Chapter 9

Respiration

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SUMMARY

ECONOMIC BOTANY*: How to Make a Better Beer*
ECONOMIC BOTANY*: Control of Respiration after Fruit harvest*

KEY CONCEPTS

1. All living cells require energy to drive the reactions of life. Respiration releases energy trapped as chemical bond energy in organic food molecules. Some of this energy is transferred to adenosine diphosphate (ADP) and nicotinamide adenine dinucleotide phosphate (NAD^+) in the formation of adenosine triphosphate (ATP) and reduced nicotinamide adenine dinucleotide phosphate (NADH). These energy carriers may then be moved in the cell and coupled to energy-requiring reactions.

2. When a six-carbon-atom sugar molecule is respired in a cell, the sugar is broken down into pyruvate in a series of enzymatic reactions called glycolysis. A small amount of ATP is formed. In the absence of molecular oxygen, the pyruvate may be enzymatically broken down to form CO_2 and ethyl alcohol, a process called alcoholic fermentation.

3. In the presence of molecular oxygen, the pyruvate is broken down in aerobic respiration, an integrated series of enzymatic reactions called the tricarboxylic acid cycle and the electron transport chain. In the tricarboxylic acid cycle, CO_2 and water are formed and some energy is trapped in the energy carriers ATP and NADH. In the electron transport chain, NADH is oxidized and some of the energy released is used to synthesize more ATP.

4. Enzymes of glycolysis and alcoholic fermentation are located in the cytoplasm; enzymes involved in aerobic respiration are located in the mitochondrial matrix and the inner membrane of the mitochondria.

5. The chemiosmotic theory of ATP formation proposes that the proton gradient that develops across the mitochondrial inner membrane during the terminal oxidation reactions drives ATP synthesis from ADP and inorganic phosphate.

6. Some cells have developed alternate pathways of respiration. The pentose phosphate pathway transfers energy from glucose to $NADP^+$, forming NADPH, and produces ribose-5-phosphate, necessary for the synthesis of nucleic acids. The glyoxylate pathway converts fat into intermediate compounds and ultimately into sucrose.

7. Environmental factors such as cell hydration, temperature, oxygen supply, and food availability affect the rate of respiration of a plant, as do factors such as cell type and the age and species of the plant.

9.1 THE RELEASE OF ENERGY FROM FOOD

To stay alive, every living cell must obtain energy in a usable form, which it does by oxidizing food molecules. Without energy from the oxidation of food, cell membranes cannot maintain differential permeability or accumulate solutes, and cytoplasm will not circulate. Cells cannot synthesize nucleic acids, proteins, or fats without the energy and intermediate carbon compounds produced by respiration. This chapter examines respiration in detail to explore why it is so fundamental to life.

Early biologists applied the term respiration to the exchange of gases between an organism and its environment. Even today, many people consider respiration and breathing in animals as synonyms. Breathing is merely the visible indication that chemical reactions are taking place in animal cells. We define **respiration** as the oxidation of organic molecules within cells, accompanied by the release of usable energy.

Digestion Converts Complex Food into Simpler Molecules

Before large insoluble food molecules can be respired, they most be broken down into smaller soluble components. Cells cannot oxidize even a simple solute food molecule such as sucrose until it is broken into simpler sugars. Digestion, the breaking apart of complex foods (carbohydrates, fats, and proteins) into simpler compounds (sugars, fatty acids, glycerin, and amino acids), occurs easily in the presence of water and specific enzymes. Large amounts of energy are not released, because the transfer of electrons through oxidation-reduction reactions is not involved. Cells use water molecules in digestion; therefore, the process is a hydrolysis reaction.

Green plants differ from animals in that they normally do not ingest complex foods but synthesize them. Plants do move food molecules from cells where they are formed or stored to cells that need them. Digestion occurs in any cell that stores complex food molecules. During photosynthesis, starch often is stored as insoluble starch granules in the chloroplasts. When the time comes for this temporary starch reserve to be used by the cells or to be transported out of the cells, it must be changed back to soluble sugar. This chemical transformation of the insoluble starch into soluble sugar is one example of digestion (Fig. 9.1).

Respiration Is an Oxidation-Reduction Process

Once the large food molecules have been digested, respiration results in the transfer of energy from food molecules to energy-carrier molecules in the cell. Respiration is an oxidation-reduction process because it involves the removal of electrons from electron donor molecules to electron acceptor molecules; nevertheless, many steps do not involve the use of molecular oxygen. Indeed, many organisms, such as yeast and some bacteria, obtain their energy from food without any reactions that involve molecular oxygen. Respiration that does not involve molecular oxygen is called **anaerobic respiration**. When molecular oxygen plays a role, we call the process **aerobic respiration**. Many biochemists restrict the definition of "respiration" to designate the final series of reactions that occur in aerobic respiration. However, this chapter used the term respiration in a broader sense to include all of the reactions involved in the oxidation of food molecules.

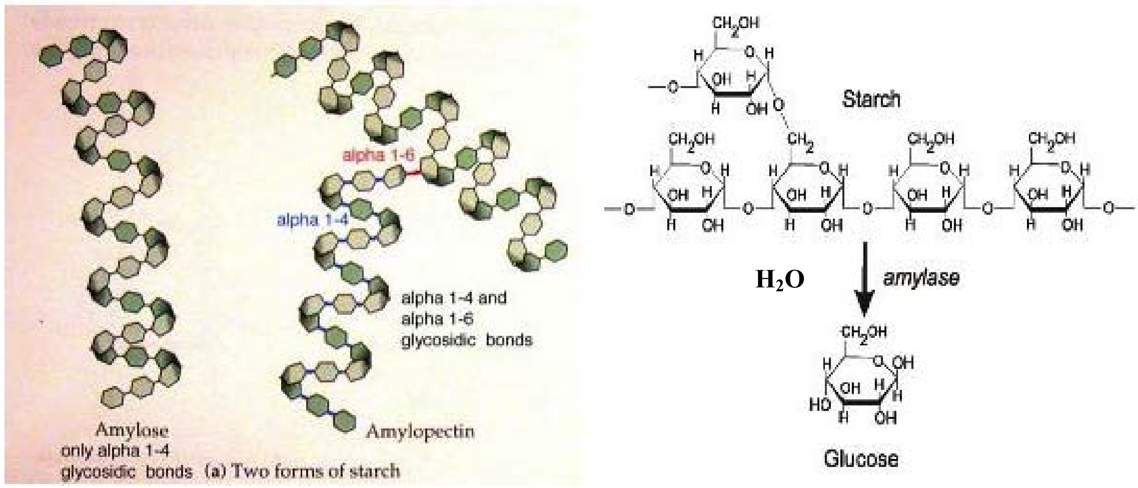


Figure 9.1. The transformation of starch to glucose, an example of digestion. (Left) Amylose and amylopectin, types of starch, are chains of sugar (glucose) units linked through oxygen atoms. (Right) Enzymatic digestion of starch by amylase enzymes involves the introduction of a water molecule between adjacent glucose units, breaking the oxygen linkages.

Respiration Is an Integrated Series of Reactions

Respiration is more than a simple oxidation reaction; it is a series of chemical reactions that supplies the energy for most cellular processes. Although the reactions of respiration might seem overwhelming if we listed them all, they fall into patterns. In particular, some consume energy as food molecules are prepared for breakdown, whereas others result in the transfer of energy to molecules that act as energy carriers in the cell. It is more important for you to have a grasp of the process of respiration as a whole and its roles in the life of a plant, than it is for you to worry about mastering each step in detail.

At the cellular level, some of the potential energy in sugars is trapped during respiration in the energy carriers ATP and NADH or NADPH, which may move throughout the cell and be used for synthesis and other energy-requiring processes (Fig. 9.2). Respiration also performs another important task in the life of the cell. During the many steps in the respiratory breakdown of food, essential intermediate compounds are formed. Living cells use many of the intermediate compounds as carbon-containing building blocks from which they synthesize other compounds such as proteins, fats, nucleic acids, and hormones. Respiration, through the oxidation of food, thus has two general functions: (1) it supplies energy in a form available to do work in the cells, and (2) it produces intermediate carbon compounds essential for the continued growth and metabolism of cells.

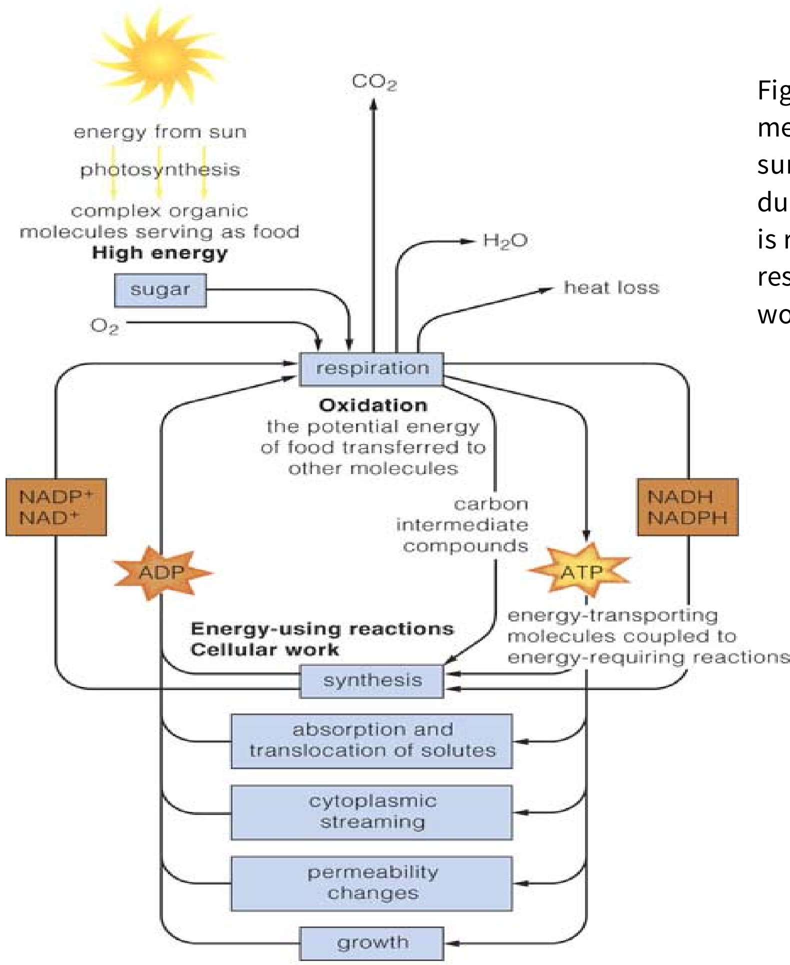


Figure 9.2. An overview of metabolism. Energy from sunlight is trapped in food during photosynthesis. Energy is released from food during respiration and is used to do work in the cell.

The Transfer of Energy Occurs through Coupled Reactions

To use the energy stored in food molecules, cells couple energy-yielding reactions with energy-consuming reactions. When 1 mole (1 gram molecular weight, 180 g) of glucose is burned in O_2, CO_2, H_2O, and 686 kcal of energy are released. This amount of energy released at once as heat would destroy living cells. Instead, energy transformations in the cells occur slowly and in small steps through a sequence of many reactions. Specific enzymes catalyze each of the reactions, allowing them to proceed at moderate rates at low temperatures. The oxidation of glucose is coupled to the formation of ATP, NADH, or NADPH, and about 40% of the energy released by the oxidation is trapped in the bond energies of these compounds. The ATP, NADH, and NADPH eventually yield energy that performs work in the cell through other coupled reactions.

Because the total amounts of ADP, NAD^+ and $NADP^+$ in a cell are quite small, a cell can never accumulate large amounts of ATP, NADH, or NADPH. Consequently, cellular energy is stored as carbohydrates, fats, and protein. A cell at rest and not using energy rapidly has converted most of its ADP to ATP. In this situation, the reactions of respiration will be slow. If such a cell is stimulated to do work---for example, to increase synthetic reactions, the rate of cytoplasmic streaming, or the rate of salt uptake--ATP is hydrolyzed to ADP and inorganic phosphate, and the ADP and phosphate become available to the respiratory pathways. Enzymes of respiration will be stimulated, and the rate of respiration will increase. This is one example of the many control mechanisms that regulate the rates of various metabolic reactions in the cell.

9.2 THE REACTIONS OF RESPIRATION

If you look at the complete oxidation of a simple six-carbon sugar in the presence of molecule oxygen, only CO_2, H_2O, and heat (energy) are the final products. This overall process is written as:

$$C_6H_{12}O_6 + 6\,O_2 \rightarrow 6\,CO_2 + 6\,H_2O + 686\text{ kcal}$$

sugar + oxygen → carbon dioxide + water + energy

The equation tells us nothing about the way the reaction occurs, the intermediate steps, or other possible products.

It took many years for scientists to discover the many steps involved in respiration. To study respiration, we separate it into phases, each of which includes reactions that produce key intermediate compounds. The first phase, **glycolysis**, breaks down a molecule of a six-carbon sugar (glucose), partially oxidizes the products, and forms two molecules of pyruvate (a three-carbon organic compound). No molecular oxygen takes part in the reactions, and the change in free energy is small. Most of the energy in the chemical bonds of the sugar being broken down still remains in the pyruvate formed; a small amount is used to form some ATP. The pyruvate is then further metabolized in either an aerobic or anaerobic pathway, depending on whether O_2 is available. In anaerobic respiration, the pyruvic acid is reduced. Alcohol and CO_2 are formed. In aerobic respiration, the pyruvate moves into the mitochondria. There, in the presence of molecular oxygen, it is oxidized to carbon dioxide and water. A large amount of energy is trapped in ATP and NADH.

Glycolysis Is the First Phase of Respiration

There are three major steps in the process of glycolysis (Fig. 9.3):

1. *Phosphorylation*: preparation of the six-carbon sugar for further reactions by the addition of phosphate from ATP.
2. *Sugar cleavage*: splitting of the sugar phosphate into two three-carbon-atom fragments.
3. *Pyruvate formation*: oxidation of the fragments to form pyruvic acid with the synthesis of NADH and ATP.

The six-carbon sugar, the most common sugar respired in the cell, is glucose. Although glucose is energy-rich, it is stable and does not react readily with oxygen at

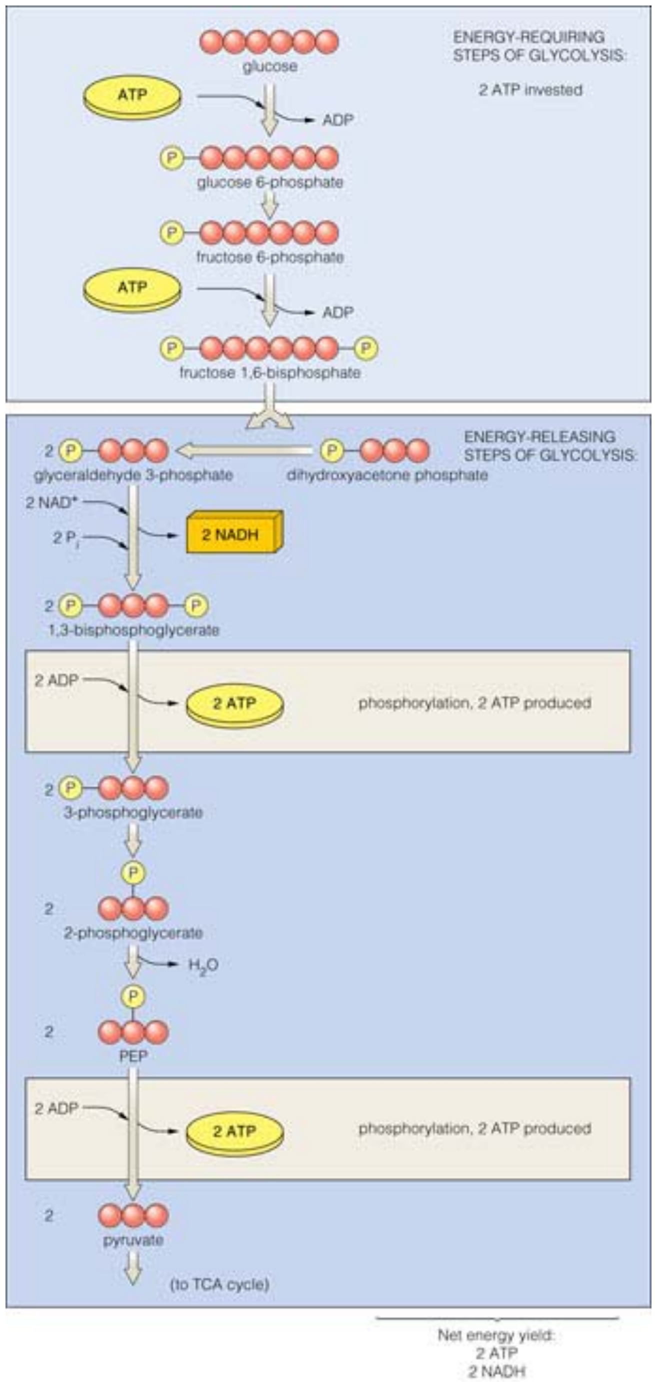


Figure 9.3. Two major phases of the process of glycolysis. The first (steps 1 and 2 described in the text) uses energy (ATP); the second returns that energy and yields more. ATP, adenosine triphosphate; ADP adenosine diphosphate; NAD^+, NADH, oxidized and reduced nicotinamide adenine dinucleotide; PEP, phosphoenolpyruvate; P, Pi, inorganic phosphate.

life-sustaining temperatures, and it is not readily broken down into intermediate products. Before a cell can break down glucose and release its stored energy, glucose must react enzymatically with ATP, which donates a phosphate group and energy. This reaction occurs in two steps, so that two ATP molecules provide energy and phosphate in the phosphorylation of the sugar molecule (Fig. 9.3). Note that ATP used in these reactions is replaced during later steps in respiration. Between the two phosphorylation steps, the sugar undergoes an internal rearrangement. The result is the formation of another six-carbon sugar phosphate, fructose-1,6-bisphosphate, which is split in the next reactions.

The next enzyme catalyzes the splitting of fructose-1,6-bisphosphate into two different three-carbon sugars, dihydroxyacetone phosphate and glyceraldehyde-3-phosphate. These three-carbon sugar phosphates are in equilibrium and may be converted enzymatically into one another. Glyceraldehyde-3-phosphate is oxidized to pyruvate (Fig. 9.3). As it is broken down, the equilibrium shifts so that more dihydroxyactone phosphate is converted into glyceraldehyde-3-phosphate. Thus, both three-carbon sugar phosphates are actually available for pyruvate formation.

The three-carbon sugar phosphate, glyceraldehyde-3-phosphate, is oxidized by the transfer of two of its electrons and hydrogen to an electron and hydrogen acceptor, NAD^+. This effectively transfers some of the energy in the chemical bonds of the three-carbon sugar to the newly formed NADH. Also, during the oxidation of each three-carbon sugar phosphate molecule to pyruvate, energy is used to form two molecules of ATP from two molecules of ADP plus two phosphates. The oxidation of the two three-carbon sugars forms a total of four ATP and two NADH molecules. However, because two molecules of ATP were originally used to phosphorylate a glucose molecule in preparation for its breakdown (Fig. 9.3), glycolysis results in a net yield to two ATP and two NADH. Glycolysis benefits the cell because usable intermediate compounds are formed, and although only a small amount (approximately 20%) of the energy of the glucose molecule is trapped in ATP and NADH, only about 3% has been lost as heat.

The fate of the pyruvate that results from glycolysis depends on the availability of molecule oxygen. If molecular oxygen is absent, anaerobic respiration (fermentation) may occur in most plant cells, forming ethyl alcohol and CO_2. If molecular oxygen is present, aerobic respiration may occur, and the pyruvate moves into the mitochondria. This activates two more phases of respiration: (1) the **tricarboxylic acid (TCA) cycle**, sometimes called the Krebs Cycle after the Nobel Laureate physiologist Hans Krebs, whose research contributed a great deal to our knowledge of respiration; and (2) the **terminal electron transport chain**.

Anaerobic Respiration Transforms Only a Small amount of Energy

Normally, higher plants cannot live long in the absence of molecular oxygen. Under anaerobic conditions, their cells have the following characteristics: (1) They do not oxidize food completely enough to yield adequate energy for their life processes; (2) They may produce poisonous products; and (3) They may not synthesize some necessary intermediate compounds.

Anaerobic respiration of pyruvate takes place in two steps (Fig. 9.4):

1. One CO_2 molecule is enzymatically split off from each pyruvate molecule. This leaves a two-carbon compound, acetaldehyde.

2. NADH, formed during glycolysis, reduces acetaldehyde to ethyl alcohol. NAD^+ is thereby regenerated and available to take part in glycolysis again.

Thus, during anaerobic respiration, the energy trapped in NADH is used to form alcohol (**alcoholic fermentation**). It is significant in the life of the cell that for each mole of glucose fermented to alcohol a net of only two moles of ATP are available to do work in the cell. This is about 14 kcal or less than 3% of the energy available in one mole of glucose. About 16% of the energy is lost as heat, whereas almost 84% is still locked in the two molecules

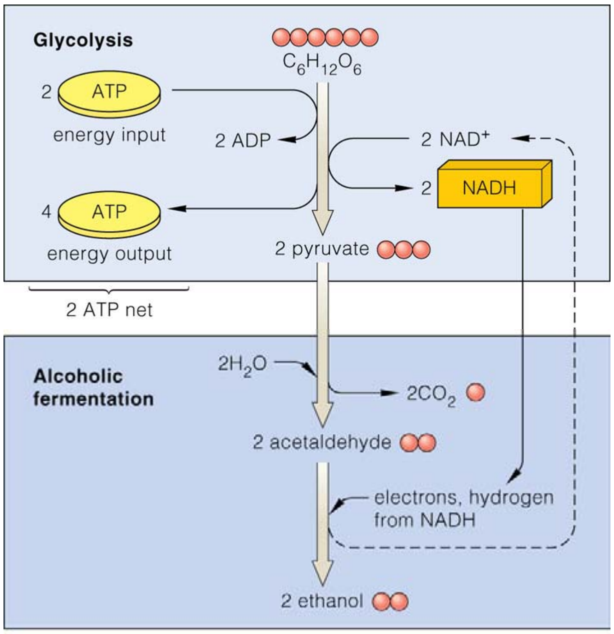


Figure 9.4. The reactions of alcoholic fermentation (anaerobic respiration). In the absence of molecular oxygen, pyruvate is converted to carbon dioxide and alcohol (ethanol).

of alcohol formed and thus is unavailable to the plant. In addition, the alcohol itself may be toxic to the plant. We can conclude that anaerobic respiration is not an efficient way of using the energy in food.

Some fruits, notably apples, may be held for long periods in an atmosphere containing small amounts of oxygen and continue to give off carbon dioxide. Yeast may live actively in an atmosphere with a low concentration of oxygen and produce relative large amounts of carbon dioxide and alcohol from the pyruvate formed during glycolysis. However, yeast has a limited tolerance for alcohol. When the alcohol concentration of the medium in which yeast is living reaches about 12%, the yeast cells stop metabolizing. Consequently, wines and other naturally fermented alcoholic beverages do not have an alcohol content greater than about 12%. Yeast grows much less vigorously under anaerobic conditions than in the presence of oxygen. Indeed, relatively few organisms grow well under strictly anaerobic conditions. Some fungi, bacteria, and animal cells may live and grow slowly under anaerobic conditions by producing products other than alcohol. When oxygen is not supplied rapidly enough to vigorously exercising muscles in your body, the muscle cells may produce not alcohol but lactic acid, which may cause cramping.

Aerobic Respiration Effectively Transforms the Energy in Food

When respiration occurs in an environment containing molecular oxygen, a plant cell oxidizes pyruvate more efficiently than it can under anaerobic conditions. Glycolysis releases about 20% of the energy contained in a glucose molecule, leaving most of the energy still locked in the bonds of two pyruvate molecules. During aerobic oxidation of pyruvate to CO_2 and H_2O, 40% to 50% of the energy in the pyruvate is converted to potentially useful forms as ATP and NADH.

The reaction steps of aerobic respiration are divided into three parts according to the intermediate molecules and end products formed (Fig. 9.5). All of these occur in the mitochondria.

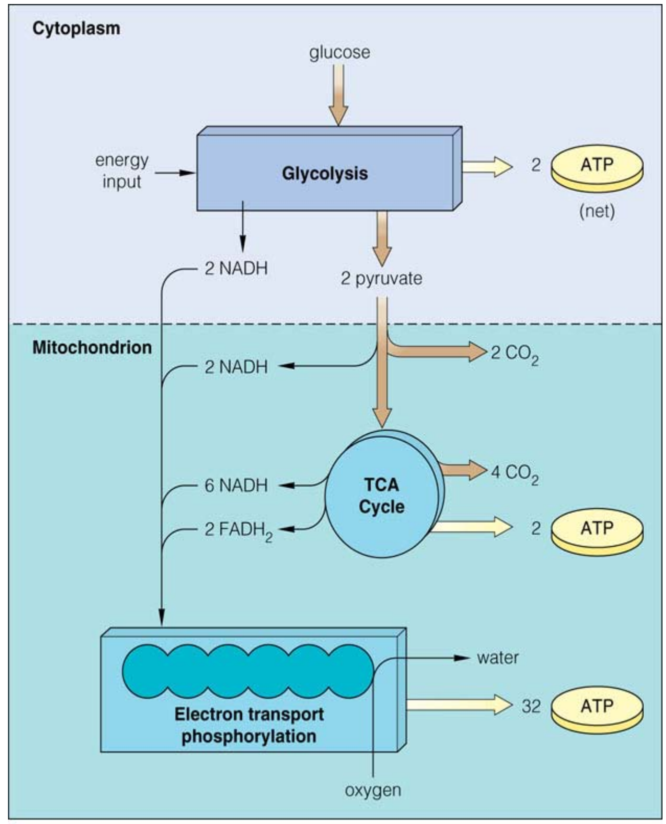


Figure 9.5. Overview of the complete oxidation of glucose during aerobic respiration.

1. *Entry of carbon into the TCA cycle of respiration.* Pyruvate releases CO_2, and the remaining two-carbon-atom fragment enters the TCA cycle, so named because several organic acids with three carboxylate groups (-COOH) play prominent roles.

2. *The TCA cycle.* The two-carbon-atom fragments release CO_2 and become oxidized. ATP and NADH are formed.

3. *Electron transport and terminal oxidation.* Oxygen accepts electrons and H^+ from the reduced coenzymes. ATP and water are formed.

ENTRY OF CARBON INTO THE TRICARBOXYLIC ACID CYCLE. One of the most complex series of reactions in respiration oxidizes pyruvate, thereby forming the carbon molecules that will enter the TCA cycle. In these reactions, several vitamins, particularly those of the vitamin B complex (niacin, thiamin, pantothenic acid), serve as coenzymes or parts of coenzymes (see Chapter 8). In the final stages, each molecule of pyruvate oxidized forms a molecule CO_2, with the transfer of two electrons and one hydrogen atom to NAD^+ to form NADH. About 53 kcal of energy are transferred from a mole of pyruvate to NAD^+ when NADH is formed.

The remaining part of pyruvate is now a two-carbon-atom fragment called an acetyl group. It is complexed with coenzyme A (CoA) to produce the reactive substance, acetyl-CoA (Fig. 9.6), the molecule that enters the TCA cycle. In the presence of a specific enzyme, acetyl-CoA transfers (donates) its acetyl group to another acceptor molecule.

In this way, each acetyl group, which still contains a high percentage of the energy originally present in the glucose molecule, may be transferred from one series of reactions to another in the cell. This transfer is analogous to the transfer of electrons and hydrogen by the coenzyme NADH. Although pyruvate is the usual donor of acetyl groups to CoA, it is not the only donor. The breakdown of fats and some amino acids forms acetyl groups that also may enter the TCA cycle.

THE TRICARBOXYLIC ACID CYCLE In the TCA cycle, acetyl-CoA donates its acetyl group to an organic acid acceptor molecule, oxaloacetate, producing citrate. The citrate is gradually broken down in a cyclic series of reactions, which involve seven other organic acids, including the acceptor, oxaloacetate (Fig. 9.6). During these reactions, pairs of electrons plus H^+ atoms are transferred to the electron carriers NAD^+ and FAD (flavin adenine dinucleotide) to form NADH and $FADH_2$. All of the atoms brought in with the acetyl group are gradually removed, and the carbon and oxygen are released as molecules of CO_2. Because the reactions form a new oxalocetate molecule capable of accepting another acetyl group, the cycle begins again.

In one turn of the cycle, some of the potential energy in one acetyl group is lost as heat to the environment, but about 66% is trapped in one molecule of ATP, three molecules of NADH, and one molecule of $FADH_2$. The ATP is free to carry its energy to other parts of the cell where work is being done. The energy in the NADH and $FADH_2$ generally is used in the synthesis of more ATP during the final stages of respiration.

The enzymes of the TCA cycle provide the following benefits to the cell.

1. They transfer electrons to energy carriers NAD^+ and FAD.

2. They produce the energy carrier ATP from ADP and inorganic phosphate.

3. They produce intermediate compounds that may act as starting materials for the synthesis of proteins and lipids.

Although all the energy originally associated with the reduced carbon atoms in the sugar molecule is released as heat or transferred to other molecules by the time the TCA cycle is completed, only about 10% is directly transferred to the ATP formed during glycolysis and the TCA cycle. About 56% is first trapped in reduced NADH and $FADH_2$. How does the cell convert the energy in these molecules to a more usable form? It uses an electron transport chain.

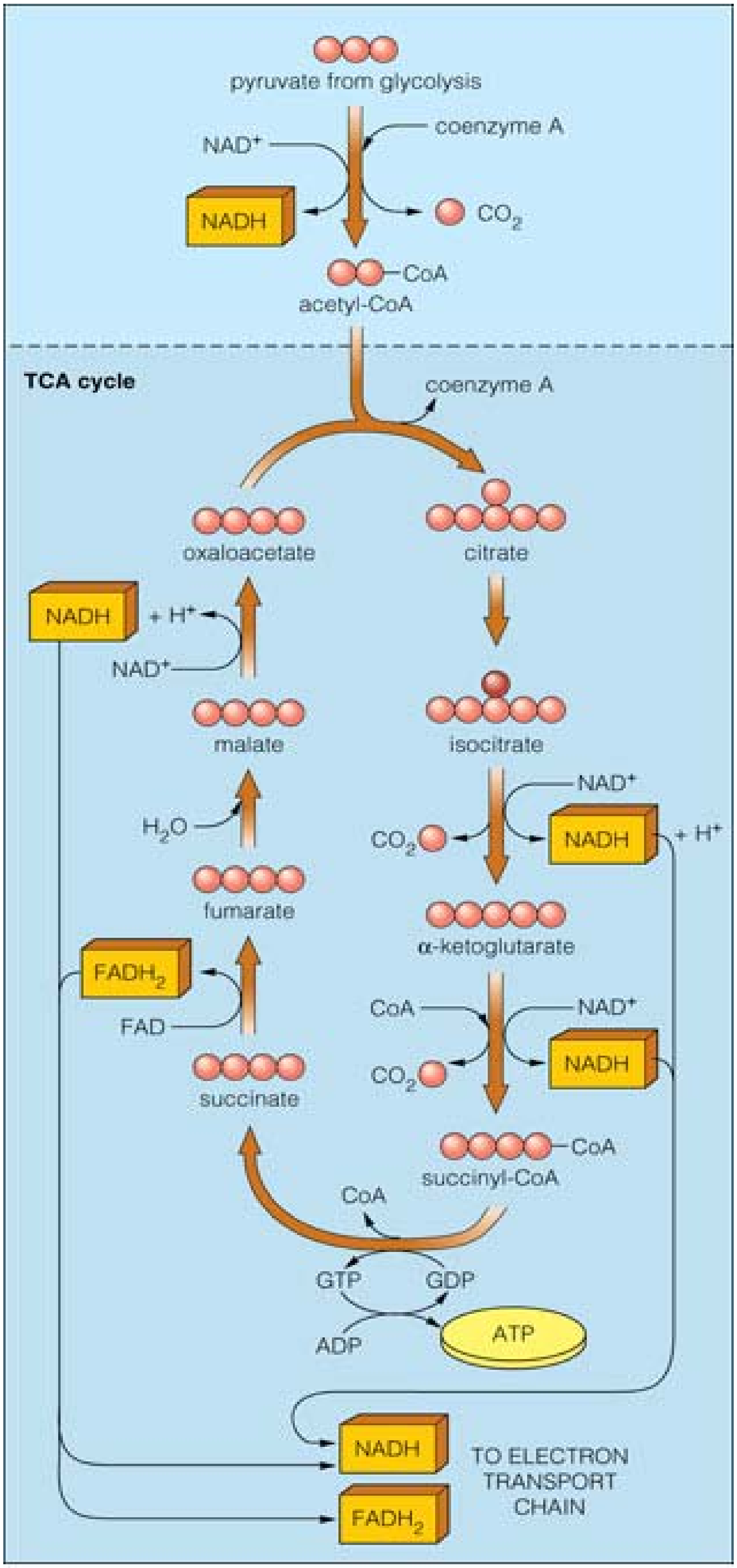


Figure 9.6. Several steps in the tricarboxylic acid (TCA) cycle leading to the production of the reduced nucleotides NADH and $FADH_2$.

THE ELECTRON TRANSPORT CHAIN During electron transport, the final series of reactions in aerobic respiration, cells do not obtain energy directly from the reduced NADH and $FADH_2$. Instead, they oxidize the nucleotides and trap part of their energy in ATP. In a stepwise series of coupled oxidations and reductions, electrons are transferred from the reduced nucleotides through an electron transport chain of carriers (Fig. 9.5). These electron carriers include a flavoprotein, coenzyme Q and several **cytochromes**. The carriers are alternately reduced (accept electrons) and oxidized (release electrons). For instance, cytochromes contain iron atoms, which are reduced to the ferrous (Fe^{2+}) form and then give up their electrons, becoming oxidized to the ferric (Fe^{3+}) form. The last step in the chain transfers a pair of electrons to an oxygen atom. Two hydrogen ions from the cellular environment then combine with the oxygen to form water. If free oxygen were not available, this last transfer of electrons could not take place. The flow of electrons would cease, and the entire electron transport sequence would stop.

As a result of electron transport, molecules of ATP are synthesized from ADP and inorganic phosphate. This process, which connects oxidation (of NADH or $FADH_2$) to phosphorylation (of ADP), is called **oxidative phosphorylation**. Complete aerobic respiration of one glucose molecule uses six molecules of oxygen and forms six CO_2 and six H_2O molecules. The process transfers about 40% of the potential energy in the glucose to some 30 molecules of ATP, which can do work in the cell. About 60% of the energy is lost as heat during the various steps of respiration.

Some Plant Cells Have Alternate Pathways of Respiration

Although aerobic respiration is the most common method plant cells use to oxidize foods, alternate pathways exist that also provide energy and organic building blocks. Two common alternate pathways are the pentose phosphate pathway (PPP) and the glyoxylate cycle.

THE PENTOSE PHOSPHATE PATHWAY The PPP occurs in the cytoplasm of some tissues. Biochemically, the PPP starts in the same way as glycolysis--that is, with the phosphorylation of a glucose molecule to produce glucose-6-phosphate. But instead of producing fructose-6-phosphate, the next steps in the PPP are the oxidation of the glucose-6-phosphate to form two molecules of NADPH, one molecule of CO_2, and one molecule of ribulose-5-phosphate (Fig. 9.7). The rest of the cycle consists of a series of sugar transformations which regenerate glucose or another intermediate in glycolysis. Young growing plant tissues appear to use the TCA cycle as the predominant pathway of glucose oxidation, whereas aerial parts of the plant and older tissues seem to use the PPP as well.

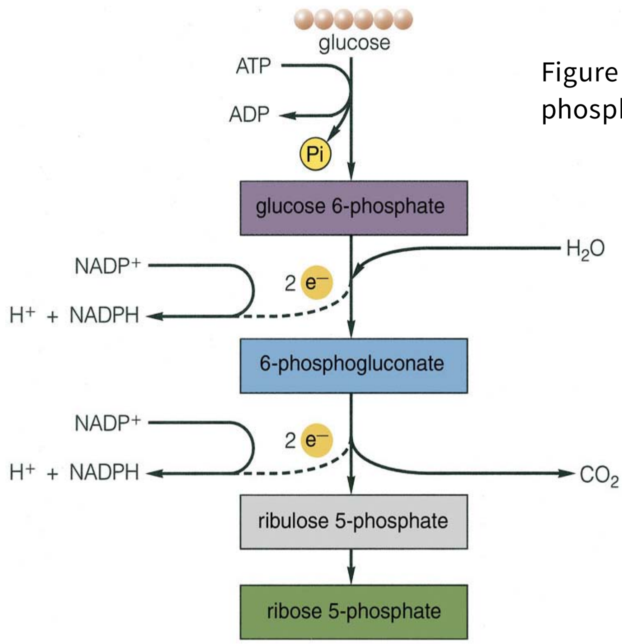


Figure 9.7. The first steps in the pentose phosphate pathway.

The PPP has two important consequences:

1. The pathway transfers energy and electrons from glucose to NADP+, forming NADPH, which is used is many biosynthetic reactions.

2. The pathway can produce the five-carbon sugar phosphate, ribose-5-phosphate, which is necessary for the synthesis of some essential compounds such as nucleic acids.

THE GLYOXYLATE CYCLE When fat-storing seeds germinate, the developing embryo requires energy and carbon compounds for growth. Because fats and oils do not dissolve in water, they are not transported from storage cells in the seed to cells in the growing seedling. Instead, the fats must be broken down to their constituent long-chain fatty acids and glycerol, which are then further metabolized. The glycerol may be converted in dihydroxyacetone phosphate, thus entering glycolysis, but this represents only a relatively small amount of the stored energy and carbon units. Fatty acids, however, yield large amount of acetyl-CoA when they are metabolized--they are the major source of energy and carbon in these germinating seeds.

The acetyl-CoA can be used to generate energy (ATP) through the TCA cycle and the electron transport chain. The use of acetyl-CoA to form glucose for cell wall synthesis, however, requires a series of reactions called the glyoxylate cycle. This is a modification of the TCA cycle involving some of the same enzymes, but rather than oxidizing the acetyl-CoA to CO_2, the glyoxylate cycle converts two acetyl-CoA molecules to a four-carbon molecule. Through a reverse of glycolysis, this molecule can be used to synthesize glucose, which in turn is a substrate for cellulose and other components of cell walls and for sucrose, which can be transported to other parts of the plant.

9.3 RESPIRATION AND CELL STRUCTURE

One cannot understand the entire process of respiration without knowing the role that cell structure plays in the process. Biochemists and plant physiologists isolate and purify the various cell organelles and membranes to determine how cellular structures function in cell metabolism. Some enzymes are confined to specific locations in the cell. Two experiments show this to be true for enzymes of respiration.

The first experiment demonstrates that when plant tissue is ground until all cells and organelles are broken apart, but the enzymes are preserved, glycolysis and alcoholic fermentation will occur in the cell-free preparation, but complete aerobic respiration will not. This means that the enzymes of glycolysis and fermentation are soluble enzymes and do not depend on intact organelles or membranes for their action.

The second experiment shows that if mitochondria are isolated from plant tissue, and pyruvate, ADP, Mg^{2+}, and inorganic phosphate are added, the pyruvate will break down, and the entire TCA cycle will occur, as well as electron transport. ATP will be synthesized, molecular oxygen will be absorbed, and H_2O and CO_2 will be produced. The results of this experiment, in light of the first, means that at least some steps in aerobic respiration require intact mitochondria.

In fact, biochemists have shown that all but one of the enzymes of the TCA cycle are present in the mitochondrial matrix, and that one is located on the inner mitochondrial membrane. The electron transport chain enzymes and the ATP synthesizing machinery are also part of the inner mitochondrial membrane (Fig. 9.8). By breaking cells and separating their parts, researchers have been able to determine where specific reactions occur in a cell (Table 9.1).

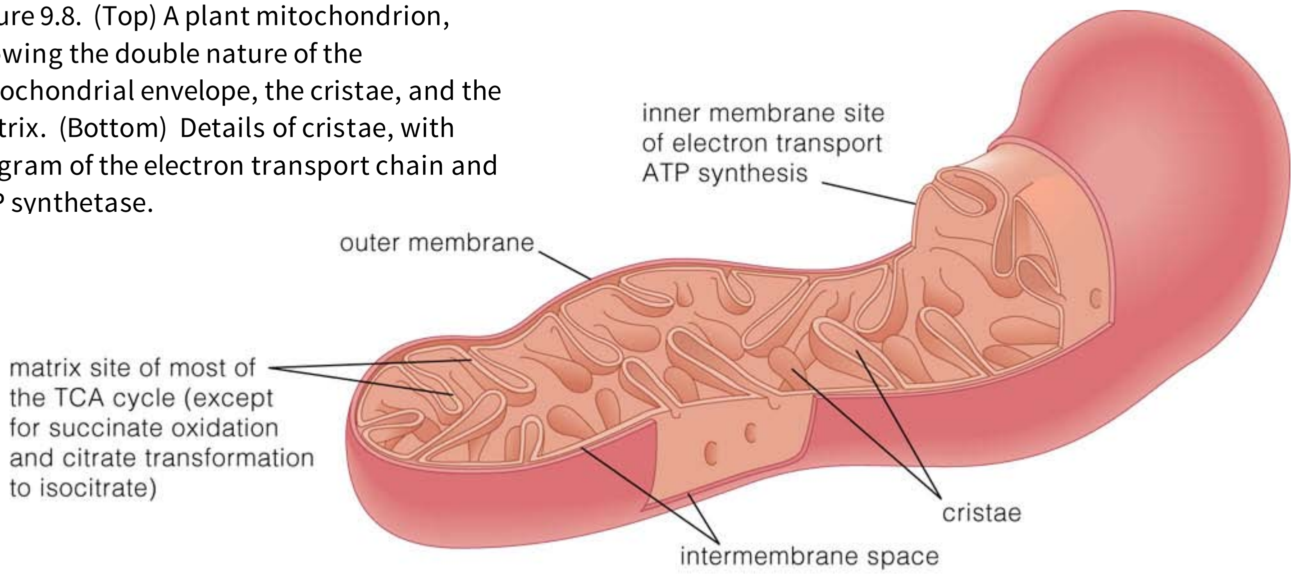
Table 9.1 Intracellular Location of Some Respiratory Reactions

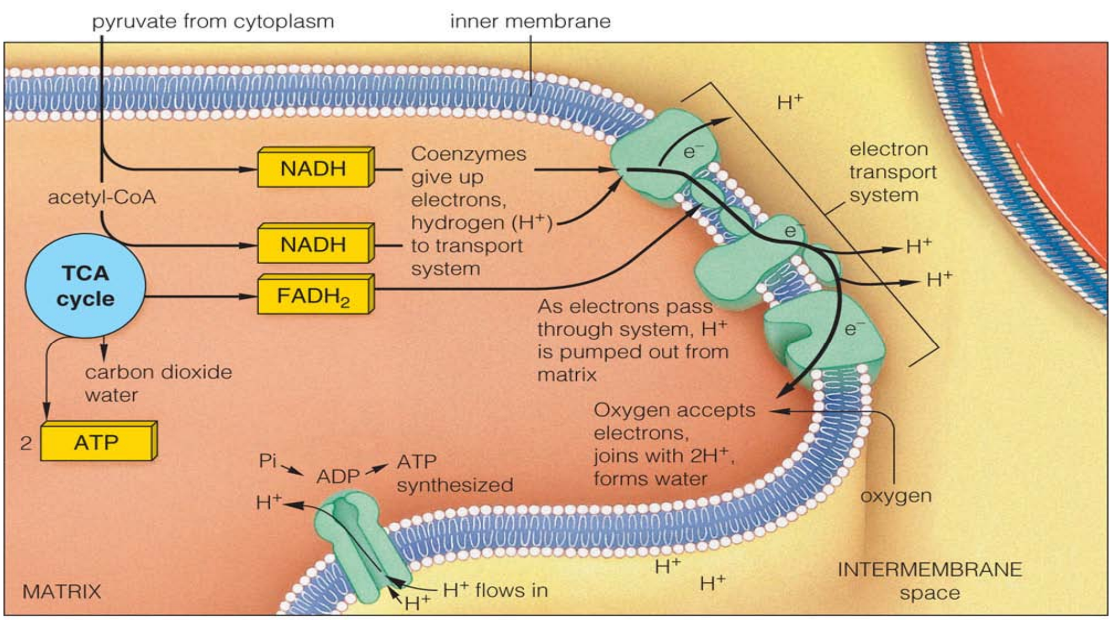
Reactions Catalyzed	Location of Reactions
Glycolysis	Cell cytoplasm
Alcoholic fermentation	Cell cytoplasm
Tricarboxylic acid cycle	Mitochondrial matrix (most reactions)
Citrate to isocitrate and succinic oxidation	Inner mitochondrial membrane
Electron transport	Inner mitochondrial membrane
Adenosine triphosphate synthesis	Inner mitochondrial membrane

Reactions in the Inner Mitochondrial Membrane Include Electron Transport and Adenosine Triphosphate Synthesis

Experimental findings indicate that a relationship exists between mitochondrial structure and respiration and that the membrane structure in mitochondria is an inner

Figure 9.8. (Top) A plant mitochondrion, showing the double nature of the mitochondrial envelope, the cristae, and the matrix. (Bottom) Details of cristae, with diagram of the electron transport chain and ATP synthetase.





important for ATP synthesis and electron transport. A mitochondrion, you will recall, and consists of a matrix surrounded by a double membrane envelope made up of an outer membrane. These membranes separate the mitochondrion from the rest of the cytoplasm (Fig. 9.8).

The final stages of respiration, electron transport and oxidative phosphorylation, occur on the inner membrane. NADH and $FADH_2$, produced during the oxidation of organic acids in the TCA cycle in the matrix, lose electrons to components of the electron transport chain on the membrane. This reaction regenerates NAD^+ and FAD.

The electron carriers making up the electron transport chain are present in aggregated clusters. Each cluster appears to consist of a fixed number of molecules of each carrier. This close organization facilitates the rapid exchange of electrons from a reduced carrier to the next oxidized carrier. Electrons flow from high free-energy levels in NADH or $FADH_2$ along the carrier chain down an energy gradient and finally are
transferred to oxygen. Hydrogen ions are taken up from the matrix, combined with the reduced oxygen, and thus water is formed

Each step in the electron transport releases energy. Some is lost as heat, but some is used to drive H^+ (protons) out of the mitochondrial matrix, through the inner mitochondrial membrane, and into the space between the two mitochondrial membranes. Because the inner membrane prevents the free diffusion of H^+ back into the matrix, a proton gradient and thus an energy gradient is established across the membrane. The energy gradient has two components:

1. an osmotic component--because there is a difference of H^+ concentration across the membrane, and

2. an electrical component--because there is an higher concentration of positive charges (H^+) outside the inner membrane relative to inside.

The proton gradient represents stored free energy, which can be used to synthesize ATP.

The Chemiosmotic Theory Explains the Synthesis of Adenosine Triphosphate in Mitochondria

In 1961, Peter Mitchell, a British biochemist, hypothesized that the proton gradient that develops across the inner mitochondrial membrane during electron transport drives ATP synthesis. This **chemiosmotic theory** (so named because the storage of energy is both chemical and osmotic) is now generally accepted as the best explanation for the mechanism of ATP synthesis in mitochondria, as well as ATP synthesis during photosynthesis in chloroplasts.

The harvest of energy from the proton gradient is accomplished by a complex enzyme known as **ATP synthetase**. Part of the enzyme is embedded in, and extends across, the inner mitochondrial membrane (Fig. 9.8); another part extends into the matrix. Protons from outside the inner membrane flow through the membrane part of enzyme and into the matrix. The flow of H^+ causes a periodic change in the shape of the matrix part of the enzyme, and this shape change drives ATP synthesis.

Thus the reactions of the TCA cycle and glycolysis are coupled, through the reduction and re-oxidation of NAD^+ and $FADH_2$ and the formation and resolution of an H^+ gradient, to the synthesis of ATP.

9.4 EFFECTS OF ENVIRONMENTAL FACTORS ON RESPIRATION

Conditions inside and outside the cell affect the rate of respiration, as well as the rates of other cellular activities, such as photosynthesis, absorption of water and mineral salts, cell division, and growth. The rate of respiration usually is expressed as the quantity of CO_2 released or O_2 absorbed per unit of cell weight per unit of time. Many factors that affect respiration rate act indirectly--for example, soil flooding decreases the availability of oxygen to respiring cells, whereas shading decreases photosynthesis and therefore reduces the amount of food available to respiring cells. Respiration rate also depends on cell type and age. Cells of different ages or from different kinds of plants may respond differently to the same environmental factor. For example, at low oxygen concentrations, CO_2 is released at a much greater rate from cells in rice seedlings than from cells in wheat seedlings. This is undoubtedly because rice seedlings have a greater capacity for fermentation than wheat seedlings, having adapted to grow under water, where O_2 concentration is low.

A few examples of the effects of environmental factors, age, and species of plant on respiration are discussed in the following section.

Cell Hydration May Indirectly Affect the Rate of Respiration

The water content of active protoplasm may be as high as 90%, and small fluctuations in water content have little effect on the rate of respiration. However, in some plants, starch is hydrolyzed to sugar during water stress--for example, when a plant wilts. In these plants, the rate of respiration may increase during wilting as a result of an increase in the amount of respirable food.

One of the most dramatic effects of the influence of cell water content on respiration occurs when seeds mature and begin to dry. When the water content of seeds decreases to between 16% and 17%, there is a sharp decrease in the rate of CO_2 released from their cells. The water content of mature seeds often becomes less than 10% of the seed weight. Growth ceases, mineral salts are not absorbed, and cell division stops, as do many, if not all, reactions that synthesize new molecules. Respiration goes on at a very slow rate; therefore, cells consume little O_2, little CO_2 is given off, and only a very little amount of heat is released. Energy is needed only to maintain a little-understood steady state in the quiescent protoplasm. In this dormant state, seeds may be kept for long periods in large storage bins such as grain elevators.

If even a little water is added to viable seeds, it is imbibed. The seeds then swell, respiration increases rapidly, and if the seeds have an adequate oxygen supply, the seeds germinate and growth begins. However, if confined too closely, as grain in a grain elevator, a rapid rate of respiration can increase the temperature inside the mass and kill the grain.

Living Cells Are Very Sensitive to Fluctuations in Temperature

Temperature has a marked effect on most biological reactions, especially those that are controlled by enzymes. In the temperature range from near freezing (0°C/32°F) to about 30°C (86°F), the rate of respiration approximately doubles for every increase of 10°C (18°F). At temperatures greater than 35 to 40°C (95 to 113°F), protein molecules, which make up enzymes, progressively unfold (become denatured), resulting in the loss of enzyme activity. The longer a cell is subjected to a high temperature, the greater the loss of enzyme activity. At first an increase in temperature of cells to 35 or 40°C may cause the respiration rate to increase, but soon the rate decreases and eventually respiration ceases.

Nevertheless, over the long periods sufficient for evolutionary adaptation to occur, certain organisms have developed characteristics that enable them to survive in otherwise hostile environments. For instance, some species of algae and bacteria are adapted to respire and grow under temperature extremes that would kill most other organisms. Particularly impressive are organisms in hot springs, where water temperatures may exceed 60°C (140°F). Others grow in snow, where the temperature remains near freezing (Fig. 9.9).

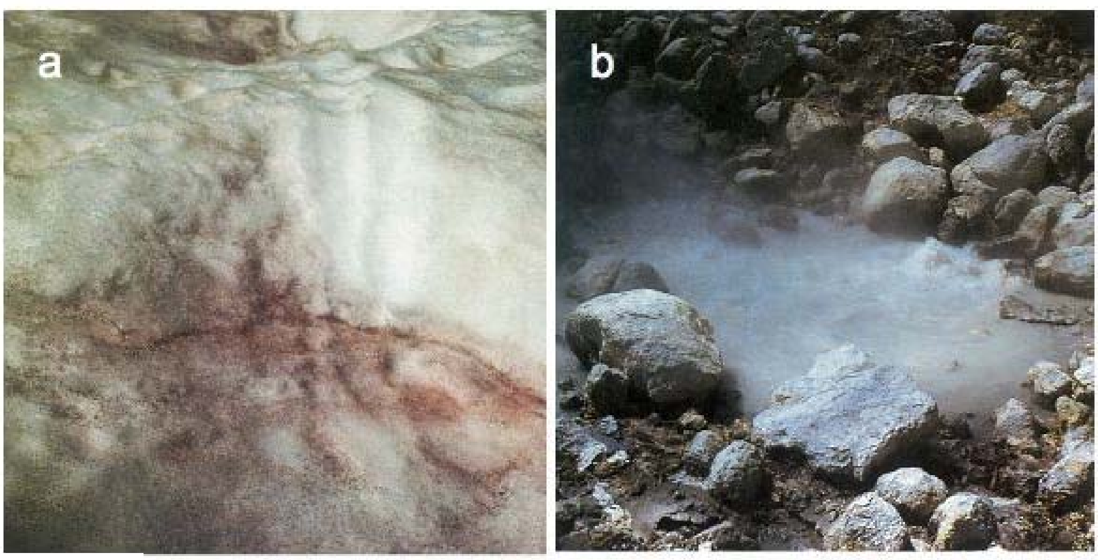


Figure 9.9. Adaptations to temperature extremes. (a) Cyanobacteria growing in a snowbank. (b) Cyanobacteria growing in hot springs.

In some plants such as potato, a decrease in temperature causes the hydrolysis of starch into sugar. Because cells cannot respire starch directly--but can respire sugar--instead of decreasing the rate of respiration, a decrease in temperature may actually increase respiration in potato cells. It has been demonstrated that more sugar accumulates in potato tubers stored at 0°C than in those stored at 4.5°C. At the same time, the respiration of potatoes stored at 0°C is markedly faster than it is in potatoes stored at 4.5°C, undoubtedly because of the greater sugar concentration. This accumulation of sugar in potatoes stored at or near freezing is of considerable importance to people who process potatoes, because high sugar levels frequently cause undesirable browning in heat-processed potato products.

Factors That Affect Photosynthesis May Indirectly Affect Respiration

Photosynthesizing cells produce their own food in the light. Under normal conditions, they must also make enough food to supply the needs of all the other living ells in the plant. All living cells of a plant kept in the dark continue to use food even though photosynthesis has stopped. The stored food reserves, particularly starch and sugars, rapidly become depleted. If this condition continues, the plant will eventually starve to death. In fact, any factor that limits photosynthesis, such as light, temperature, and CO_2 level in the air, limits food availability and must indirectly influence respiration.

Oxygen Gas Must Be Available for Aerobic Respiration

Higher plants require a supply of molecular oxygen. Rarely does the concentration of oxygen in the atmosphere deviate from the normal 21% enough to affect the rate of respiration in above-ground organs. However, underground stems, seeds, and roots may be oxygen-poor, because microorganisms and the plant parts themselves may use the oxygen in the soil atmosphere faster than it is replaced from the air. Under these conditions, respiration in the cells of these organs may decrease. Similar conditions of low oxygen and high carbon dioxide concentrations may occur in the internal cells of bulky plant organs such as large fleshy fruits. However, in general the diffusion of O_2 through the intercellular spaces is rapid enough that aerobic respiration occurs even within bulky plant parts.

Although the absence of oxygen is detrimental to the cells of most plants, modified atmospheres containing low concentrations of O_2 and greater levels of inert gases, coupled with low temperatures, effectively prolong storage life and improve the quality of many fruit and vegetable products. Shippers and handlers of fresh produce have developed controlled atmosphere containers and storage bins to take advantage of these effects (see sidebar "ECONOMIC BOTANY: Control of Respiration after Fruit Harvest").

KEY TERMS

adenosine triphosphate (ATP)
synthetase
aerobic respiration
alcoholic fermentation
anaerobic respiration
chemiosmotic theory
cytochromes
digestion
glycolysis
Krebs cycle
oxidative phosphorylation
respiration
terminal electron transport chain
tricarboxylic acid (TCA) cycle

SUMMARY

1. The two major functions of respiration are (a) the transformation of chemical bond energy stored in food into useful forms through the production of ATP and reduced nucleotides and (b) the production of intermediate products that are used in the synthetic reactions of the cell.

2. Energy-yielding oxidative reactions may be coupled to energy-requiring reactions.

3. Phosphorylation prepares sugar for oxidation. During phosphorylation, phosphorus from ATP is transferred to the sugar molecule. Sugar cleavage then occurs, resulting in the production of two three-carbon sugar phosphate intermediates. These, in turn, are oxidized to pyruvic acid. This entire sequence is glycolysis.

4. In the absence of O_2, plant cells usually metabolize pyruvate to alcohol and carbon dioxide. Only small amounts of ATP are generated by this process.

5. In the presence of O_2, pyruvate is oxidized to CO_2 and H_2O by enzymes of the TCA cycle. Large amounts of energy are stored in ATP and reduced nucleotides (NADH, $FADH_2$). Reduced nucleotides are oxidized in the electron transport chain, and ATP is produced.

6. Respiration is carried out through an integration of reactions going on in the cytoplasm (glycolysis) and mitochondria (TCA cycle and electron transport chain).

7. The synthesis of ATP from ADP and inorganic phosphate depends on the proton gradient formed across the inner mitochondrial membrane during electron transport. The movement of protons through the ATP synthetase drives ATP synthesis.

8. Some cells exhibit alternate pathways of respiration. One alternate pathway, the PPP, transfers energy from glucose to NADP, forming NADPH, and produces ribose-5-phosphate, which is used in the synthesis of nucleic acids. Another pathway, the glyoxylate cycle, converts fat into sugars (glucose, sucrose).

9. Cell hydration, temperature, oxygen supply, food availability, plant type, and plant age all affect the rate of respiration.

Questions

1. Define "digestion."

2. What two major roles does respiration play in the life of a plant?

3. Why does the rate of respiration increase when a cell is stimulated to do work such as salt absorption or cytoplasmic streaming?

4. Name and summarize the three major reaction blocks of respiration.

5. How would you determine whether a plant was carrying out aerobic or anaerobic respiration?

6. Why is anaerobic respiration not an effective way of using food?

7. Describe an experiment showing that some of the enzymes involved in aerobic respiration are soluble and others are attached to mitochondrial membranes.

8. What is the chemiosmotic theory of ATP synthesis? What is an alternative way of making ATP?

9. Explain how you would expect the rate of respiration in root cells to change when a plant that was kept dark for a period is moved to the light.

10. Show on a graph how you would expect the rate of respiration to change if the temperature was increased from 10 to 20, 30, 40, 50, and 60°C.

ECONOMIC BOTANY: *How to Make a Better Beer*

Beer making is a complex art, one that has developed over centuries. Although the techniques were originally perfected by serendipity and trial and error, they currently are based on important scientific principles. The major steps are:

- ♦ Germinating the grain (barley, *Hordeum vulgare* L.)
- ♦ Roasting the germinated grain to produce malt
- ♦ Extracting the malt sugars; boiling the wort
- ♦ Adding hops during boiling to extract their essential oils
- ♦ Inoculating the wort with yeast and incubating it to allow fermentation
- ♦ Bottling

The success of these steps depends greatly on the enzymes of respiration in the yeast. The methods of culturing pure yeast strains and some methods of studying enzymes were developed at research laboratory of the Carlsberg brewery in Copenhagen.

Moistening the grain to start germination activates enzymes that break down starch, stored in the endosperm, into sugars. The enzymes are a-amylase and maltase. a-Amylase hydrolyzes starch, cutting the chain of glucose molecules into single glucose and maltose molecules. Maltose is formed of two glucoses connected, and maltase hydrolyzes the maltose, separating the glucoses. At the same time, seedlings start to grow, using the glucose as an energy source. Once the hydrolysis is almost finished, roasting kills the seedlings and inactivates the enzymes, leaving the glucose available to support yeast fermentation. Some over-roasting, which oxidizes a portion of the glucose, provides caramel flavor and color and some sweetness, because the modified glucose cannot be used by the yeast.

The roasted malt is ground into a powder and boiled with water to dissolve the sugars and to kill unwanted microorganisms. (Amateur brewers may buy a concentrated malt extract, which they add to boiling water.) Hop flowers or extracts of the flowers may be added to provide bitter flavor, aroma, and some protection against infection by unwanted microorganisms. Then the liquid, called wort, is cooled, and a culture of yeast (*Saccharomyces carlsbergensis*) is added ("pitched") into the liquid. The mixture is incubated for several days at 4°C (for lagers) or 20°C (for ales) until the fermentation is complete. It is during this time that enzymes of glycolysis in the yeast break down the glucose. Because the liquid is not stirred (or air is prevented from entering), oxygen is used up, and most of the glucose is metabolized by alcoholic fermentation, which produces ethanol and CO_2. Sometimes much of the CO_2 escapes; therefore, for proper fizziness, more CO_2 must be added after the beer is bottled. In commercial beers, this often is done by injecting purified CO_2 gas into pasteurized beer as the beer is bottled, but it can also be done by adding a little sugar to unpasteurized beer just before the bottle is sealed. In the latter method, the yeast will produce a little more CO_2 (and ethanol), and there will be a layer of yeast at the bottom of the bottle.

What gives different beers their unique taste? It is a combination of the four ingredients: water, malt, yeast, and hops. The malt is critical--how much, what kind, and how much it has been roasted. The light Pilsner lagers are made from lightly roasted malt; the brown ales and stouts include some varieties of malt that are roasted at greater temperatures for longer times. The amounts and varieties of hops are also major factors. Most American lagers are very lightly hopped; pale ales, in contrast, are more "hoppy", bitter, and aromatic.

Beers, like all foods, should be appreciated for the art and science that go into their creation and for the subtle nuances of taste that they can provide to a meal.

ECONOMIC BOTANY: *Control of Respiration after Fruit Harvest*

An understanding of the process of respiration--and of means to control and modify it--is critically important to the producers, handlers, and shippers of fruits and vegetables. High rates of respiration are associated with a complex series of biochemical changes during the ripening and maturation of fruit. If the rate of respiration is reduced, the rate of maturation is delayed, and storage life is extended.

One of the most effects methods for reducing the rate of respiration is to cool the produce. Most long-range shipping of fruit is done in refrigerated railcars and trucks. Because all living cells respire--and because respiration produces heat--the amount of refrigeration required to ship and store fruit is considerable. For example, 40,000 boxes of apples at 0°C (32°F) produce enough heat to melt 2.8 tons of ice in 24 hours; at 4°C (39°F), respiratory heat would melt 4.8 tons of ice; and at 16°C (61°F), respiratory heat would melt 19 tons of ice. Temperature control represents a considerable cost in marketing plant produce.

Fruits and vegetables vary greatly in their susceptibility to low temperature and in the optimum conditions they require for successful storage. Bananas, tomatoes, and summer squash, all of tropical origin, suffer chilling injury when stored at temperatures less than 10 to 13°C (50-55°F) but greater than freezing. (Chilling injury, which shows up when the produce is taken out of cold storage, can include internal browning, scalding, and pitting.) Cold weather fruits and vegetables such as pears, some apples, and onions, by contrast, tolerate storage at near-freezing temperature for extended periods. A shipper's objective is to store the produce at the lowest economical temperature that still maintains the quality of the particular fruit or vegetable being shipped.

Grocery stores have tasty, crisp apples from Washington and Oregon year-round, even though the apple harvest lasts only a few weeks. If the apples are kept at room temperature after harvest, their rate of respiration rapidly increases, and production of ethylene (a gaseous plant hormone) increases. Ethylene stimulates the aging process and decreases fruit quality. Within a few weeks, the apples become soft, mealy, and undesirable. Cold storage significantly prolongs the storage life of apples.

Also, oxygen is necessary for aerobic respiration. If the oxygen level in the air in the storage room is reduced from about 21% to about 1 to 3%, and if CO_2 level is increased from 0.03% to 1 to 3%, storage life is increased. Reducing the oxygen level to zero is not desirable, because this stimulates anaerobic respiration and may result in a more rapid deterioration of the apples. A large fraction of Washington and Oregon apples are stored under refrigeration in rooms with modified atmospheres. These apples can be stored for as long as 9 months.

In addition to the other changes that accompany respiration, there is a loss of the sugar that is respired. The loss can be considerable. Consider sugar beets: At harvest, the tops of the sugar beets are removed, and the roots are mechanically dug up and loaded into trucks for transport to the sugar processing plant. At the plant, beets often are stored in large piles on a cement apron. If the pile loses 1% of its weight in 20 days, a reasonable figure, a 100-ton pile loses 1 ton of its weight. Of course, part of the weight loss is water, but a significant portion is sugar. No practical means of reducing the rate of respiration in harvested sugar beets exists, but the faster the beets are processed after harvest, the smaller the amount of sugar that will be lost.

Figure credits

CO. Jeff Austink, University of California

Figure 9-8b. Raychael Ciemma

Sidebar (beer). Meek Brewing Co.

Sidebar (fruit). Sandstein, 2009