATP formed by fermentation.
 d. is the energy source for facultative anaerobes under anaerobic conditions.

15. Fermentation produces less ATP than cellular respiration because
 a. NAD^+ is regenerated by alcohol or lactate production, without the electrons of NADH passing through the electron transport chain.
 b. pyruvate still contains most of the "hilltop" electrons that were present in glucose.
 c. its starting reactant is pyruvate and not glucose.
 d. Both **a** and **b** are correct.

16. Muscle cells in oxygen deprivation gain which of the following from the reduction of pyruvate?
 a. ATP
 b. ATP and NAD^+
 c. CO_2 and NAD^+
 d. ATP and CO_2

17. Glucose made from six radioactively labeled carbon atoms is fed to yeast cells in the absence of oxygen. How many molecules of radioactive alcohol (C_2H_5OH) are formed from each molecule of glucose?
 a. 0 c. 2
 b. 1 d. 3

18. Glycolysis is considered one of the first metabolic pathways to have evolved because it
 a. relies on fermentation, which is characteristic of archaea and bacteria.
 b. is found only in prokaryotes, whereas eukaryotes use mitochondria to produce ATP.
 c. produces ATP only by substrate-level phosphorylation and does not involve redox reactions.
 d. is nearly universal, is located in the cytosol, and does not involve O_2.

19. Which of the following substances produces the *most* ATP per gram?
 a. glucose, because it is the starting place for glycolysis
 b. glycogen or starch, because they are polymers of glucose
 c. fats, because they are highly reduced compounds
 d. proteins, because of the energy stored in their tertiary structure

20. Fats and proteins can be used as fuel in the cell because they
 a. can be converted to glucose by enzymes.
 b. can be converted to intermediates of glycolysis or the citric acid cycle.
 c. can pass through the mitochondrial membrane to enter the citric acid cycle.
 d. contain more energy than glucose.

21. Which of the following statements is *false* concerning the enzyme phosphofructokinase?
 a. It is an allosteric enzyme.
 b. It is inhibited by citrate.
 c. It is inhibited by AMP.
 d. It is an early enzyme in the glycolytic pathway.

22. Brown fat, which is found in newborn infants and hibernating mammals, has uncoupler proteins that, when activated, make the inner mitochondrial membrane leaky to H^+. What is the function of brown fat?
 a. It produces more ATP than does regular fat and is also found in the flight muscles of ducks and geese, providing more energy for long-distance migrations.
 b. It lowers the pH of the intermembrane space, which results in the production of more ATP per gram than is produced by the oxidation of glucose or regular fat tissue.
 c. Because it dissipates the proton gradient, it generates heat through cellular respiration without producing ATP, thereby raising the body temperature of hibernating mammals or newborn infants.
 d. Its main function is insulation in the endothermic animals in which is it common.