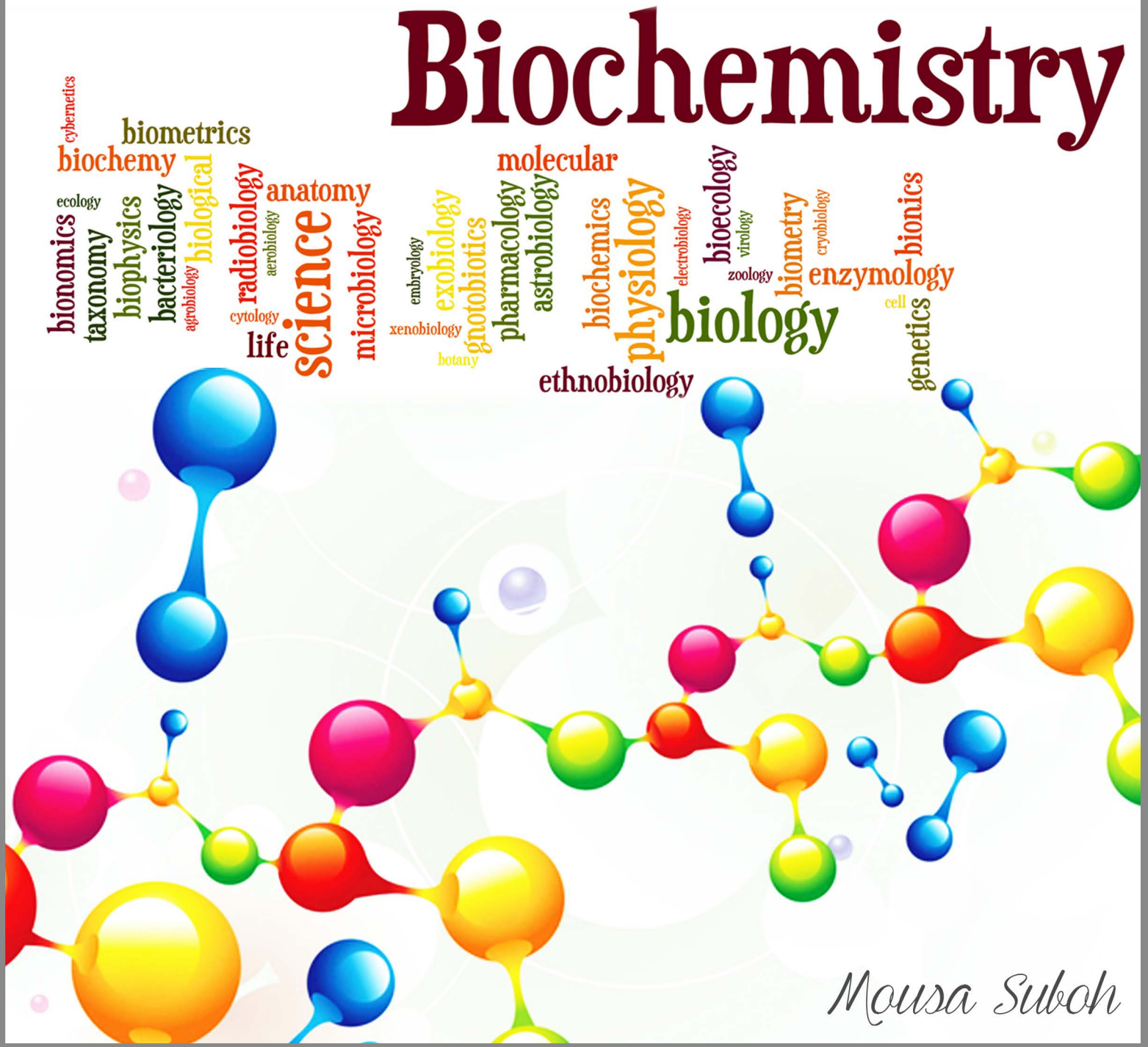
Chapter :.....8 , 9 , 10.....

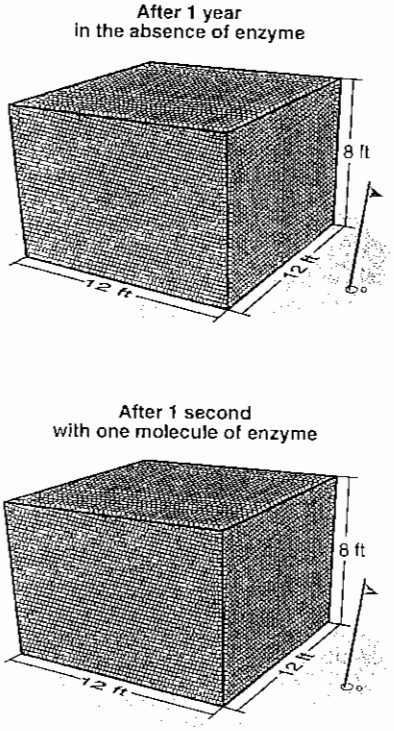
Book:.....Mark's Midical Biochemistry.....

The University of Jordan



Mousa Suboh

8 Enzymes as Catalysts



Enzymes increase the rate of a chemical reaction by a factor of 10^{11} or higher. To appreciate an increase in reaction rate by this order of magnitude, consider a room-sized box of golf balls that "react" by releasing energy and turning brown. The 12 ft × 12 ft × 8 ft box contains 380,000 golf balls. If the rate of the reaction in the absence of enzyme were 100 golf balls per year, the presence of 1 molecule of enzyme would turn the entire box of golf balls brown in 1 second (assuming a 10^{11} increase in reaction rate).

Enzymes are proteins that act as ***catalysts****, compounds that increase the rate of chemical reactions (Fig. 8.1). Enzyme catalysts bind reactants (substrates), convert them to products, and release the products. Although enzymes may be modified during their participation in this reaction sequence, they return to their original form at the end. In addition to increasing the speed of reactions, enzymes provide a means for regulating the rate of metabolic pathways in the body. This chapter describes the properties of enzymes that allow them to function as catalysts. The next chapter explains the mechanisms of enzyme regulation.*

Enzyme-Binding Sites. *An enzyme binds the* ***substrates*** *of the reaction and converts them to* ***products****. The substrates are bound to specific* ***substrate-binding sites*** *on the enzyme through interactions with the amino acid residues of the enzyme. The spatial geometry required for all the interactions between the substrate and the enzyme makes each enzyme* ***selective for its substrates*** *and ensures that only* ***specific products*** *are formed.*

Active Catalytic Sites. *The substrate-binding sites overlap in the* ***active catalytic site*** *of the enzyme, the region of the enzyme where the reaction occurs. Within the catalytic site,* ***functional groups*** *provided by* ***coenzymes, tightly bound metals,*** *and, of course,* ***amino acid residues*** *of the enzyme participate in catalysis.*

Activation Energy and the Transition State. *The functional groups in the catalytic site of the enzyme activate the substrate and decrease the energy needed to form the high-energy intermediate stage of the reaction known as the* ***transition-state complex****. Some of the catalytic strategies employed by enzymes, such as* ***general acid–base catalysis****, formation of* ***covalent intermediates****, and* ***stabilization of the transition state****, are illustrated by chymotrypsin.*

pH and Temperature Profiles. Enzymes have a functional pH range determined by the pK_a *of the functional groups in the active site and the interactions required for three-dimensional structure. Increases of temperature, which do not lead to protein denaturation, increase the reaction rate.*

Mechanism-Based Inhibitors. *The effectiveness of many* ***drugs*** *and* ***toxins*** *depends on their ability to inhibit an enzyme. The strongest inhibitors are* ***covalent inhibitors****, compounds that form covalent bonds with a reactive group in the enzyme active site, or* ***transition-state analogs*** *that mimic the transition-state complex.*

Enzyme Names. *Most enzyme names end in "-ase." Enzymes usually have both a common name and a systematic classification that includes a name and an Enzyme Commission (EC) number.*

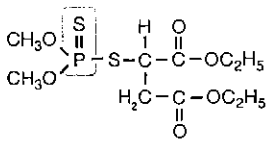
THE WAITING ROOM

A year after recovering from salicylate poisoning (see Chapter 4), **Dennis "the Menace" Veere** was playing in his grandfather's basement. Dennis drank an unknown amount of the insecticide malathion, which is sometimes used for killing fruit flies and other insects (Fig. 8.2). Sometime later, when he was not feeling well, Dennis told his grandfather what he had done. Mr. Veere retrieved the bottle and rushed Dennis to the emergency room of the local hospital. On the way, Dennis vomited repeatedly and complained of abdominal cramps. At the hospital, he began salivating and had an uncontrollable defecation.

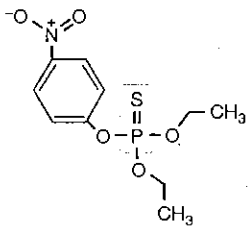
In the emergency room, physicians passed a nasogastric tube for stomach lavage, started intravenous fluids, and recorded vital signs. Dennis's pulse rate was 48 beats/minute (slow), and his blood pressure was 78/48 mm Hg (low). The physicians noted involuntary twitching of the muscles in his extremities.

Lotta Topaigne was diagnosed with acute gouty arthritis involving her right great toe (see Chapter 5). The presence of insoluble urate crystals within the joint space confirmed the diagnosis. Several weeks after her acute gout attack subsided, Ms. Topaigne was started on allopurinol therapy in an oral dose of 150 mg twice per day.

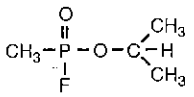
Al Martini, a 44-year-old man who has been an alcoholic for the past 5 years, had a markedly diminished appetite for food. One weekend, he became unusually irritable and confused after drinking two-fifths of scotch and eating very little. His landlady convinced him to visit his doctor. Physical examination indicated a heart rate of 104 beats/minute. His blood pressure was slightly low, and he was in early congestive heart failure. He was poorly oriented to time, place, and person.



Malathion



Parathion



Sarin

FIG. 8.2. Organophosphorous compounds. Malathion and parathion are organophosphorous insecticides. Nausea, coma, convulsions, respiratory failure, and death have resulted from the use of parathion by farmers who have gotten it on their skin. Malathion is similar in structure to parathion but is not nearly as toxic. The nerve gas sarin, another organophosphorus compound, was used in a terrorist attack in a Japanese subway.

I. THE ENZYME-CATALYZED REACTION

Enzymes, in general, provide speed, specificity, and regulatory control to reactions in the body. Enzymes are usually proteins that act as catalysts, compounds that increase the rate of chemical reactions. Enzyme-catalyzed reactions have three basic steps:

1. Binding of substrate (a reactant): $E + S \leftrightarrow ES$
2. Conversion of bound substrate to bound product: $ES \leftrightarrow EP$
3. Release of product: $EP \leftrightarrow E + P$

An enzyme binds the substrates of the reaction it catalyzes and brings them together at the right orientation to react. The enzyme then participates in the making and breaking of bonds required for product formation, releases the products, and returns to its original state once the reaction is completed.

Enzymes do not invent new reactions; they simply make reactions occur faster. The catalytic power of an enzyme (the rate of the catalyzed reaction divided by the rate of the uncatalyzed reaction) is usually in the range of 10^6 to 10^{14}. Without the catalytic power of enzymes, reactions such as those involved in nerve conduction, heart contraction, and digestion of food would occur too slowly for life to exist.

Each enzyme usually catalyzes a specific biochemical reaction. The ability of an enzyme to select just one substrate and distinguish this substrate from a group of very similar compounds is referred to as *specificity* (Fig. 8.3). The enzyme converts

Most, if not all, of the tissues and organs in the body are adversely affected by chronic ingestion of excessive amounts of alcohol, including the liver, pancreas, heart, reproductive organs, central nervous system, and the fetus. Some of the effects of alcohol ingestion, such as the psychotropic effects on the brain or inhibition of vitamin transport, are direct effects caused by ethanol itself. However, many of the acute and chronic pathophysiologic effects of alcohol relate to the pathways of ethanol metabolism (see Chapter 25).

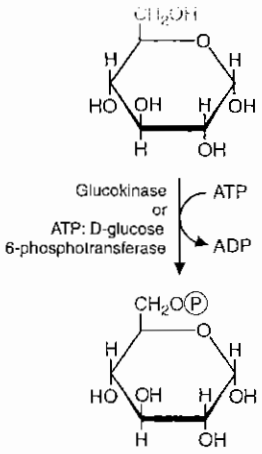


FIG. 8.3. Reaction catalyzed by glucokinase, an example of enzyme reaction specificity. Glucokinase catalyzes the transfer of a phosphate from adenosine triphosphate (ATP) to carbon 6 of glucose. It cannot rapidly transfer a phosphate from other nucleotides to glucose, or from ATP to closely related sugars such as galactose, or from ATP to any other carbon on glucose. The only products formed are glucose 6-phosphate and adenosine diphosphate (ADP).

this substrate to just one product. The specificity, as well as the speed, of enzyme-catalyzed reactions results from the unique sequence of specific amino acids that form the three-dimensional structure of the enzyme.

A. The Active Site

To catalyze a chemical reaction, the enzyme forms an enzyme–substrate complex in its active catalytic site (Fig. 8.4). The active site is usually a cleft or crevice in the enzyme formed by one or more regions of the polypeptide chain. Within the active site, cofactors and functional groups from the polypeptide chain participate in transforming the bound substrate molecules into products.

Initially, the substrate molecules bind to their substrate-binding sites, also called the substrate recognition sites (see Fig. 8.4B). The three-dimensional arrangement of binding sites in a crevice of the enzyme allows the reacting portions of the substrates to approach each other from the appropriate angles. The proximity of the bound substrate molecules and their precise orientation toward each other contribute to the catalytic power of the enzyme.

The active site also contains functional groups that participate directly in the reaction (see Fig. 8.4C). The functional groups are donated by the polypeptide chain or by bound cofactors (metals or complex organic molecules called *coenzymes*). As the substrate binds, it induces conformational changes in the enzyme that promote further interactions between the substrate molecules and the enzyme functional groups. (For example, a coenzyme might form a covalent intermediate with the substrate, or an amino acid side chain might abstract a proton from the reacting substrate.) The activated substrates and the enzyme form a *transition-state complex*, an unstable high-energy complex with a strained electronic configuration that is intermediate between substrate and product. Additional bonds with the enzyme stabilize the transition-state complex and decrease the energy required for its formation.

The transition-state complex decomposes to products, which dissociate from the enzyme (see Fig. 8.4D). The enzyme generally returns to its original form. The free enzyme then binds another set of substrates and repeats the process.

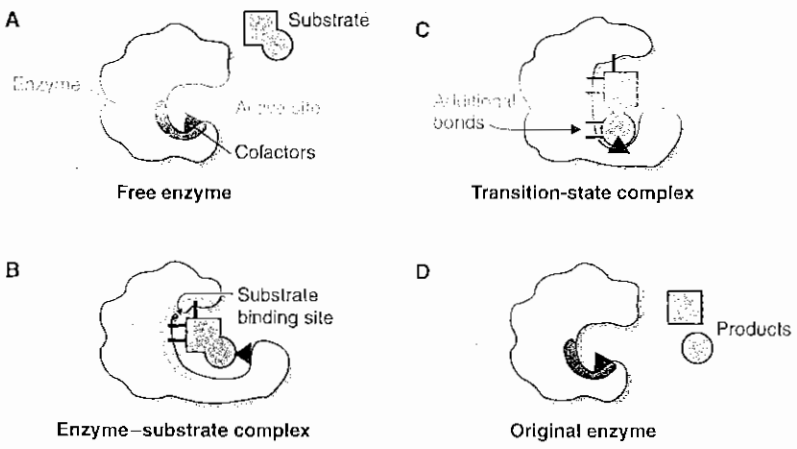


FIG. 8.4. Reaction in the enzyme active catalytic site. **A.** The enzyme contains an active catalytic site, shown in *dark red*, with a region or domain where the substrate binds. The active site also may contain cofactors, nonprotein components that assist in catalysis. **B.** The substrate forms bonds with amino acid residues in the substrate-binding site. Substrate binding induces a conformational change in the active site. **C.** Functional groups of amino acid residues and cofactors in the active site participate in forming the transition-state complex, which is stabilized by additional noncovalent bonds with the enzyme, shown in *red*. **D.** Because the products of the reaction dissociate, the enzyme returns to its original conformation.

B. Substrate-Binding Sites

Enzyme specificity (the enzyme's ability to react with just one substrate) results from the three-dimensional arrangement of specific amino acid residues in the enzyme that form binding sites for the substrates and activate the substrates during the course of the reaction. The "lock-and-key" and the "induced-fit" models for substrate binding describe two aspects of the binding interaction between the enzyme and substrate.

1. LOCK-AND-KEY MODEL FOR SUBSTRATE BINDING

The substrate-binding site contains amino acid residues arranged in a complementary three-dimensional surface that "recognizes" the substrate and binds it through multiple hydrophobic interactions, electrostatic interactions, or hydrogen bonds (Fig. 8.5). The amino acid residues that bind the substrate can come from very different parts of the linear amino acid sequence of the enzyme, as seen in glucokinase. The binding of compounds with a structure that differs from the substrate even to a small degree may be prevented by steric hindrance and charge repulsion. In the lock-and-key model, the complementarity between the substrate and its binding site is compared to that of a key fitting into a rigid lock.

2. INDUCED-FIT MODEL FOR SUBSTRATE BINDING

Complementarity between the substrate and the binding site is only part of the picture. As the substrate binds, enzymes undergo a conformational change ("induced fit") that repositions the side chains of the amino acids in the active site and increases the number of binding interactions (see Fig. 8.4). The induced-fit model for substrate binding recognizes that the substrate-binding site is not a rigid "lock" but rather a dynamic surface created by the flexible overall three-dimensional structure of the enzyme.

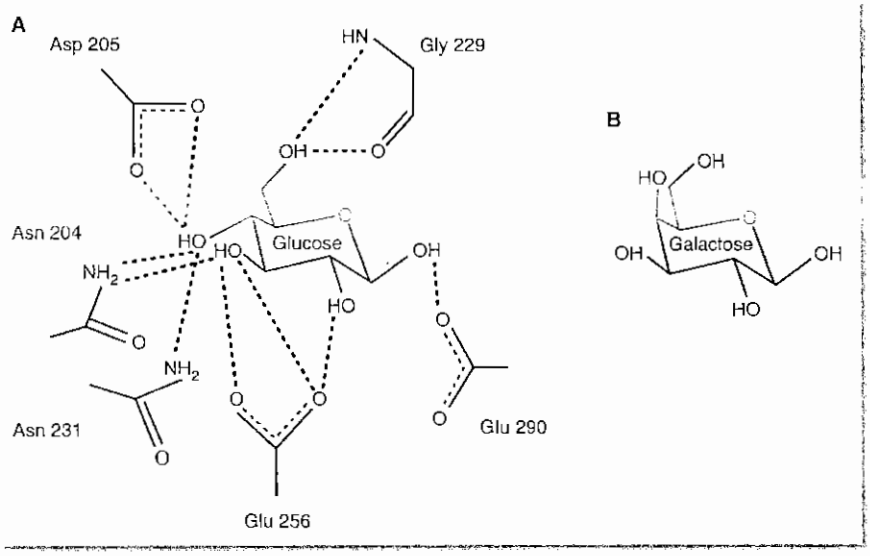


FIG. 8.5. Glucose-binding site in glucokinase. **A.** Glucose, shown in *red*, is held in its binding site by multiple hydrogen bonds between each hydroxyl group and polar amino acids from different regions of the enzyme amino acid sequence in the actin fold (see Chapter 7). The position of the amino acid residue in the linear sequence is given by its number. The multiple interactions enable glucose to induce large conformational changes in the enzyme (induced fit). (Modified from Pilkis SJ, Weber IT, Harisson RW, et al. Glucokinase: structural analysis of a protein involved in susceptibility to diabetes. *J Biol Chem.* 1994;269:21925–21928.) **B.** Enzyme specificity is illustrated by the comparison of galactose and glucose. Galactose differs from glucose only in the position of the –OH group, shown in *red*. However, it is not phosphorylated at a significant rate by the enzyme. Cells therefore require a separate galactokinase for the metabolism of galactose.

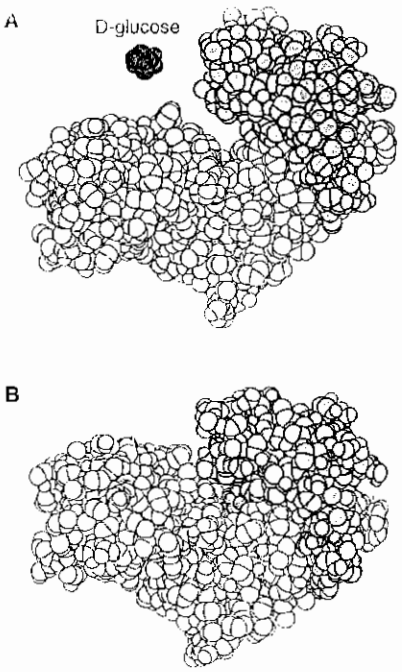


FIG. 8.6. Conformational change resulting from the binding of glucose to hexokinase. (The figure is actually yeast hexokinase, which is more similar to human glucokinase than it is to the other human hexokinase isozymes.) The shaded and unshaded areas show the two domains (four subdomains) that form the actin fold with its adenosine triphosphate (ATP)-binding cleft. **A.** Free enzyme. **B.** With glucose bound, the cleft closes, forming the ATP-binding site. The closure of the cleft when glucose binds to hexokinase (or human glucokinase) is one of the largest "induced fits" known. The combination of secondary structures in the actin fold that give hexokinase the flexibility required for this shift are discussed in Chapter 7, Section IV.B.1, "The Actin Fold." (From Bennett WS, Steitz TA. Structure of a complex between yeast hexokinase A and glucose. II. Detailed comparisons of conformation and active site configuration with the native hexokinase B monomer and dimer. *J Mol Biol.* 1980;140:211–230.)

The function of the conformational change induced by substrate binding, th induced fit, is usually to reposition functional groups in the active site in a way tha promotes the reaction, improves the binding site of a cosubstrate, or activates a adjacent subunit through cooperativity. For example, consider the large conforma tional changes that occur in the actin fold of glucokinase when glucose binds. Th induced fit involves changes in the conformation of the whole enzyme that clos the cleft of the fold, thereby improving the binding site for adenosine triphosphat (ATP) and excluding water (which might interfere with the reaction) from th active site (Fig. 8.6). Thus, the multiple interactions between the substrate an the enzyme in the catalytic site serve both for substrate recognition and for initiatin the next stage of the reaction—formation of the transition-state complex.

C. The Transition-State Complex

For a reaction to occur, the substrates undergoing the reaction need to be activatec If the energy levels of a substrate are plotted as the substrate is progressively con verted to product, the curve will show a maximum energy level that is higher tha that of either the substrate or the product (Fig. 8.7). This high energy level occur at the transition state. For some enzyme-catalyzed reactions, the transition stat is a condition in which bonds in the substrate are maximally strained. For othe enzyme-catalyzed reactions, the electronic configuration of the substrate become very strained and unstable as it enters the transition state. The highest energy leve corresponds to the most unstable substrate configuration, and the condition i which the changing substrate molecule is most tightly bound to participating func tional groups in the enzyme. The difference in energy between the substrate and th transition-state complex is called the *activation energy*.

According to transition-state theory, the overall rate of the reaction is determine by the number of molecules acquiring the activation energy necessary to form th transition-state complex. Enzymes increase the rate of the reaction by decreasin this activation energy. They use various catalytic strategies, such as electroni stabilization of the transition-state complex or acid–base catalysis, to obtain thi decrease.

Once the transition-state complex is formed, it can collapse back to substrates o decompose to form products. The enzyme does not change the initial energy leve of the substrates or the final energy level of the products.

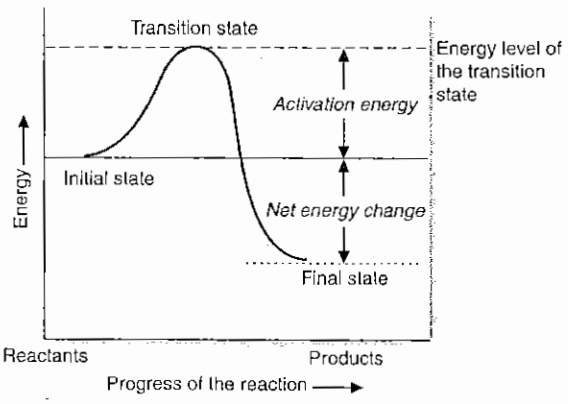


FIG. 8.7. Energy diagram showing the energy levels of the substrates as they progres toward products in the absence of enzyme. The substrates must pass through the high-energy transition state during the reaction. Although a favorable loss of energy occurs during the reaction, the rate of the reaction is slowed by the energy barrier to form the transition state The energy barrier is referred to as the activation energy.

Because the transition-state complex binds more tightly to the enzyme than does the substrate, compounds that resemble its electronic and three-dimensional surface (transition-state analogs) are more potent inhibitors of an enzyme than are substrate analogs. Consequently, a drug developed as a transition-state analog would be highly specific for the enzyme it is designed to inhibit. Nevertheless, transition-state analogs are highly unstable when they are not bound to the enzyme, and would have great difficulty making it from the digestive tract or injection site to the site of action. Some of the approaches in drug design that are being used to deal with the instability problem include designing drugs that are almost transition-state analogs but have a stable modification, designing a prodrug that is converted to a transition-state analog at the site of action, and using the transition-state analog to design a complementary antibody.

If the structure of a transition state can be modeled, it can be used as an antigen for the production of *abzymes* (catalytic antibodies). These antibodies have an arrangement of amino acid side chains in their variable regions that is similar to the active site of the enzyme in the transition state. Consequently, they can act as artificial enzymes. For example, abzymes have been developed against analogs of the transition-state complex of cocaine esterase, the enzyme that degrades cocaine in the body. These abzymes have esterase activity, and monthly injections of the abzyme drug can be used to rapidly destroy cocaine in the blood, thereby decreasing the dependence of addicted individuals. (See Chapter 7 for antibody structure.)

II. CATALYTIC MECHANISM OF CHYMOTRYPSIN

The enzyme chymotrypsin provides a good example of the strategies and amino acid side chains used by enzymes to lower the amount of activation energy required. *Chymotrypsin* is a digestive enzyme released into the intestine that catalyzes the hydrolysis of specific peptide bonds in denatured proteins. *Hydrolysis* is the use of water to lyse (break) a bond. Chymotrypsin is a member of the serine protease superfamily, enzymes that use a serine in the active site to form a covalent intermediate during proteolysis (the hydrolysis of a peptide bond in a protein). In the overall hydrolysis reaction, an OH^- from water is added to the carbonyl carbon of the peptide bond, and an H^+ to the N, thereby cleaving the bond (Fig. 8.8). The bond that is cleaved is called the *scissile bond.*

A. The Reaction in the Absence of Enzyme

In the reaction carried out in the absence of enzyme, the negatively charged hydroxyl group of water attacks the carbonyl carbon, which carries a partial positive charge. An unstable oxyanion tetrahedral transition-state complex is formed in which the oxygen atom carries a full negative charge. The rate of the chemical reaction in the absence of chymotrypsin is slow because there are too few OH^- molecules in H_2O with enough energy to form the transition-state complex and too few OH^- molecules colliding with the substrate at the right orientation.

B. Catalytic Strategies in the Reaction Catalyzed by Chymotrypsin

In the reaction catalyzed by chymotrypsin, the same oxyanion intermediate is formed by using the hydroxyl group of a serine residue for the attack instead of a free hydroxyl anion. The rate of the chymotrypsin-catalyzed reaction is faster because functional groups in the enzyme active site activate the attacking hydroxyl group, stabilize the oxyanion transition-state complexes, form a covalent intermediate, and destabilize the leaving group. The reaction takes place in two stages: (1) cleavage of the peptide bond in the denatured substrate protein and formation of a covalent acyl–enzyme intermediate (Fig. 8.9, steps 1 through 5), and (2) hydrolysis of the acyl–enzyme intermediate to release the remaining portion of the

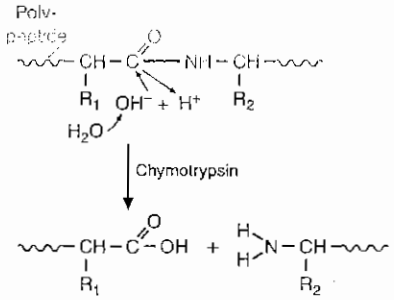
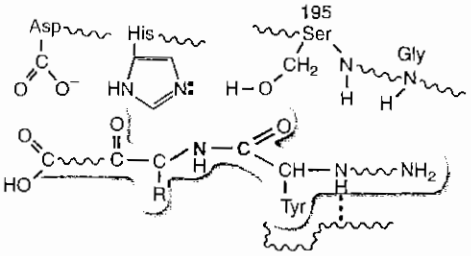
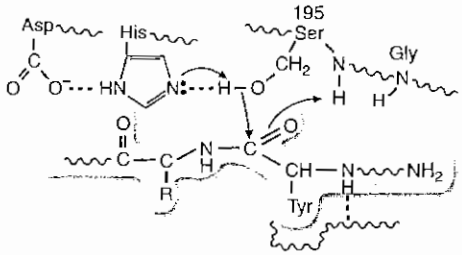


FIG. 8.8. Chymotrypsin hydrolyzes certain peptide bonds in proteins. The scissile bond is shown in *red.* The carbonyl carbon, which carries a partial positive charge, is attacked by a hydroxyl group from water. An unstable tetrahedral oxyanion intermediate is formed, which is the transition-state complex. As the electrons return to the carbonyl carbon, it becomes a carboxylic acid, and the remaining proton from water adds to the leaving group to form an amine.

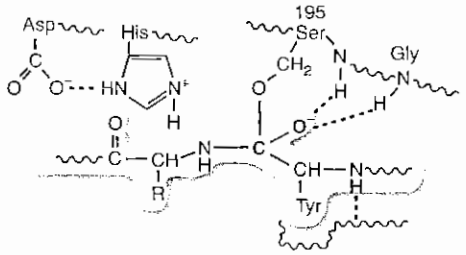
1. Substrate binding



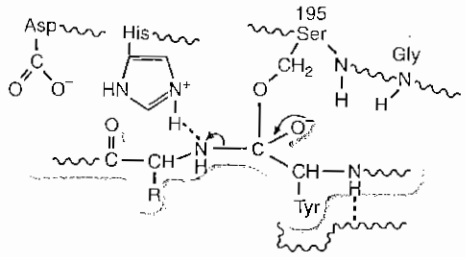
2. Histidine activates serine for nucleophilic attack



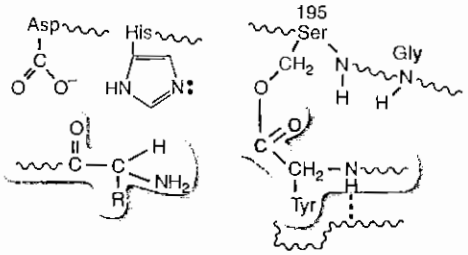
3. The oxyanion tetrahedral intermediate is stabilized by hydrogen bonds



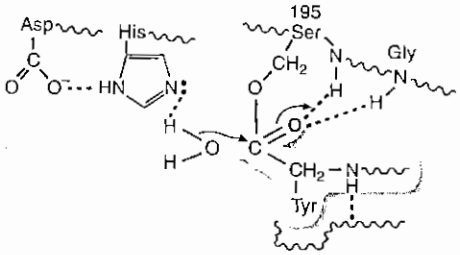
4. Cleavage of the peptide bond



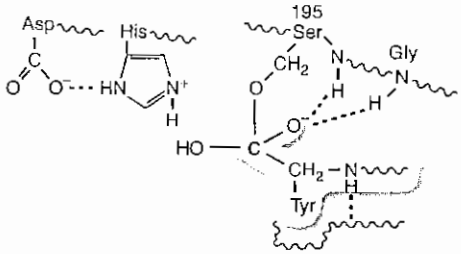
5. The covalent acyl–enzyme intermediate



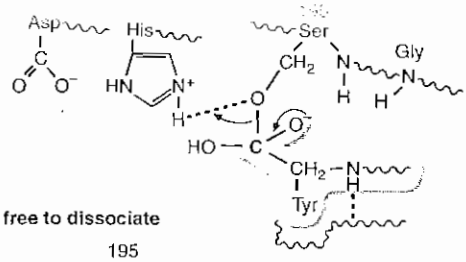
6. Water attacks the carbonyl carbon



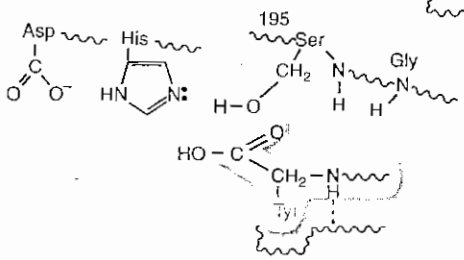
7. Second oxyanion tetrahedral intermediate



8. Acid catalysis breaks the acyl–enzyme covalent bond



9. The product is free to dissociate



substrate protein (see Fig. 8.9, steps 6 through 9). The names of the catalytic strategies employed in the various steps are in italics in the following paragraphs.

1. SPECIFICITY OF BINDING TO CHYMOTRYPSIN

Chymotrypsin hydrolyzes the peptide bond on the carbonyl side of a phenylalanine, tyrosine, or tryptophan in a denatured protein. The substrate recognition site consists of a hydrophobic binding pocket that holds the hydrophobic amino acid contributing the carbonyl group of the scissile bond (see Fig. 8.9, step 1). The substrate protein must be denatured to fit into the pocket and be held rigidly in place by glycines in the enzyme peptide backbone. Scissile-bond specificity is also provided by the subsequent steps of the reaction, such as moving serine 195 into attacking position (*proximity* and *orientation*).

2. FORMATION OF THE ACYL–ENZYME INTERMEDIATE IN CHYMOTRYPSIN

In the first stage of the reaction, the peptide bond of the denatured protein substrate is cleaved as an active-site serine hydroxyl group attacks the carbonyl carbon of the scissile bond (*nucleophilic catalysis—a nucleophile is a chemical group that is attracted to the positively charged nucleus*) (see Fig. 8.9, step 2). Aspartate and histidine cooperate in converting this hydroxyl group (with a partial negative charge on the oxygen) into a better nucleophilic attacking group by giving it a more negative charge. An active-site histidine acts as a base and abstracts a proton from the serine hydroxyl (*acid–base catalysis*). The protonated histidine is stabilized by the negative charge of a nearby aspartate. This step is necessary because the "normal" p*K* for the serine hydroxyl group is very high; at physiologic pH, conditions within the enzyme structure must be established that aid in removal of the proton and stabilization of the resulting oxygen anion.

The aspartate–histidine–serine combination, referred to as the *catalytic triad*, is an example of cooperative interactions between amino acid residues in the active site. The strong nucleophilic attacking group created by this charge-relay system has the same general effect on reaction rate as increasing the concentration of hydroxyl ions available for collision in the uncatalyzed reaction. This same combination of active-site residues is found in many serine proteases, particularly those proteins involved in protein digestion and blood clotting (see Chapter 44).

In the next step of the reaction sequence, an oxyanion tetrahedral transition-state complex is formed that is stabilized by hydrogen bonds with —NH groups in the peptide backbone (see Fig. 8.9, step 3). The original view of the way enzymes form transition-state complexes was that they stretched the bonds or distorted the bond angles of the reacting substrates. Nevertheless, most transition-state complexes, such as the oxyanion tetrahedral complex, are better described as showing "electronic strain," an electrostatic surface that would be highly improbable if it

Q: In the stomach, gastric acid decreases the pH to 1 to 2 in order to denature proteins through disruption of hydrogen bonding. The protease in the stomach, pepsin, is a member of the aspartate protease superfamily, enzymes that use two aspartate residues in the active site for acid–base catalysis of the peptide bond. Why can they not use histidine like chymotrypsin?

FIG. 8.9. Catalytic mechanism of chymotrypsin. The substrate (a denatured protein) is in the *shaded area*. **1.** As the substrate protein binds to the active site, serine 195 and histidine (his 57) are moved closer together and at the right orientation for the nitrogen electrons on histidine to attract the hydrogen of serine. Without this change of conformation on substrate binding, the catalytic triad cannot form. **2.** Histidine serves as a general base catalyst because it abstracts a proton from the serine, increasing the nucleophilicity of the serine-oxygen, which attacks the carbonyl carbon. **3.** The electrons of the carbonyl group form the oxyanion tetrahedral intermediate. The oxyanion is stabilized by the N–H groups of serine 195 and glycine in the chymotrypsin peptide backbone. **4.** The amide nitrogen in the peptide bond is stabilized by interaction with the histidine proton. Here, the histidine acts as a general acid catalyst. Because the electrons of the carbon–nitrogen peptide bond withdraw into the nitrogen, the electrons of the carboxyanion return to the substrate carbonyl carbon, resulting in cleavage of the peptide bond. **5.** The cleavage of the peptide bond results in formation of the covalent acyl–enzyme intermediate, and the amide half of the cleaved protein dissociates. **6.** The nucleophilic attack by H_2O on the carbonyl carbon is activated by histidine, whose nitrogen electrons attract a proton from water. **7.** The second tetrahedral oxyanion intermediate (the transition-state complex) is formed. It is again stabilized by hydrogen bonds with the peptide backbone bonds of glycine and serine. **8.** Because the histidine proton is donated to the electrons of the bond between the serine oxygen and the substrate carbonyl group, the electrons from the oxyanion return to the substrate carbon to form the carboxylic acid, and the acyl–enzyme bond is broken. **9.** The enzyme, as it releases substrate, returns to its original state.

To participate in general acid–base catalysis, the amino acid side chain must be able to extract a proton at one stage of the reaction and donate it back at another. Histidine (pK_a 6.0) would be protonated at this low pH and could not extract a proton from a potential nucleophile. However, aspartic acid, with a pK_a of about 2, can release protons at a pH of 2. The two aspartates work together to activate water through the removal of a proton to form the hydroxyl nucleophile.

were not stabilized by bonds with functional groups on the enzyme. *Stabilization of the transition-state complex* lowers its energy level and increases the number of molecules that reach this energy level.

Subsequently, the serine in the active site forms a full covalent bond with the carbon of the carbonyl group as the peptide bond is cleaved (*covalent catalysis*). The formation of a stable covalent intermediate is a catalytic strategy employed by many enzymes and often involves serine or cysteine residues. The covalent intermediate is subsequently hydrolyzed (*acid–base catalysis*). The dissociating products of an enzyme-catalyzed reaction are often "destabilized" by some degree of charge repulsion in the active site. In the case of chymotrypsin, the amino group formed after peptide-bond cleavage is destabilized or "uncomfortable" in the presence of the active-site histidine (*destabilization of developing product*).

3. HYDROLYSIS OF THE ACYL–CHYMOTRYPSIN INTERMEDIATE

The next sequence of events hydrolyzes the acyl–enzyme intermediate to release the bound carbonyl-side peptide (see Fig. 8.9, steps 6 through 9). The active-site histidine activates water to form an OH^- for a nucleophilic attack, resulting in a second oxyanion transition-state complex. When the histidine adds the proton back to serine, the reaction is complete, and the product dissociates.

C. Energy Diagram in the Presence of Chymotrypsin

The number of steps in real enzymatic reactions results in a "multibump" energy diagram (Fig. 8.10). At the initial stage of the reaction, a dip occurs because energy is provided by formation of the initial multiple weak bonds between the substrate and enzyme. As the reaction progresses, the curve rises because additional energy is required for formation of the transition-state complex. This

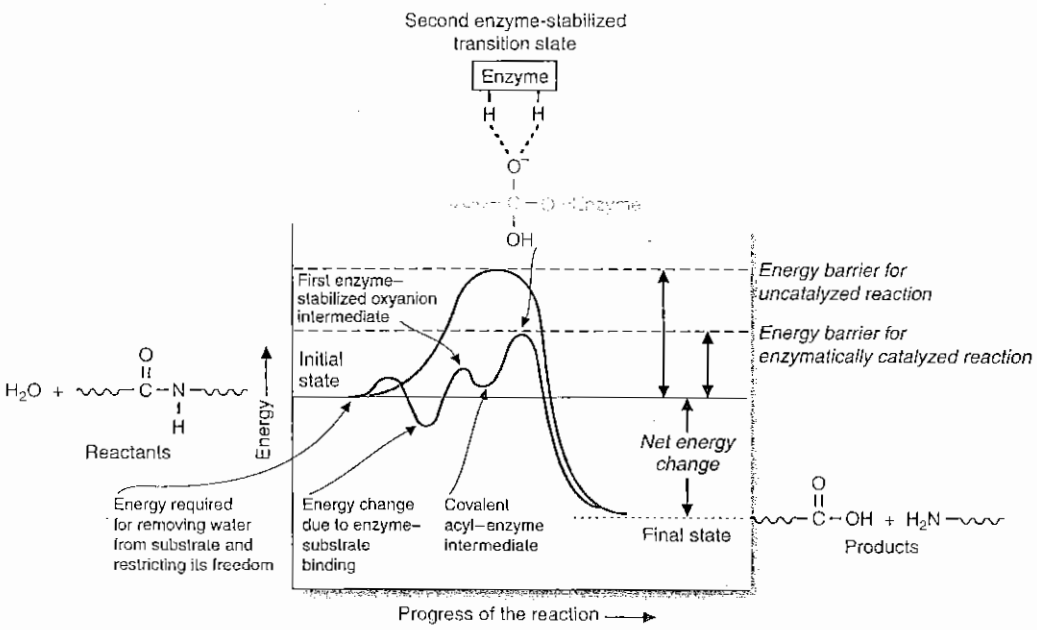


FIG. 8.10. A postulated energy diagram for the reaction catalyzed by chymotrypsin. In the presence of enzyme (*red*); in the absence of enzyme (*blue*). The energy barrier to the transition state is lowered in the enzyme-catalyzed reaction by the formation of additional bonds between the substrate and enzyme in the transition-state complex. The energy is provided by substrate binding to the enzyme. The enzyme does not, however, change the energy levels of the substrate or product.

energy is provided by the subsequent steps in the reaction, replacing the initial weak bonds with progressively tighter bonds. Semistable covalent intermediates of the reaction have lower energy levels than do the transition-state complexes and are present in the reaction diagram as dips in the energy curve. The final transition-state complex has the highest energy level in the reaction and is therefore the most unstable state. It can collapse back to substrates or decompose to form products.

III. FUNCTIONAL GROUPS IN CATALYSIS

The catalytic strategies employed by chymotrypsin to increase the reaction rate are common to many enzymes. One of these catalytic strategies, proximity and orientation, is an intrinsic feature of substrate binding and part of the catalytic mechanism of all enzymes. All enzymes also stabilize the transition state by electrostatic interactions, but not all enzymes form covalent intermediates.

Great variety occurs in the functional groups employed by different enzymes to carry out these catalytic strategies. Some enzymes, such as chymotrypsin, rely on amino acid residues within the active site. Other enzymes increase their repertoire by employing cofactors (nonprotein compounds that participate in the catalytic process) to provide a functional group with the right size, shape, and properties. They are generally divided into three categories: coenzymes, metal ions (e.g., Fe^{2+}, Mg^{2+}, or Zn^{2+}), and metallocoenzymes (similar to the Fe^{2+}-heme in hemoglobin, see Chapter 7).

A. Functional Groups on Amino Acid Side Chains

Almost all of the polar amino acids participate directly in catalysis in one or more enzymes (Table 8.1). Serine, cysteine, lysine, and histidine can participate in covalent catalysis. Histidine, because it has a pK_a that can donate and accept a proton at neutral pH, often participates in acid–base catalysis. Most of the polar amino acid side chains are nucleophilic and participate in nucleophilic catalysis by stabilizing more positively charged groups that develop during the reaction.

B. Coenzymes in Catalysis

Coenzymes (cofactors) are complex nonprotein organic molecules that participate in catalysis by providing functional groups, much like the amino acid side chains. In the human, they are usually (but not always) synthesized from vitamins. Each coenzyme is involved in catalyzing a specific type of reaction for a class of substrates with certain structural features. Coenzymes can be divided into two general classes: activation-transfer coenzymes and oxidation–reduction coenzymes.

Because most vitamins function as coenzymes, the symptoms of vitamin deficiencies reflect the loss of specific enzyme activities that depend on the coenzyme form of the vitamin. Thus, drugs and toxins that inhibit proteins required for coenzyme synthesis (e.g., vitamin transport proteins or biosynthetic enzymes) can cause the symptoms of a vitamin deficiency. This type of deficiency is called a *functional deficiency*, whereas an inadequate intake is called a *dietary deficiency*.

Most coenzymes are tightly bound to their enzymes and do not dissociate during the course of the reaction. However, a functional or dietary vitamin deficiency that decreases the level of a coenzyme will result in the presence of the apoenzyme in cells (an enzyme devoid of cofactor).

Ethanol is an "antivitamin" that decreases the cellular content of almost every coenzyme. For example, ethanol inhibits the absorption of thiamine, and acetaldehyde produced from ethanol oxidation displaces pyridoxal phosphate from its protein-binding sites, thereby accelerating its degradation.

Table 8.1 Some Functional Groups in the Active Site

Function of Amino Acid	*Enzyme Example*
Covalent intermediates	
Cysteine–SH	Glyceraldehyde 3-phosphate dehydrogenase
Serine–OH	Acetylcholinesterase
Lysine–NH_2	Aldolase
Histidine–NH	Phosphoglucomutase
Acid–base catalysis	
Histidine–NH	Chymotrypsin
Aspartate–COOH	Pepsin
Stabilization of anion formed during the reaction	
Peptide backbone–NH	Chymotrypsin
Arginine–NH	Carboxypeptidase A
Serine–OH	Alcohol dehydrogenase
Stabilization of cation formed during the reaction	
Aspartate–COO^-	Lysozyme

Although coenzymes look as though they should be able to catalyze reactions autonomously (on their own), they have almost no catalytic power when not bound to the enzyme. Why?

1. ACTIVATION-TRANSFER COENZYMES

The activation-transfer coenzymes usually participate directly in catalysis by forming a covalent bond with a portion of the substrate; the tightly held substrate moiety is then activated for transfer, addition of water, or some other reaction. The portion of the coenzyme that forms a covalent bond with the substrate is its functional group. A separate portion of the coenzyme binds tightly to the enzyme.

Thiamine pyrophosphate provides a good illustration of the manner in which coenzymes participate in catalysis (Fig. 8.11). It is synthesized in human cells from the vitamin thiamine by the addition of a pyrophosphate. This pyrophosphate provides negatively charged oxygen atoms that chelate Mg^{2+}, which then binds tightly to the enzyme. The functional group that extends into the active site is the reactive carbon atom with a dissociable proton (see Fig. 8.11). In all of the enzymes that use thiamine pyrophosphate, this reactive thiamine carbon forms a covalent bond with a substrate keto group while cleaving the adjacent carbon–carbon bond. However, each thiamine-containing enzyme catalyzes the cleavage of a different substrate (or group of substrates with very closely related structures).

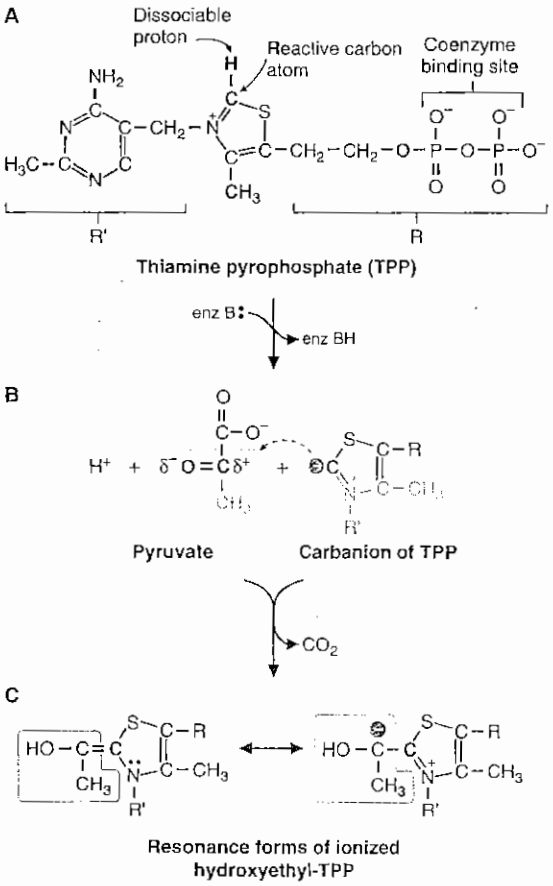


FIG. 8.11. The role of the functional group of thiamine pyrophosphate (the reactive carbon shown in *red*) in formation of a covalent intermediate. **A.** A base on the enzyme (*B*) abstracts a proton from thiamine, creating a carbanion (general acid–base catalysis). **B.** The carbanion is a strong nucleophile and attacks the partially positively charged keto group on the substrate. **C.** A covalent intermediate is formed, which, after decarboxylation, is stabilized by resonance forms. The uncharged intermediate is the stabilized transition-state complex.

Coenzymes have very little activity in the absence of the enzyme and very little specificity. The enzyme provides specificity, proximity, and orientation in the substrate recognition site, as well as other functional groups for stabilization of the transition state, acid–base catalysis, and so on. For example, thiamine is made into a better nucleophilic attacking group by a basic amino acid residue in the enzyme that removes the dissociable proton (EnzB in Fig. 8.11), thereby generating a negatively charged thiamine carbon anion. Later in the reaction, the enzyme returns the proton.

Coenzyme A (CoA), biotin, and pyridoxal phosphate are also activation-transfer coenzymes synthesized from vitamins. CoA (CoASH), which is synthesized from the vitamin pantothenate, contains an adenosine 3′,5′-bisphosphate that binds reversibly, but tightly, to a site on an enzyme (Fig. 8.12A). Its functional group, a sulfhydryl group at the other end of the molecule, is a nucleophile that always attacks carbonyl groups and forms acyl thioesters (in fact, the "A" in Co*A* stands for the *a*cyl group that becomes attached). Most coenzymes, such as functional groups on the enzyme amino acids, are regenerated during the course of the reaction. However, CoASH and a few of the oxidation–reduction coenzymes are transformed during the reaction into products that dissociate from the enzyme at the end of the reaction (e.g., CoASH is converted to an acyl-CoA derivative, and nicotinamide adenine dinucleotide [NAD^+] is reduced to NADH). These dissociating coenzymes are nonetheless classified as coenzymes rather than substrates because they are common to so many reactions, the original form is regenerated by subsequent reactions in a metabolic pathway, they are synthesized from vitamins, and the amount of coenzyme in the cell is nearly constant.

Biotin, which does not contain a phosphate group, is covalently bonded to a lysine in enzymes called *carboxylases* (see Fig. 8.12B). Its functional group is a nitrogen atom that covalently binds a CO_2 group in an energy-requiring reaction. This bound CO_2 group is activated for addition to another molecule. In the human, biotin functions only in carboxylation reactions.

Pyridoxal phosphate is synthesized from the vitamin pyridoxine, which is also called vitamin B_6 (see Fig. 8.12C). The reactive aldehyde group usually functions in enzyme-catalyzed reactions by forming a covalent bond with the amino groups on amino acids. The positively charged ring nitrogen withdraws electrons from a bond in the bound amino acid, resulting in cleavage of that bond. The enzyme participates by removing protons from the substrate and by keeping the amino acid and the pyridoxal group in a single plane to facilitate shuttling of electrons.

These coenzymes illustrate three features all activation-transfer coenzymes have in common: (1) a specific chemical group involved in binding to the enzyme, (2) a separate and different functional or reactive group that participates directly in the catalysis of one type of reaction by forming a covalent bond with the substrate, and (3) dependence on the enzyme for additional specificity of substrate and additional catalytic power.

2. OXIDATION–REDUCTION COENZYMES

A large number of coenzymes are involved in oxidation–reduction reactions catalyzed by enzymes categorized as *oxidoreductases*. When a compound is oxidized, it loses electrons. As a result, the oxidized carbon has fewer H atoms or gains an O atom. The reduction of a compound is the gain of electrons, which shows in its structure as the gain of H or loss of O. Some coenzymes, such as NAD^+ and flavin adenine dinucleotide (FAD), can transfer electrons together with hydrogen and have unique roles in the generation of ATP from the oxidation of fuels. Other oxidation–reduction coenzymes work with metals to transfer single electrons to oxygen. Vitamin E and vitamin C (ascorbic acid) are oxidation–reduction coenzymes that can act as antioxidants and protect against oxygen free radical injury. The different functions of oxidation–reduction coenzymes in metabolic pathways are explained in Chapters 19 through 22. A subclass of oxidoreductases is given the

For a substrate to react with a coenzyme, it must collide with a coenzyme at exactly the right angle. The probability of the substrate and coenzyme in free solution colliding in exactly the right place at the exactly right angle is very small. In addition to providing this proximity and orientation, enzymes contribute in other ways, such as activating the coenzyme by extracting a proton (e.g., thiamine pyrophosphate and coenzyme A) or polarizing the substrate to make it more susceptible to nucleophilic attack.

Many alcoholics like **Al Martini** develop thiamine deficiency because alcohol inhibits the transport of thiamine through the intestinal mucosal cells. In the body, thiamine is converted to thiamine pyrophosphate (TPP). TPP acts as a coenzyme in the decarboxylation of α-keto acids such as pyruvate and α-ketoglutarate (see Fig. 8.11) and in the utilization of pentose phosphates in the pentose phosphate pathway. As a result of thiamine deficiency, the oxidation of α-keto acids is impaired. Dysfunction occurs in the central and peripheral nervous system, the cardiovascular system, and other organs.

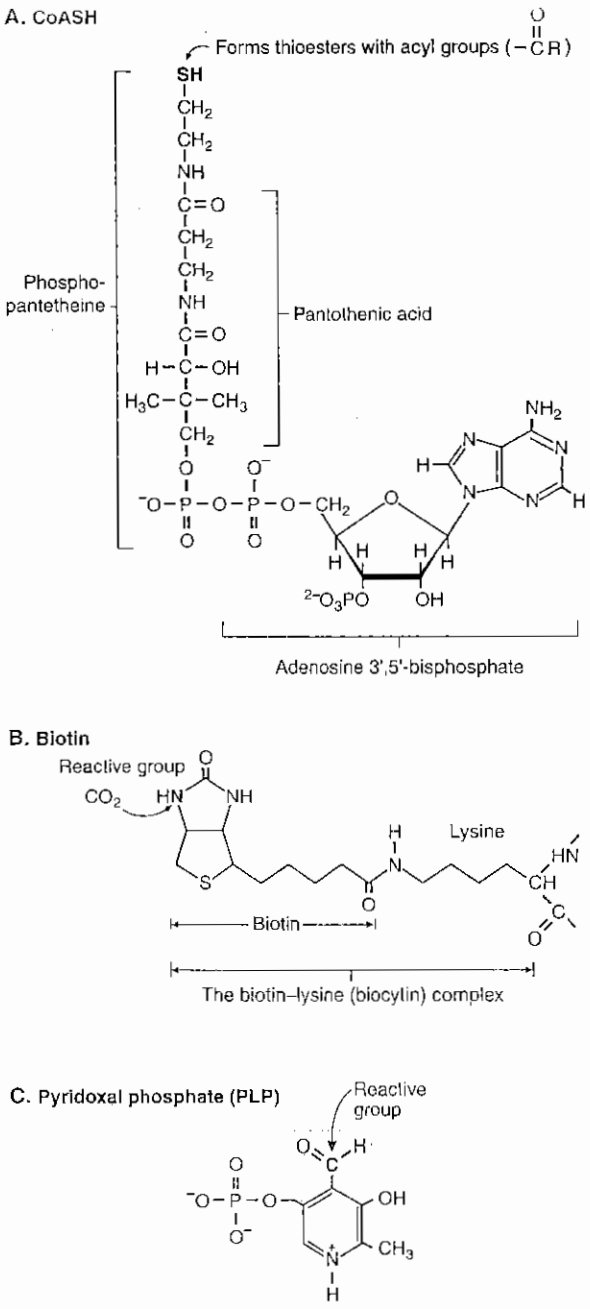


FIG. 8.12. Activation-transfer coenzymes. **A.** Coenzyme A (CoA or CoASH) and phos phopantetheine are synthesized from the vitamin pantothenate (pantothenic acid). The activ sulfhydryl group, shown in *red*, binds to acyl groups (e.g., acetyl, succinyl, or fatty acyl) t form thioesters. **B.** Biotin activates and transfers CO_2 to compounds in carboxylation reac tions. The reactive *N* is shown in *red*. Biotin is covalently attached to a lysine residue i the carboxylase enzyme. **C.** Reactive sites of pyridoxal phosphate. The functional group o pyridoxal phosphate is a reactive aldehyde (shown in the *yellow box*) that forms a covalen intermediate with amino groups of amino acids (a Schiff base). The positively charged pyri dine ring is a strong electron-withdrawing group that can pull electrons into it (electrophili catalysis).

name *dehydrogenases* because they transfer hydrogen (hydrogen atoms or hydride ions) from the substrate to an electron-accepting coenzyme such as NAD^+.

Oxidation–reduction coenzymes follow the same principles as activation-transfer coenzymes, except that they do not form covalent bonds with the substrate. Each coenzyme has a unique functional group that accepts and donates electrons and is specific for the form of electrons it transfers (e.g., hydride ions, hydrogen atoms, oxygen). A different portion of the coenzyme binds the enzyme. Like activation-transfer coenzymes, oxidation–reduction coenzymes are not good catalysts without participation from amino acid side chains on the enzyme.

The enzyme lactate dehydrogenase, which catalyzes the transfer of electrons from lactate to NAD^+, illustrates these principles (Fig. 8.13). The coenzyme NAD^+ is synthesized from the vitamin niacin (which forms the nicotinamide ring) and from ATP (which contributes an adenosine monophosphate [AMP]). The adenosine diphosphate (ADP) portion of the molecule binds tightly to the enzyme and causes conformational changes in the enzyme. The functional group of NAD^+ is the carbon on the nicotinamide ring opposite the positively charged nitrogen. This carbon atom accepts the hydride ion (a hydrogen atom that has two electrons) transferred from a specific carbon atom on the substrate. The H^+ from the substrate alcohol (-OH) group then dissociates, and a keto group (C=O) is formed. One of the roles

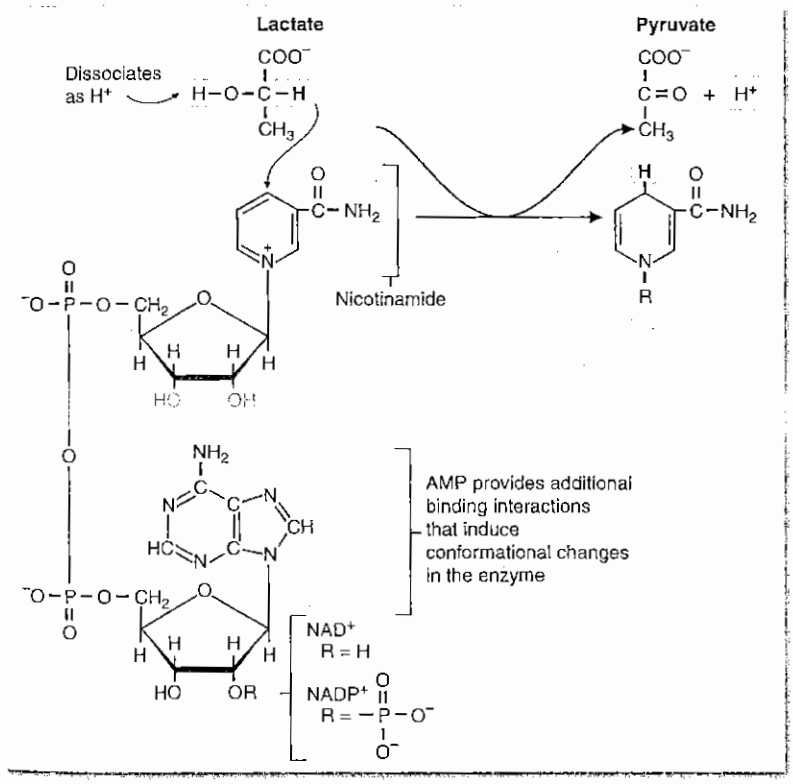


FIG. 8.13. The coenzyme NAD^+ accepting a hydride ion, shown in *red*, from lactate. NAD^+-dependent dehydrogenases catalyze the transfer of a hydride ion (H^-) from a carbon to NAD^+ in oxidation reactions such as the oxidation of alcohols to ketones or aldehydes to acids. The positively charged pyridine ring nitrogen of NAD^+ increases the electrophilicity of the carbon opposite it in the ring. This carbon then accepts the negatively charged hydride ion. The proton from the alcohol group is released into water. $NADP^+$ functions by the same mechanism, but it is usually involved in pathways of reductive synthesis.

In humans, most of ingested ethanol is oxidized to acetaldehyde in the liver by alcohol dehydrogenase (ADH):

$$\text{Ethanol} + NAD^+ \leftrightarrow \text{acetaldehyde} + NADH + H^+$$

ADH is active as a dimer, with an active site containing zinc present in each subunit. The human has at least seven genes that encode isozymes of ADH, each with a slightly different range of specificities for the alcohols it oxidizes.

The acetaldehyde produced from ethanol is highly reactive, toxic, and immunogenic. In **Al Martini** and other patients with chronic alcoholism, acetaldehyde is responsible for much of the liver injury associated with chronic alcoholism.

of the enzyme is to contribute a histidine nitrogen that can bind the dissociable proton on lactate, thereby making it easier for NAD^+ to pull off the other hydrogen with both electrons. Finally, NADH dissociates.

C. Metal Ions in Catalysis

Metal ions, which have a positive charge, contribute to the catalytic process by acting as electrophiles (electron-attracting groups). They assist in binding of the substrate, or they stabilize developing anions in the reaction. They can also accept and donate electrons in oxidation–reduction reactions.

The ability of certain metals to bind multiple ligands in their coordination sphere enables them to participate in binding substrates or coenzymes to enzymes. For example, Mg^{2+} plays a role in the binding of the negatively charged phosphate groups of thiamine pyrophosphate to anionic or basic amino acids in the enzyme (see Fig. 8.11). The phosphate groups of ATP are usually bound to enzymes through Mg^{2+} chelation.

The metals of some enzymes bind anionic substrates or intermediates of the reaction to alter their charge distribution, thereby contributing to catalytic power. The enzyme alcohol dehydrogenase (ADH), which transfers electrons from ethanol to NAD^+ to generate acetaldehyde and NADH, illustrates this role (Fig. 8.14). In the active site of ADH, an activated serine pulls a proton off the ethanol –OH group, leaving a negative charge on the oxygen that is stabilized by zinc. This electronic configuration allows the transfer of a hydride ion to NAD^+. Zinc is essentially fulfilling the same function in ADH that histidine fulfills in lactate dehydrogenase.

In the clinical laboratory, ethanol (in serum) is usually analyzed by coupling its oxidation to the formation of NADH using the enzyme alcohol dehydrogenase. NAD^+ has a low extinction coefficient at 340 nm; NADH has a much higher one. Thus, the increase in absorbance at 340 nm that occurs during the reaction indicates how much NADH was produced, which is directly proportional to the ethanol concentration in the serum. These reactions have been fully automated for the clinical lab.

D. Noncatalytic Roles of Cofactors

Cofactors sometimes play a noncatalytic structural role in certain enzymes, binding different regions of the enzyme together to form the tertiary structure. They can also serve as substrates that are cleaved during the reaction.

IV. OPTIMAL pH AND TEMPERATURE

If the activity of most enzymes is plotted as a function of the pH of the reaction, an increase of reaction rate is usually observed as the pH goes from a very acidic level to the physiologic range; a decrease of reaction rate occurs as the pH goes from the

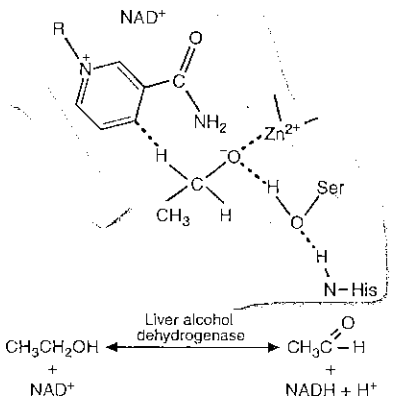


FIG. 8.14. Liver alcohol dehydrogenase (ADH) catalyzes the oxidation of ethanol (shown in *red*) to acetaldehyde. The active site of liver ADH contains a bound zinc atom, a serine side chain –OH, and histidine nitrogen that participate in the reaction. The histidine pulls an H+ off the active site serine, which pulls the H+ off of the substrate –OH group, leaving the oxygen with a negative charge that is stabilized by zinc.

physiologic range to a very basic range (Fig. 8.15). The shape of this curve in the acid region usually reflects the ionization of specific functional groups in the active site (or in the substrate) by the increase of pH, and the more general formation of hydrogen bonds important for the overall conformation of the enzyme. The loss of activity on the basic side usually reflects the inappropriate ionization of amino acid residues in the enzyme.

Most human enzymes function optimally at a temperature of approximately 37°C. An increase of temperature from 0° to 37°C increases the rate of the reaction by increasing the vibrational energy of the substrates. The maximum activity for most human enzymes occurs near 37°C because denaturation (loss of secondary and tertiary structure) occurs at higher temperatures.

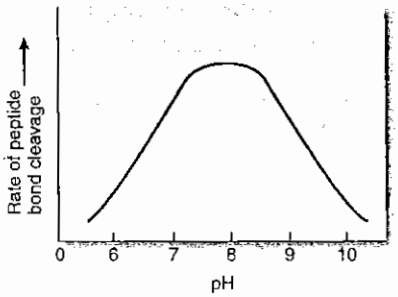


FIG. 8.15. pH profile of an enzyme. The rate of the reaction increases as the pH increases from 6 to 7.4. The exact shape of the curve depends on the protonation state of active-site amino acid residues or on the hydrogen bonding required for maintenance of three-dimensional structure in the enzyme. For the enzyme shown in the figure, the increase of reaction rate corresponds to deprotonation of the active-site histidine. At a pH >8.5, deprotonation of an amino-terminal $-NH_3^+$ alters the conformation at the active site and the activity decreases. Other enzymes might have a lower pH maximum, a broader peak, or retain their activity in the basic side of the curve.

V. MECHANISM-BASED INHIBITORS

Inhibitors are compounds that decrease the rate of an enzymatic reaction. Mechanism-based inhibitors mimic or participate in an intermediate step of the catalytic reaction. The term includes transition state analogs (see Section I.C) and compounds that can react irreversibly with functional groups in the active site.

A. Covalent Inhibitors

Covalent inhibitors form covalent or extremely tight bonds with functional groups in the active catalytic site. These functional groups are activated by their interactions with other amino acid residues and are therefore far more likely to be targeted by drugs and toxins than amino acid residues outside the active site.

The lethal compound *diisopropylphosphofluoridate* (diisopropylfluorophosphate [DFP]) is an organophosphorus compound that served as a prototype for the development of the nerve gas sarin and other organophosphorus toxins such as the insecticides malathion and parathion (Fig. 8.16). DFP exerts its toxic effect by forming a covalent intermediate in the active site of acetylcholinesterase, thereby preventing the enzyme from degrading the neurotransmitter acetylcholine. Once the covalent bond is formed, the inhibition by DFP is essentially irreversible, and activity can only be recovered as new enzyme is synthesized. DFP also inhibits many other enzymes that use serine for hydrolytic cleavage (such as the serine proteases), but the inhibition is not as lethal.

Aspirin (acetylsalicylic acid) provides an example of a pharmacologic drug that exerts its effect through the covalent acetylation of an active site serine in the enzyme prostaglandin endoperoxide synthase (cyclooxygenase). Aspirin resembles a portion of the prostaglandin precursor that is a physiologic substrate for the enzyme.

B. Transition-State Analogs and Compounds that Resemble Intermediate Stages of the Reaction

Transition-state analogs are extremely potent and specific inhibitors of enzymes because they bind more tightly to the enzyme than do substrates or products. Drugs cannot be designed that precisely mimic the transition state because of its highly unstable structure. However, substrates undergo progressive changes in their overall electrostatic structure during the formation of a transition-state complex, and effective drugs often resemble an intermediate stage of the reaction more closely than they resemble the substrate. Medical literature often refers to such compounds as substrate analogs, even though they bind more tightly than substrates.

1. PENICILLIN

The antibiotic penicillin is a transition-state analog that binds very tightly to glycopeptidyl transferase, an enzyme required by bacteria for synthesis of the cell wall (Fig. 8.17). Glycopeptidyl transferase catalyzes a partial reaction with penicillin

The symptoms experienced by **Dennis Veere** resulted from inhibition of acetylcholinesterase. Acetylcholinesterase cleaves the neurotransmitter acetylcholine to acetate and choline in the postsynaptic terminal, thereby terminating the transmission of the neural signal (see Fig. 8.16). Malathion is metabolized in the liver to a toxic derivative (malaoxon) that binds to the active-site serine in acetylcholinesterase and other enzymes, an action similar to that of diisopropylfluorophosphate. As a result, acetylcholine accumulates and overstimulates the autonomic nervous system (the involuntary nervous system, including the heart, blood vessels, and glands), thereby accounting for Dennis's vomiting, abdominal cramps, salivation, and sweating. Acetylcholine is also a neurotransmitter for the somatic motor nervous system, where its accumulation resulted in Dennis's involuntary muscle twitching (muscle fasciculations).

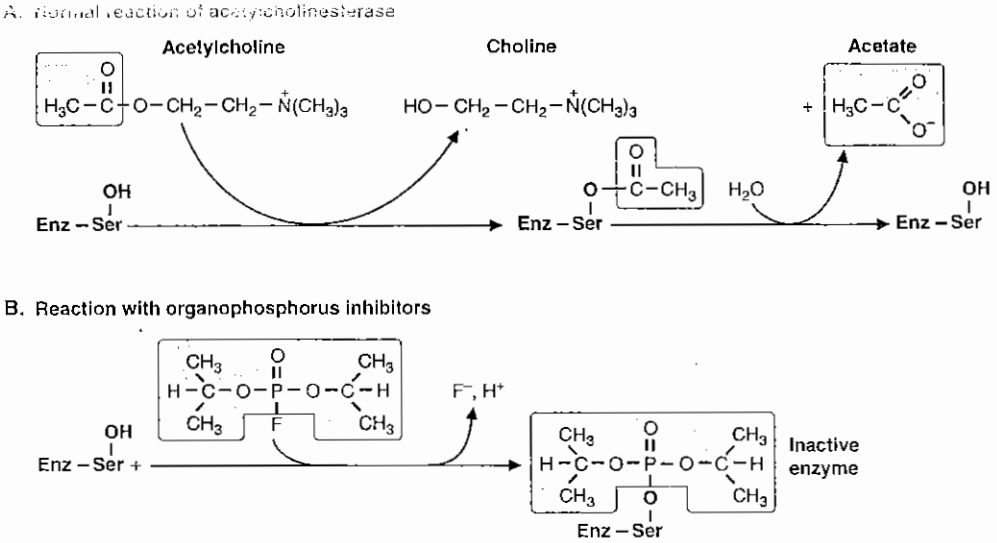


FIG. 8.16. **A.** Acetylcholinesterase normally catalyzes inactivation of the neurotransmitter acetylcholine in a hydrolysis reaction. The active-site serine forms a covalent intermediate with a portion of the substrate during the course of the reaction. **B.** Diisopropylphosphofluoridate (DFP), the ancestor of current organophosphorus nerve gases and pesticides, inactivates acetylcholinesterase by forming a covalent complex with the active-site serine that cannot be hydrolyzed by water. The result is that the enzyme cannot carry out its normal reaction, and acetylcholine accumulates.

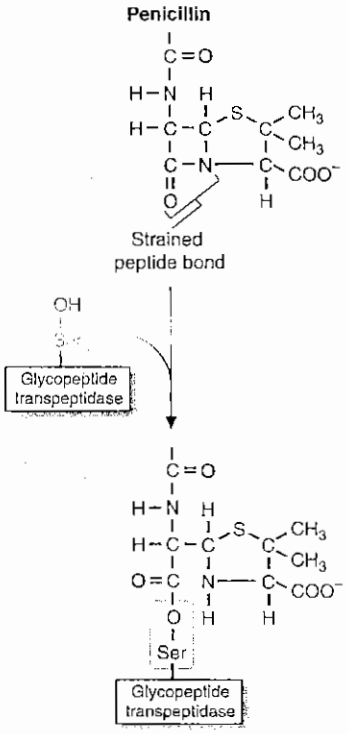


FIG. 8.17. The antibiotic penicillin inhibits the bacterial enzyme glycopeptide transpeptidase. The transpeptidase is a serine protease involved in cross-linking components of bacterial cell walls and is essential for bacterial growth and survival. It normally cleaves the peptide bond between two D-alanine residues in a polypeptide. Penicillin contains a strained peptide bond within the β-lactam ring that resembles the transition state of the normal cleavage reaction, and thus penicillin binds very readily in the enzyme active site. As the bacterial enzyme attempts to cleave this penicillin peptide bond, penicillin becomes irreversibly covalently attached to the enzyme's active-site serine, thereby inactivating the enzyme.

that covalently attaches penicillin to its own active-site serine. The reaction is favored by the strong resemblance between the peptide bond in the β-lactam ring of penicillin and the transition-state complex of the natural transpeptidation reaction. Active-site inhibitors such as penicillin that undergo partial reaction to form irreversible inhibitors in the active site are sometimes termed *suicide inhibitors*.

2. ALLOPURINOL

Allopurinol, a drug used to treat gout, decreases urate production by inhibiting xanthine oxidase. This inhibition provides an example of an enzyme that commits suicide by converting a drug to a transition-state analog. The normal physiologic function of xanthine oxidase is the oxidation of hypoxanthine to xanthine and xanthine to uric acid (urate) in the pathway for degradation of purines (Fig. 8.18). The enzyme contains a molybdenum–sulfide (Mo-S) complex that binds the substrates and transfers the electrons required for the oxidation reactions. Xanthine oxidase oxidizes the drug allopurinol to oxypurinol, a compound that binds very tightly to a molybdenum–sulfide complex in the active site. As a result, the enzyme has committed suicide and is unable to carry out its normal function—the generation of uric acid (urate).

Lotta Topaigne is being treated with allopurinol for gout, which is caused by an accumulation of sodium urate crystals in joints and joint fluid, particularly in the ankle and great toe. Allopurinol is a suicide inhibitor of the enzyme xanthine oxidase, which is involved in the degradation of purine nucleotides adenosine monophosphate (AMP) and guanosine monophosphate (GMP) to uric acid (urate). Although hypoxanthine levels increase in the presence of allopurinol, hypoxanthine does not participate in urate crystal formation and precipitation at this concentration. It is excreted in the urine.

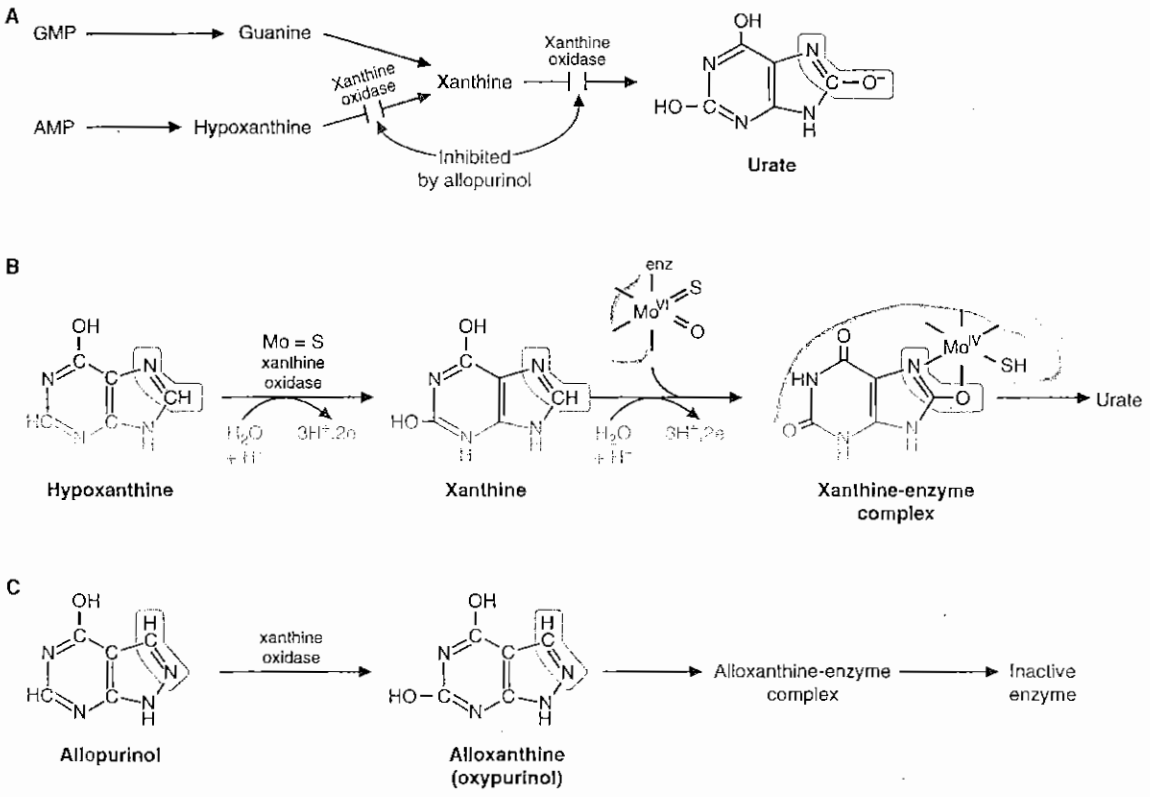


FIG. 8.18. Allopurinol is a suicide inhibitor of xanthine oxidase. **A.** Xanthine oxidase catalyzes the oxidation of hypoxanthine to xanthine and xanthine to uric acid (urate) in the pathway for degradation of purine nucleotides. **B.** The oxidations are performed by a molybdenum–oxo–sulfide coordination complex in the active site that complexes with the group being oxidized. Oxygen is donated from water. The enzyme can work either as an oxidase (O_2 accepts the $2e^-$ and is reduced to H_2O_2) or as a dehydrogenase (NAD^+ accepts the $2e^-$ and is reduced to NADH). The figure only indicates that $2e^-$ is generated during the course of the reaction. **C.** Xanthine oxidase is able to perform the first oxidation step and convert allopurinol to alloxanthine (oxypurinol). As a result, the enzyme has committed suicide; the oxypurinol remains bound in the molybdenum coordination sphere, where it prevents the next step of the reaction. The portion of the purine ring in *green* indicates the major structural difference between hypoxanthine, xanthine, and allopurinol.

C. Heavy Metals

Heavy-metal toxicity is caused by tight binding of a metal such as mercury (Hg), lead (Pb), aluminum (Al), or iron (Fe) to a functional group in an enzyme. Heavy metals are relatively nonspecific for the enzymes they inhibit, particularly if the metal is associated with high-dose toxicity. Mercury, for example, binds to so many enzymes, often at reactive sulfhydryl groups in the active site, that it has been difficult to determine which of the inhibited enzymes is responsible for mercury toxicity. Lead provides an example of a metal that inhibits through replacing the normal functional metal in an enzyme, such as calcium, iron, or zinc. Its developmental and neurologic toxicity may be caused by its ability to replace Ca^{2+} in several regulatory proteins that are important in the central nervous system and other tissues.

CLINICAL COMMENTS

Dennis Veere. Dennis Veere survived his malathion intoxication because he had ingested only a small amount of the chemical, vomited shortly after the agent was ingested, and was treated rapidly in the emergency room. Lethal doses of oral malathion are estimated at 1 g/kg of body weight for humans. Once it has been ingested, the liver converts malathion to the toxic reactive compound, malaoxon, by replacing the sulfur with oxygen. Malaoxon then binds to the active site of acetylcholinesterase and reacts to form the covalent intermediate. Unlike the complex formed between diisopropylfluorophosphate and acetylcholinesterase, this initial acylenzyme intermediate is reversible. However, with time, the enzyme-inhibitor complex "ages" (dealkylation of the inhibitor and enzyme modification) to form an irreversible complex. Emergency room physicians used the drug pralidoxime (an oxime) to reactivate the acetylcholinesterase in Dennis before the aged complex formed. They also used intravenous atropine, an anticholinergic (antimuscarinic) agent, to antagonize the action of the excessive amounts of acetylcholine accumulating in cholinergic receptors throughout his body.

After several days of intravenous therapy, the signs and symptoms of acetylcholine excess abated, and therapy was slowly withdrawn. Dennis made an uneventful recovery.

Lotta Topaigne. Within several days of starting allopurinol therapy, Ms. Topaigne's serum uric acid level began to decrease. Several weeks later, the level in her blood was normal. Nevertheless, while Lotta was adapting to allopurinol therapy, she experienced a mild gout attack, which was treated with a low dose of colchicine (see Chapter 10).

At low concentrations of ethanol, liver alcohol dehydrogenase is the major route of ethanol oxidation to acetaldehyde, a highly toxic chemical. Acetaldehyde not only damages the liver, it can enter the blood and potentially damage the heart and other tissues. At low ethanol intakes, much of the acetaldehyde produced is safely oxidized to acetate in the liver by acetaldehyde dehydrogenases.

Al Martini. Al Martini was admitted to the hospital after intravenous thiamine was initiated at a dose of 100 mg/day (compared with an RDA of 1.4 mg/day). His congestive heart failure was believed to be the result, in part, of the cardiomyopathy (heart muscle dysfunction) of acute thiamine deficiency known as *beriberi heart disease*. This nutritional cardiac disorder and the peripheral nerve dysfunction usually respond to thiamine replacement. However, an alcoholic cardiomyopathy can also occur in well-nourished patients with adequate thiamine levels. Exactly how ethanol, or its toxic metabolite acetaldehyde, causes alcoholic cardiomyopathy in the absence of thiamine deficiency is unknown.

BIOCHEMICAL COMMENTS

Basic Reactions and Classes of Enzymes. In the following chapters, students will be introduced to various reaction pathways and enzyme names. Although it may seem that the number of reactions is infinite, many of these reactions are similar and occur frequently in different pathways.

Recognition of the type of reaction can aid in remembering the pathways and enzyme names, thereby reducing the amount of memorization required. You may wish to use this section for reference as you go through your first biochemical pathways.

The Enzyme Commission (EC) has divided the basic reaction types and the enzymes catalyzing them into six broad-numbered classes: (1) oxidoreductases, (2) transferases, (3) hydrolases, (4) lyases, (5) isomerases, and (6) ligases. Each broad class of enzymes includes subsets of enzymes with a systematic name and a common name (e.g., dehydrogenases and kinases). For example, glucokinase (common name) has the systematic name ATP: D-hexose 6-phosphotransferase, and its EC number is EC 2.7.1.2. The first "2" refers to the general class (transferase) of enzyme, followed by a period. The "7" refers to the specific number of subclasses within the transferase family of enzymes (in this case, the class that transfers a phosphate). The "1" denotes transfer to an alcohol receptor, and the final "2" yields a specific enzyme number for glucokinase.

Oxidoreductases. Oxidation–reduction reactions are very common in biochemical pathways and are catalyzed by a broad class of enzymes called *oxidoreductases*. Whenever an oxidation–reduction reaction occurs, at least one substrate gains electrons and becomes reduced, and another substrate loses electrons and becomes oxidized. One subset of reactions is catalyzed by dehydrogenases, which accept and donate electrons in the form of hydride ions (H^-) or hydrogen atoms. Usually, an electron-transferring coenzyme, such as NAD^+/NADH, acts as an electron donor or acceptor (e.g., see Figs. 8.13 and 8.14).

In another subset of reactions, O_2 donates either one or both of its oxygen atoms to an acceptor (e.g., see xanthine oxidase, Fig. 8.18). When this occurs, O_2 becomes reduced, and an electron donor is oxidized. Enzymes participating in reactions with O_2 are called *hydroxylases* and *oxidases* when one oxygen atom is incorporated into a substrate and the other oxygen atom into water, or both atoms are incorporated into water. They are called *oxygenases* when both atoms of oxygen are incorporated into the acceptor. Most hydroxylases and oxidases require metal ions, such as Fe^{2+}, for electron transfer.

Transferases. Transferases catalyze group transfer reactions—the transfer of a functional group from one molecule to another. If the transferred group is a high-energy phosphate (as shown in Fig. 8.3), the enzyme is a kinase; if the transferred group is a carbohydrate residue, the enzyme is a glycosyltransferase; and if it is a fatty acyl group, the enzyme is an acyltransferase. A common feature of these reactions is that the group being transferred exists as a good leaving group on the donor molecule.

Another subset of group transfer reactions consists of transaminations (Fig. 8.19A). In this type of reaction, the nitrogen group from an amino acid is donated to an α-keto acid, forming a new amino acid and the α-keto acid corresponding to the donor amino acid. Enzymes that catalyze this last type of reaction are called *transaminases* or *aminotransferases*. The coenzyme pyridoxal phosphate is required for all transaminases (see Fig. 8.12C).

When the physiologically important aspect of the reaction is the compound synthesized, the transferase may be called a *synthase*. For example, the enzyme commonly called *glycogen synthase* transfers a glucosyl residue from UDP-glucose to the end of a glycogen molecule. Its systematic name is UDP-glucose-glycogen glycosyltransferase.

Hydrolases. In hydrolysis reactions, C–O, C–N, or C–S bonds are cleaved by the addition of H_2O in the form of OH^- and H^+ to the atoms forming the bond (see, e.g., Fig. 8.8). The enzyme class names specify the group being cleaved (e.g., the enzyme commonly named chymotrypsin is a protease, a hydrolase that cleaves peptide bonds in proteins).

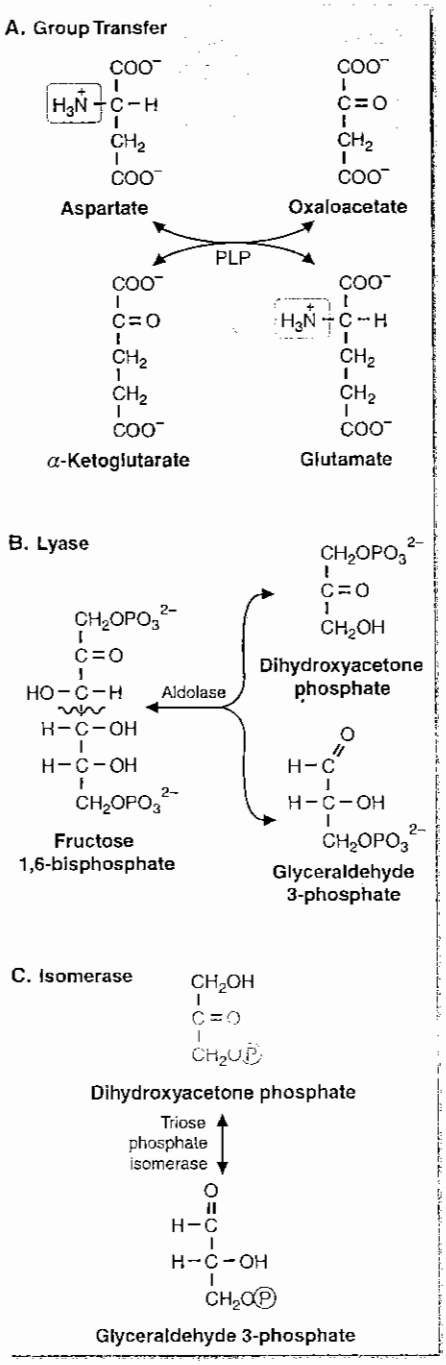


FIG. 8.19. A. An example of a group transfer reaction—a transamination. Pyridoxal phosphate (PLP) on aspartate aminotransferase transfers an amino group from aspartate to the α-keto acid (α-ketoglutarate) to form a new amino acid (glutamate). The enzyme was formerly called *glutamate-oxaloacetate transaminase*. B. An example of a lyase. Aldolases catalyze carbon–carbon bond cleavage in reactions that are usually reversible. In glycolysis, the enzyme fructose 1,6-bisphosphate aldolase cleaves a carbon–carbon bond in fructose 1,6-bisphosphate. Aldolases have a lysine ε-amino group in the active site that participates in the reaction. C. An example of an isomerase. Isomerases rearrange atoms within a molecule. In the pathway of glycolysis, triose phosphate isomerase converts dihydroxyacetone phosphate to glyceraldehyde 3-phosphate by rearranging hydrogen atoms. No other substrates or products of the reaction exist.

Lyases. The lyase class of enzymes consists of a diverse group of enzymes that cleave C–C, C–O, and C–N bonds by means other than hydrolysis or oxidation. Some of the enzymes that catalyze C–C bond cleavage are called aldolases, decarboxylases (when carbon dioxide is released from a substrate), and thiolases (when the sulfur-containing nucleophile of cysteine or CoASH is used to break a carbon–carbon bond) (see Fig. 8.19B). The structures amenable to carbon–carbon bond cleavage usually require a carbonyl carbon that can act as an electron sink to stabilize the carbanion formed transiently when the carbon–carbon bond breaks.

This broad class of enzymes also includes dehydratases and many synthases. Dehydratases remove the elements of water from two adjacent carbon–carbon bonds to form a double bond. Certain enzymes in this group, such as certain group transferases, are commonly called synthases when the physiologically important direction of the reaction favors the formation of a carbon–carbon bond (e.g., citrate synthase).

Isomerases. Many biochemical reactions simply rearrange the existing atoms of a molecule, that is, create isomers of the starting material (see Fig. 8.19C). Enzymes that rearrange the bond structure of a compound are called *isomerases*, whereas enzymes that catalyze movement of a phosphate from one atom to another are called *mutases*.

Ligases. Ligases synthesize C–C, C–S, C–O, and C–N bonds in reactions coupled to the cleavage of a high-energy phosphate bond in ATP or another nucleotide. Carboxylases, for example, add CO_2 to another compound in a reaction that requires ATP cleavage to provide energy (see Fig. 8.12B). Most carboxylases require the coenzyme biotin. Other ligases are named *synthetases* (e.g., fatty acyl CoA synthetase). Synthetases differ from the synthases mentioned under "Lyases" and "Transferases" in that synthetases derive the energy for new bond formation from cleavage of high-energy phosphate bonds, and synthases use a different source of energy.

Key Concepts

- Enzymes are proteins that act as catalysts—molecules that can accelerate the rate of a reaction.
- Enzymes are specific for various substrates due to the selective nature of the binding sites on the enzyme.
- The catalytic (active) site is the portion of the enzyme molecule at which the reaction occurs.
- Enzymes accelerate reaction rates by decreasing the amount of energy required to reach a high-energy intermediate stage of the reaction known as the transition-state complex. This is referred to as lowering the energy of activation.
- Enzymes use functional groups at the active site provided by coenzymes, metals, or amino acid residues to perform catalysis.
- Enzymes use general acid–base catalysis, formation of covalent intermediates, and transition-state stabilization as various mechanisms to accelerate reaction rates.
- Many drugs and toxins act by inhibiting enzymes.
- Enzymes can be regulated to control reaction rates through various mechanisms.
- Diseases discussed in this chapter are summarized in Table 8.2.

Table 8.2 Diseases Discussed in Chapter 8

Disorder or Condition	*Genetic or Environmental*	*Comments*
Malathion poisoning	Environmental	Inhibition of acetylcholinesterase at neuromuscular junctions, leading to acetylcholine accumulation at the junction and overstimulation of the autonomic nervous system.
Gout	Both	Accumulation of uric acid in blood, leading to precipitation in joints and severe pain and discomfort.
Thiamin deficiency (beriberi heart disease)	Environmental	Leads to lack of energy production due to reduced activity of key enzymes, and can lead to the Wernicke-Korsakoff syndrome. Brought about by alcoholism, as manifest by a poor diet, and ethanol inhibition of thiamine transport through the intestinal mucosa.

REVIEW QUESTIONS—CHAPTER 8

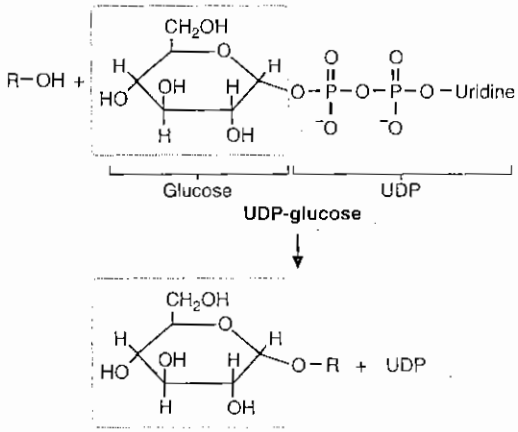
The following questions cover material from Chapters 6 and 7 as well as Chapter 8 (including Biochemical Comments).

1. A patient was born with a congenital mutation in an enzyme that severely affected its ability to bind an activation-transfer coenzyme. As a consequence, which one of the following is most likely to occur?
   A. The enzyme will be unable to bind the substrate of the reaction.
   B. The enzyme will be unable to form the transition-state complex.
   C. The enzyme will normally use a different activation-transfer coenzyme.
   D. The enzyme will normally substitute the functional group of an active-site amino acid residue for the coenzyme.
   E. The reaction may be carried out by the free coenzyme, provided the diet carries an adequate amount of its vitamin precursor.

2. An individual had a congenital mutation in glucokinase in which a proline was substituted for a leucine on a surface helix far from the active site but within the hinge region of the actin fold. This mutation would be expected to have which one of the following effects?
   A. It would have no effect on the rate of the reaction because it is not in the active site.
   B. It would have no effect on the rate of the reaction because proline and leucine are both nonpolar amino acids.
   C. It would have no effect on the number of substrate molecules reaching the transition state.
   D. It would probably affect the binding of ATP or a subsequent step in the reaction sequence.
   E. It would probably cause the reaction to proceed through an alternate mechanism.

3. Lysozyme is an enzyme that cleaves glycosidic linkages in bacterial cell walls. The pH optimum of the purified enzyme is 5.2. There are two acidic residues at the active site of lysozyme (E35 and D52) that are required for enzyme activity. The pK_a of E35 is 5.9, whereas the pK_a of D52 is 4.5. What are the primary ionization states of these two residues at the pH optimum of the enzyme?
   A. E35 is protonated, D52 is ionized.
   B. E35 is protonated, D52 is protonated.
   C. E35 is ionized, D52 is protonated.
   D. E35 is ionized, D52 is ionized.
   E. This cannot be determined from the information provided.

Questions 4 and 5 refer to the following reaction:



4. The type of reaction shown fits into which of the following classifications?
   A. Group transfer
   B. Isomerization
   C. Carbon–carbon bond breaking
   D. Carbon–carbon bond formation
   E. Oxidation–reduction

5. The type of enzyme that catalyzes this reaction is which one of the following?
   A. Kinase
   B. Dehydrogenase
   C. Glycosyltransferase
   D. Transaminase
   E. Isomerase

9 Regulation of Enzymes

In the human body, thousands of diverse enzymes are regulated to fulfill their individual functions without waste of dietary components. Thus, with changes in our physiologic state, time of eating, environment, diet, or age, the rates of some enzymes must increase and those of others decrease. In this chapter, we describe the mechanisms for regulating enzyme activity and the strategies employed to regulate the metabolic pathways in which they reside.

Regulation Matches Function. *Changes in the rate of a metabolic pathway occur because at least one enzyme in that pathway, the regulatory enzyme, has been activated or inhibited, or the amount of enzyme has increased or decreased. Regulatory enzymes usually catalyze the rate-limiting, or slowest, step in the pathway, so that increasing or decreasing their rate changes the rate of the entire pathway (Fig. 9.1). The mechanisms used to regulate the rate-limiting enzyme in a pathway reflect the function of the pathway.*

Substrate Concentration. *The rate of all enzymes depends on substrate concentration. Enzymes exhibit* **saturation kinetics**; *their rate increases with increasing substrate concentration [S], but it reaches a maximum velocity (V_{max}) when the enzyme is saturated with substrate. For many enzymes, the* **Michaelis-Menten equation** *describes the relationship among v_i (the initial velocity of a reaction), [S], V_{max}, and the K_m (the substrate concentration at which $v_i = \frac{1}{2}V_{max}$).*

Reversible Inhibition. *Enzymes are reversibly inhibited by* **structural analogs** *and* **products**. *These inhibitors are classified as* **competitive**, **noncompetitive**, *or* **uncompetitive**, *depending on their effect on formation of the* **enzyme–substrate complex**.

Allosteric Enzymes. **Allosteric activators** *or* **inhibitors** *are compounds that bind at sites other than the active catalytic site and regulate the enzyme by* [illegible] *conformational changes that affect the catalytic site.*

Covalent Modification. *Enzyme activity also may be regulated by a covalent modification, such as* **phosphorylation** *of a serine, threonine, or tyrosine residue by a* **protein kinase**.

Protein–Protein Interactions. *Enzyme activity can be modulated through the reversible binding of a* **modulator protein**, *such as* **Ca^{2+} calmodulin**. **Monomeric G proteins** *(guanosine triphosphate [GTP]-binding proteins) activate target proteins through reversible binding.*

Zymogen Cleavage. *Some enzymes are synthesized as inactive precursors, called zymogens, which are activated by* **proteolysis** *(e.g., the digestive enzyme chymotrypsin).*

Changes in Enzyme Concentration. *The concentration of an enzyme can be regulated by changes in the rate of enzyme synthesis (e.g., induction of gene transcription) or the rate of degradation.*

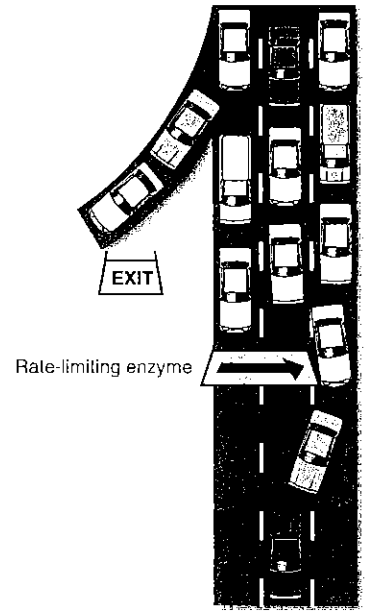


FIG. 9.1. The flux of substrates down a metabolic pathway is analogous to cars traveling down a highway. The rate-limiting enzyme is the portion of the highway that is narrowed to one lane by a highway barrier. This single portion of the highway limits the rate at which cars can arrive at their final destination miles later. Cars will back up before the barrier (similar to the increase in concentration of a precursor when a rate-limiting enzyme is inhibited). Some cars may exit and take an alternate route (similar to precursors entering another metabolic pathway). Moving the barrier just a little to open an additional lane is like activating a rate-limiting enzyme: It increases flow through the entire length of the pathway.

Regulation of Metabolic Pathways. The regulatory mechanisms for the rate-limiting enzyme of a pathway always reflect the function of the pathway in a particular tissue. In ***feedback regulation****, the end product of a pathway directly or indirectly controls its own rate of synthesis; in* ***feed-forward regulation****, the substrate controls the rate of the pathway.* ***Biosynthetic and degradative*** *pathways are controlled through different but* ***complementary regulation****. Pathways are also regulated through* ***compartmentation*** *of enzymes unique to specific pathways.*

When **Al Martini** was stopped by police, he was required to take a Breathalyzer test. The Breathalyzer analyzes ethanol levels in expired air (assuming a ratio of 1 part ethanol in expired air to 2,000 parts ethanol in the blood). Once the sample enters the Breathalyzer, it is reacted with sulfuric acid, silver nitrate, and potassium dichromate. If ethanol is present, it will react with the potassium dichromate to form potassium sulfate, chromium sulfate, acetic acid, and water. Potassium dichromate generates a reddish brown color, whereas chromium sulfate is light green. In the Breathalyzer device, the reacted sample is compared with a nonreacted sample, and the difference in light absorption (due to the different colors) is converted to an electrical current. The extent of current generated will lead to a determination of blood alcohol level (i.e., if there is no ethanol in the sample, no current will be generated). Other forms of gaseous ethanol determination use infrared spectroscopy or a fuel cell.

Al Martini was not able to clear his blood ethanol rapidly enough to stay within the legal limit for driving. Ethanol is cleared from the blood at about 0.5 oz/h (15 mg/dL/h). Liver metabolism accounts for more than 90% of ethanol clearance from the blood. The major route of ethanol metabolism in the liver is the enzyme liver alcohol dehydrogenase (ADH), which oxidizes ethanol to acetaldehyde with generation of NADH.

$$\text{Ethanol} + NAD^+ \rightarrow \text{acetaldehyde} + NADH + H^+$$

The multienzyme complex MEOS (microsomal ethanol oxidizing system), which is also called cytochrome P450-2E1, provides an additional route for ethanol oxidation to acetaldehyde in the liver.

THE WAITING ROOM

Al Martini is a 44-year-old man who has been an alcoholic for the past 5 years. He was recently admitted to the hospital for congestive heart failure (see Chapter 8). After being released from the hospital, he continued to drink. One night, he arrived at a friend's house at 7:00 P.M. Between his arrival and 11:00 P.M., he drank four beers and five martinis (for a total ethanol consumption of 9.5 oz). His friends encouraged him to stay an additional hour and drink coffee to sober up. Nevertheless, he ran his car off the road on his way home. He was taken to the emergency room of the local hospital and arrested for driving under the influence of alcohol. His blood alcohol concentration at the time of his arrest was 240 mg/dL, compared with the legal limit of ethanol for driving of 80 mg/dL.

Ann O'Rexia, a 23-year-old woman, 5 ft 7 in tall, is being treated for anorexia nervosa (see Chapters 1 through 3). She has been gaining weight and is now back to 99 lb from a low of 85 lb. Her blood glucose is still below normal (fasting blood glucose of 72 mg/dL, compared with a normal range of 80 to 100 mg/dL). She complains to her physician that she feels tired when she jogs, and she is concerned that the "extra weight" she has gained is slowing her down.

I. GENERAL OVERVIEW

Although the regulation of metabolic pathways is an exceedingly complex subject, dealt within most of the subsequent chapters of this text, several common themes are involved. Physiologic regulation of a metabolic pathway depends on the ability to alter flux through the pathway by activating the enzyme catalyzing the rate-limiting step in the pathway (see Fig. 9.1). The type of regulation employed always reflects the function of the pathway and the need for that pathway in a particular tissue or cell type. Pathways that produce a necessary product are usually feedback-regulated through a mechanism that involves concentration of product (e.g., allosteric inhibition or induction/repression of enzyme synthesis), either directly or indirectly. The concentration of the product signals when enough of the product has been synthesized. Storage and toxic disposal pathways are usually regulated directly or indirectly through a feed-forward mechanism that reflects the availability of precursor. Regulatory enzymes are often tissue-specific isozymes whose properties reflect the different functions of a pathway in particular tissues. Pathways are also regulated through compartmentation, collection of enzymes with a common function within a particular organelle or at a specific site in the cell.

The mechanisms employed to regulate enzymes have been organized into three general categories: regulation by compounds that bind reversibly in the active site (including the dependence of velocity on substrate concentration and product levels); regulation by changing the conformation of the active site (including allosteric regulators, covalent modification, protein–protein interactions, and zymogen cleavage); and regulation by changing the concentration of enzyme (enzyme synthesis and degradation). We will generally be using the pathways of fuel oxidation to illustrate the role of various mechanisms of enzyme regulation in metabolic pathways (see Chapters 1 through 3 for a general overview of these pathways).

II. REGULATION BY SUBSTRATE AND PRODUCT CONCENTRATION

A. Velocity and Substrate Concentration

The velocity of all enzymes is dependent on the concentration of substrate. This dependence is reflected in conditions such as starvation, in which several pathways are deprived of substrate. In contrast, storage pathways (e.g., glucose conversion to glycogen in the liver) and toxic waste disposal pathways (e.g., the urea cycle, which prevents NH_4^+ toxicity by converting NH_4^+ to urea) are normally regulated to speed up when more substrate is available. In the following sections, we use the Michaelis-Menten equation to describe the response of an enzyme to changes in substrate concentration, and we use glucokinase to illustrate the role of substrate supply in regulating enzyme activity.

1. THE MICHAELIS-MENTEN EQUATION

The equations of enzyme kinetics provide a quantitative way of describing the dependence of enzyme rate on substrate concentration. The simplest of these equations, the Michaelis-Menten equation, relates the initial velocity (v_i) to the concentration of substrate [S] and the two parameters K_m and V_{max} (Equation 9.1). The V_{max} of the enzyme is the maximal velocity that can be achieved at an infinite concentration of substrate, and the K_m of the enzyme for a substrate is the concentration of substrate required to reach $\frac{1}{2}V_{max}$. The Michaelis-Menten model of enzyme kinetics applies to a simple reaction in which the enzyme and substrate form an enzyme–substrate complex (ES) that can dissociate back to the free enzyme and substrate. The initial velocity of product formation, v_i, is proportional to the concentration of [ES]. As substrate concentration is increased, the concentration of [ES] increases, and the reaction rate increases proportionately. The total amount of enzyme present is represented by E_t.

Equation 9.1. The Michaelis-Menten equation.

For the reaction,

$$E + S \underset{k_2}{\overset{k_1}{\rightleftharpoons}} ES \xrightarrow{k_3} E + P$$

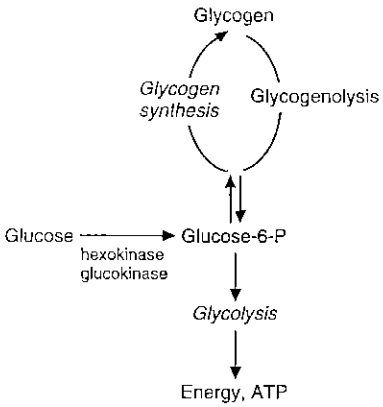
the Michaelis-Menten equation is given by

$$v_i = \frac{V_{max}[S]}{K_m + [S]}$$

where $K_m = (k_2 + k_3)/k_1$ and $V_{max} = k_3 [E_t]$.

The graph of the Michaelis-Menten equation (v_i as a function of substrate concentration) is a rectangular hyperbola that approaches a finite limit, V_{max}, as the fraction of total enzyme present as ES increases (Fig. 9.2). At a hypothetical infinitely high substrate concentration, all of the enzyme molecules contain bound substrate, and the reaction rate is at V_{max}. The approach to the finite limit of V_{max} is called *saturation kinetics* because velocity cannot increase any further once the enzyme is saturated with substrate. Saturation kinetics is a characteristic property of all rate processes that depend on the binding of a compound to a protein.

One of the fuels used by Ann O'Rexia's skeletal muscles for jogging is glucose, which is converted to glucose 6-phosphate (glucose 6-P) by the enzymes hexokinase (HK) and glucokinase (GK). Glucose 6-P is metabolized in the pathway of glycolysis to generate adenosine triphosphate (ATP). This pathway is feedback-regulated, so that as her muscles use ATP, the rate of glycolysis will increase to generate more ATP.



When she is resting, her muscles and liver will convert glucose 6-P to glycogen (a fuel storage pathway, shown in *red*). Glycogen synthesis is feed-forward–regulated by the supply of glucose and by insulin and other hormones that signal glucose availability. Glycogenolysis (glycogen degradation) is activated during exercise to supply additional glucose 6-P for glycolysis. Unless Ann consumes sufficient calories, her glycogen stores will not be replenished after exercise, and she will fire easily.

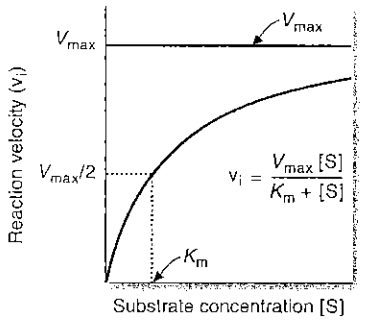


FIG. 9.2. A graph of the Michaelis-Menten equation. V_{max} (*solid red line*) is the initial velocity extrapolated to infinite [S]. K_m (*dotted red line*) is the concentration of S at which $v_i = V_{max}/2$.

Patients with maturity-onset diabetes of the young (MODY) have a rare genetic form of diabetes mellitus in which the amount of insulin being secreted from the pancreas is too low, resulting in hyperglycemia. One class of MODY is caused by mutations in the gene for pancreatic glucokinase (a closely related isozyme of liver glucokinase) that affect its kinetic properties (K_m or V_{max}). Glucokinase is part of the mechanism that controls release of insulin from the pancreas. Decreased glucokinase activity results in lower insulin secretion for a given blood glucose level.

Q: As **Ann O'Rexia** eats a high-carbohydrate meal, her blood glucose will rise to approximately 20 mM in the portal vein, and much of the glucose from her carbohydrate meal will enter the liver. How will the activity of glucokinase in the liver change as glucose is increased from 4 mM to 20 mM? (Hint: Calculate v_i as a fraction of V_{max} for both conditions, using a K_m for glucose of 5 mM and the Michaelis-Menten equation).

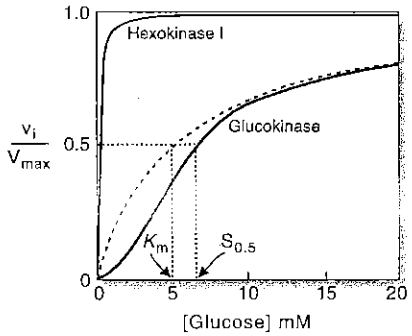


FIG. 9.3. A comparison between hexokinase I and glucokinase. The initial velocity (v_i) as a fraction of V_{max} is graphed as a function of glucose concentration. The plot for glucokinase (*heavy blue line*) is slightly sigmoidal (S-shaped), possibly because the rate of an intermediate step in the reaction is so slow that the enzyme does not follow Michaelis-Menten kinetics. The *dashed blue line* has been derived from the Michaelis-Menten equation fitted to the data for concentrations of glucose >5 mM. For S-shaped curves, the concentration of substrate required to reach half V_{max}, or half-saturation, is sometimes called the $S_{0.5}$ or $K_{0.5}$ rather than K_m. At $v_i/V_{max} = 0.5$, for glucokinase, the K_m is 5 mM and the $S_{0.5}$ is 6.7 mM.

The K_m of the enzyme for a substrate is defined as the concentration of substrate at which v_i equals $\frac{1}{2}V_{max}$. The velocity of an enzyme is most sensitive to changes in substrate concentration over a concentration range below its K_m (see Fig. 9.2). At substrate concentrations less than one-tenth of the K_m, a doubling of substrate concentration nearly doubles the velocity of the reaction; at substrate concentrations 10 times the K_m, doubling the substrate concentration has little effect on the velocity.

The K_m of an enzyme for a substrate is related to the dissociation constant, K_d, which is the rate of substrate release divided by the rate of substrate binding (k_2/k_1). For example, a genetic mutation that decreases the rate of substrate binding to the enzyme decreases the affinity of the enzyme for the substrate and increases the K_d and K_m of the enzyme for that substrate. The higher the K_m, the higher is the substrate concentration required to reach $\frac{1}{2}V_{max}$.

2. HEXOKINASE ISOZYMES HAVE DIFFERENT K_m VALUES FOR GLUCOSE

A comparison between the isozymes of hexokinase found in red blood cells and in the liver illustrates the significance of the K_m of an enzyme for its substrate. Hexokinase catalyzes the first step in glucose metabolism in most cells: the transfer of a phosphate from adenosine triphosphate (ATP) to glucose to form glucose 6-phosphate. Glucose 6-phosphate may then be metabolized in glycolysis, which generates energy in the form of ATP, or it can be converted to glycogen, a storage polymer of glucose. Hexokinase I, the isozyme in red blood cells (erythrocytes), has a K_m for glucose of approximately 0.05 mM (Fig. 9.3). The isozyme of hexokinase, called *glucokinase*, which is found in the liver and pancreas, has a much higher K_m of approximately 5 to 6 mM. The red blood cell is totally dependent on glucose metabolism to meet its needs for ATP. At the low K_m of the erythrocyte hexokinase, blood glucose could fall drastically below its normal fasting level of approximately 5 mM, and the red blood cell could still phosphorylate glucose at rates near V_{max}. The liver, however, stores large amounts of "excess" glucose as glycogen or converts it to fat. Because glucokinase has a K_m of approximately 5 mM, the rate of glucose phosphorylation in the liver will tend to increase as blood glucose increases after a high-carbohydrate meal and decreases as blood glucose levels fall. The high K_m of hepatic glucokinase, thus, promotes the storage of glucose as liver glycogen or as fat, but only when glucose is in excess supply.

3. VELOCITY AND ENZYME CONCENTRATION

The rate of a reaction is directly proportional to the concentration of enzyme; if you double the amount of enzyme, you will double the amount of product produced per minute, whether you are at low or at saturating concentrations of substrate. This important relationship between velocity and enzyme concentration is not immediately apparent in the Michaelis-Menten equation because the concentration of total enzyme present (E_t) has been incorporated into the term V_{max} (i.e., V_{max} is equal to the rate constant k_3 times E_t). However, V_{max} is most often expressed as product produced per minute per milligram of enzyme and is meant to reflect a property of the enzyme that is not dependent on its concentration.

4. MULTISUBSTRATE REACTIONS

Most enzymes have more than one substrate, and the substrate-binding sites overlap in the catalytic (active) site. When an enzyme has more than one substrate, the sequence of substrate binding and product release affect the rate equation. As a consequence, an apparent value of K_m ($K_{m,app}$) depends on the concentration of cosubstrate or product present.

5. RATES OF ENZYME-CATALYZED REACTIONS IN THE CELL

Equations for the initial velocity of an enzyme-catalyzed reaction such as the Michaelis-Menten equation can provide useful parameters for describing or comparing

enzymes. However, many multisubstrate enzymes, such as glucokinase, have kinetic patterns that do not fit the Michaelis-Menten model (or do so under nonphysiologic conditions). The Michaelis-Menten model is also inapplicable to enzymes present in a higher concentration than their substrates. Nonetheless, the term K_m is still used for these enzymes to describe the approximate concentration of substrate at which velocity equals $\frac{1}{2}V_{max}$.

B. Reversible Inhibition within the Active Site

One of the ways of altering enzyme activity is through compounds binding in the active site. If these compounds are not part of the normal reaction, they inhibit the enzyme. An *inhibitor of an enzyme* is defined as a compound that decreases the velocity of the reaction by binding to the enzyme. It is a reversible inhibitor if it is not covalently bound to the enzyme and can dissociate at a significant rate. Reversible inhibitors are generally classified as competitive, noncompetitive, or uncompetitive with respect to their relationship to a substrate of the enzyme. In most reactions, the products of the reaction are reversible inhibitors of the enzyme that produces them.

1. COMPETITIVE INHIBITION

A competitive inhibitor "competes" with a substrate for binding at the enzyme's substrate-recognition site and therefore is usually a close structural analog of the substrate (Fig. 9.4A). An increase of substrate concentration can overcome competitive inhibition; when the substrate concentration is increased to a sufficiently high level, the substrate-binding sites are occupied by substrate and inhibitor molecules cannot bind. Competitive inhibitors, therefore, increase the apparent K_m of the enzyme ($K_{m,app}$) because they raise the concentration of substrate necessary to saturate the enzyme. They have no effect on V_{max}.

2. NONCOMPETITIVE AND UNCOMPETITIVE INHIBITION

If an inhibitor does not compete with a substrate for its binding site, the inhibitor is either a noncompetitive or an uncompetitive inhibitor with respect to that particular substrate (see Fig. 9.4B). Uncompetitive inhibition is almost never encountered in medicine and will not be discussed further. To illustrate noncompetitive inhibition, consider a multisubstrate reaction in which substrates A and B react to form a product. An inhibitor (NI) that is a structural analog of substrate B would fit into substrate B's binding site, but the inhibitor would be a noncompetitive inhibitor with regard to the other substrate, substrate A. An increase of A will not prevent the inhibitor from binding to substrate B's binding site. The inhibitor, in effect, lowers the concentration of the active enzyme and therefore changes the V_{max} of the enzyme. If the inhibitor has absolutely no effect on the binding of substrate A, it will not change the K_m for A (a pure noncompetitive inhibitor).

Some inhibitors, such as metals, might not bind at either substrate-recognition site. In this case, the inhibitor would be noncompetitive with respect to both substrates.

3. SIMPLE PRODUCT INHIBITION IN METABOLIC PATHWAYS

All products are reversible inhibitors of the enzymes that produce them and may be competitive or noncompetitive relative to a particular substrate. Simple product inhibition, a decrease in the rate of an enzyme caused by the accumulation of its own product, plays an important role in metabolic pathways: It prevents one enzyme in a sequence of reactions from generating a product faster than it can be used by the next enzyme in that sequence. As an example, product inhibition of hexokinase by glucose 6-phosphate conserves blood glucose for tissues that need it. Tissues take up glucose from the blood and phosphorylate it to glucose 6-phosphate, which can then enter several different pathways (including glycolysis and glycogen

A: Glucokinase, which has a high K_m for glucose, phosphorylates glucose to glucose 6-phosphate about twice as fast after a carbohydrate meal as during fasting. Substitute the values for S and K_m into the Michaelis-Menten equation. The initial velocity will be 0.44 times V_{max} when blood glucose is at 4 mM and about 0.80 times V_{max} when blood glucose is at 20 mM. In the liver, glucose 6-phosphate is a precursor for both glycogen and fat synthesis. Thus, these storage pathways are partially regulated through a direct effect of substrate supply. They are also partially regulated through an increase of insulin and a decrease of glucagon, two hormones that signal the supply of dietary fuel.

The liver alcohol dehydrogenase that is most active in oxidizing ethanol has a very low K_m for ethanol, approximately 0.04 mM, and is at more than 99% of its V_{max} at the legal limit of blood alcohol concentration for driving (80 mg/dL or about 17 mM). In contrast, the microsomal ethanol oxidizing system (MEOS) isozyme that is most active toward ethanol has a K_m of approximately 11 mM. Thus, MEOS makes a greater contribution to ethanol oxidation and clearance from the blood at higher ethanol levels than at lower ones. Liver damage such as cirrhosis results partly from toxic by-products of ethanol oxidation generated by MEOS. **Al Martini**, who has a blood alcohol level of 240 mg/dL (approximately 52 mM), is drinking enough to potentially cause liver damage, as well as his car accident and arrest for driving under the influence of alcohol. The various isozymes and polymorphisms of alcohol dehydrogenase and MEOS are discussed in more detail in Chapter 25.

Some of **Al Martini's** problems have arisen from product inhibition of liver alcohol dehydrogenase by NADH. As ethanol is oxidized in liver cells, NAD^+ is reduced to NADH and the NADH/NAD^+ ratio rises. NADH is an inhibitor of alcohol dehydrogenase, competitive with respect to NAD^+, so the increased NADH/NAD^+ ratio slows the rate of ethanol oxidation and ethanol clearance from the blood.

NADH is also a product inhibitor of enzymes in the pathway that oxidizes fatty acids. Consequently, these fatty acids accumulate in the liver, eventually contributing to the alcoholic fatty liver.

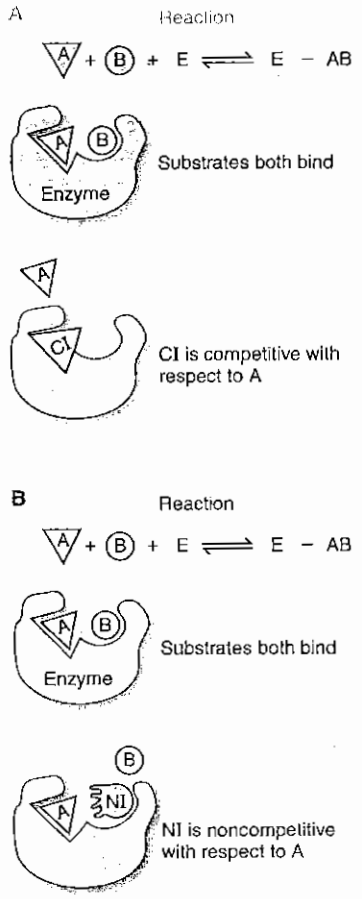


FIG. 9.4. **A.** Competitive inhibition with respect to substrate *A*. *A* and *B* are substrates for the reaction that forms the enzyme–substrate complex (*E–AB*). The enzyme has separate binding sites for each substrate, which overlap in the active site. The competitive inhibitor (*CI*) competes for the binding site of *A*, the substrate it most closely resembles. **B.** *NI* is a noncompetitive inhibitor with respect to substrate *A*. *A* can still bind to its binding site in the presence of *NI*. However, *NI* is competitive with respect to *B* because it binds to the B-binding site. In contrast, an inhibitor that is uncompetitive with respect to *A* might also resemble *B*, but it could bind to the *B* site only after *A* is bound.

synthesis). As these pathways become more active, glucose-6-phosphate concentration decreases, and the rate of hexokinase increases. When these pathways are less active, glucose-6-phosphate concentration increases, hexokinase is inhibited, and glucose remains in the blood for other tissues.

III. REGULATION THROUGH CONFORMATIONAL CHANGES

In substrate response and product inhibition, the rate of the enzyme is affected principally by the binding of a substrate or a product within the catalytic site. Most rate-limiting enzymes are also controlled through regulatory mechanisms that change the conformation of the enzyme in a way that affects the catalytic site. These regulatory mechanisms include (1) allosteric activation and inhibition; (2) phosphorylation or other covalent modification; (3) protein–protein interactions between regulatory and catalytic subunits or between two proteins; and (4) proteolytic cleavage. These types of regulation can rapidly change an enzyme from an inactive form to a fully active conformation.

In the following sections, we describe the general characteristics of these regulatory mechanisms and illustrate the first three with glycogen phosphorylase, glycogen phosphorylase kinase, and protein kinase A.

A. Conformational Changes in Allosteric Enzymes

Allosteric activators and inhibitors (allosteric effectors) are compounds that bind to the allosteric site (a site separate from the catalytic site) and cause a conformational change that affects the affinity of the enzyme for the substrate. Usually, an allosteric enzyme has multiple interacting subunits that can exist in active and inactive conformations, and the allosteric effector promotes or hinders conversion from one conformation to another.

1. COOPERATIVITY IN SUBSTRATE BINDING TO ALLOSTERIC ENZYMES

Allosteric enzymes usually contain two or more subunits and exhibit positive cooperativity; the binding of substrate to one subunit facilitates the binding of substrate to another subunit (Fig. 9.5). The first substrate molecule has difficulty in binding to the enzyme because all of the subunits are in the conformation with a low affinity for substrate (the taut "T" conformation) (see oxygen binding to hemoglobin in Chapter 7, Section VII.B). The first substrate molecule to bind changes its own subunit and at least one adjacent subunit to the high-affinity conformation (the relaxed "R" state). In the example of the tetramer hemoglobin, discussed in Chapter 7, the change in one subunit facilitated changes in all four subunits, and the molecule generally changed to the new conformation in a concerted fashion. However, most allosteric enzymes follow a more stepwise (sequential) progression through intermediate stages (see Fig. 9.5).

2. ALLOSTERIC ACTIVATORS AND INHIBITORS

Allosteric enzymes bind activators at the allosteric site, a site that is physically separate from the catalytic site. The binding of an allosteric activator changes the conformation of the catalytic site in a way that increases the affinity of the enzyme for the substrate.

In general, activators of allosteric enzymes bind more tightly to the high-affinity R state of the enzyme than the T state (i.e., the allosteric site is open only in the R enzyme) (Fig. 9.6). Thus, the activators increase the amount of enzyme in the active state, thereby facilitating substrate binding in their own and other subunits. In contrast, allosteric inhibitors bind more tightly to the T state, so either substrate concentration or activator concentration must be increased to overcome the effects of the allosteric inhibitor. Allosteric inhibitors might have their own binding site on the enzyme, or they might compete with the substrate at the active site and prevent

cooperativity. Thus, the term *allosteric inhibitor* is more generally applied to any inhibitor of an allosteric enzyme.

In the absence of activator, a plot of velocity versus substrate concentration for an allosteric enzyme usually results in a sigmoid or S-shaped curve (rather than the rectangular hyperbola of Michaelis-Menten enzymes) as the successive binding of substrate molecules activates additional subunits (see Fig. 9.6). In plots of velocity versus substrate concentration, the effect of an allosteric activator generally makes the sigmoidal S-shaped curve more like the rectangular hyperbola, with a substantial decrease in the $S_{0.5}$ (K_m) of the enzyme, because the activator changes all of the subunits to the high-affinity state. Such allosteric effectors are "*K* effectors"; they change the K_m but not the V_{max} of the enzyme. An allosteric inhibitor makes it more difficult for substrate or activators to convert the subunits to the most active conformation, and therefore inhibitors generally shift the curve to the right, either increasing the $S_{0.5}$ alone or increasing it together with a decrease in the V_{max}.

Some of the rate-limiting enzymes in the pathways of fuel oxidation (e.g., muscle glycogen phosphorylase in glycogenolysis, phosphofructokinase-1 in glycolysis, and isocitrate dehydrogenase in the tricarboxylic acid [TCA] cycle) are allosteric enzymes regulated by changes in the concentration of adenosine

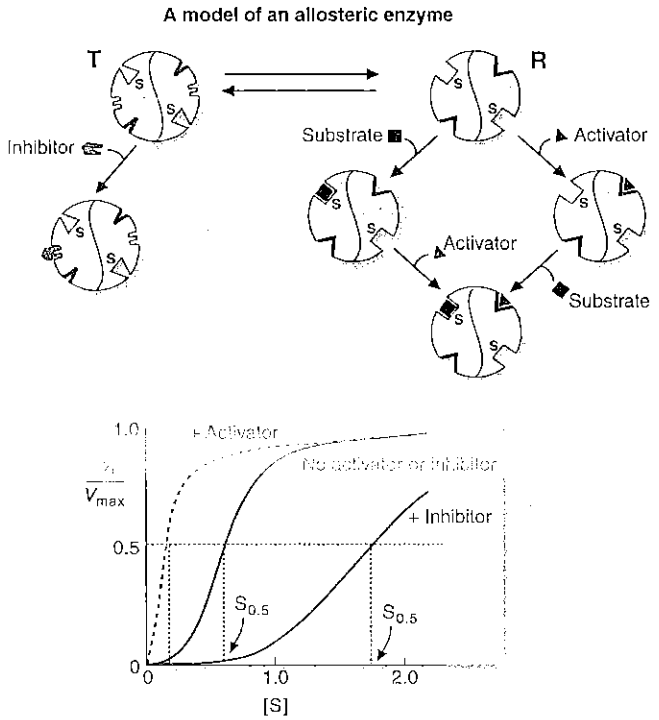


FIG. 9.6. Activators and inhibitors of an allosteric enzyme (simplified model). This enzyme has two identical subunits, each containing three binding sites: one for the substrate (*S*), one for the allosteric activator (*green triangle*), and one for the allosteric inhibitor (*two-pronged red shape*). The enzyme has two conformations: a relaxed active conformation (*R*) and an inactive conformation (*T*). The activator binds only to its activator site when the enzyme is in the R configuration. The inhibitor-binding site is open only when the enzyme is in the T state. A plot of velocity (v_i/V_{max}) versus substrate concentration reveals that binding of the substrate at its binding site stabilizes the active conformation so that the second substrate binds more readily, resulting in an S (sigmoidal)-shaped curve. The graph of v_i/V_{max} becomes hyperbolic in the presence of activator (which stabilizes the high-affinity R form), and more sigmoidal with a higher $S_{0.5}$ in the presence of inhibitor (which stabilizes the low-affinity form).

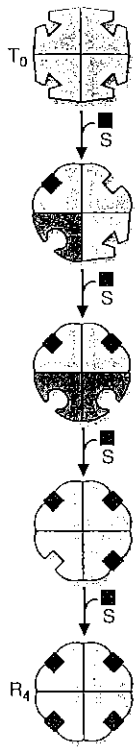


FIG. 9.5. A sequential model for an allosteric enzyme. The sequential model is actually the preferred path from the T_0 (taut, with 0 substrate bound) low-affinity conformation to the R_4 (relaxed, with four substrate molecules bound) conformation, taken from an array of all possible equilibrium conformations that differ by the conformation of only one subunit. The final result is a stepwise path in which intermediate conformations exist, and subunits may change conformations independently, depending on their geometric relationship to the subunits already containing bound substrate.

diphosphate (ADP) or adenosine monophosphate (AMP), which are allosteric activators. The function of fuel oxidation pathways is the generation of ATP. When the concentration of ATP in a muscle cell begins to decrease, ADP and AMP increase; ADP activates isocitrate dehydrogenase, and AMP activates glycogen phosphorylase and phosphofructokinase-1. The response is very fast, and small changes in the concentration of activator can cause large changes in the rate of the reaction.

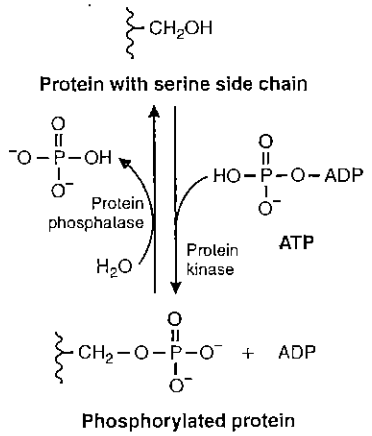


FIG. 9.7. Protein kinases and protein phosphatases.

3. ALLOSTERIC ENZYMES IN METABOLIC PATHWAYS

Regulation of enzymes by allosteric effectors provides several advantages over other methods of regulation. Allosteric inhibitors usually have a much stronger effect on enzyme velocity than competitive and noncompetitive inhibitors in the active catalytic site. Because allosteric effectors do not occupy the catalytic site, they may function as activators. Thus, allosteric enzymes are not limited to regulation through inhibition. Furthermore, the allosteric effector need not bear any resemblance to substrate or product of the enzyme. Finally, the effect of an allosteric effector is rapid, occurring as soon as its concentration changes in the cell. These features of allosteric enzymes are often essential for feedback regulation of metabolic pathways by end products of the pathway or by signal molecules that coordinate multiple pathways.

B. Conformational Changes from Covalent Modification

1. PHOSPHORYLATION

The activity of many enzymes is regulated through phosphorylation by a protein kinase or dephosphorylation by a protein phosphatase (Fig. 9.7). Serine/threonine protein kinases transfer a phosphate from ATP to the hydroxyl group of a specific serine (and sometimes threonine) on the target enzyme; tyrosine kinases transfer a phosphate to the hydroxyl group of a specific tyrosine residue. Phosphate is a bulky, negatively charged residue that interacts with other nearby amino acid residues of the protein to create a conformational change at the catalytic site. The conformational change is caused by alterations in ionic interactions and/or hydrogen bond patterns due to the presence of the phosphate group. The conformational change makes certain enzymes more active and other enzymes less active. The effect is reversed by a specific protein phosphatase that removes the phosphate by hydrolysis.

2. MUSCLE GLYCOGEN PHOSPHORYLASE

Muscle glycogen phosphorylase, the rate-limiting enzyme in the pathway of glycogen degradation, degrades glycogen to glucose 1-phosphate. It is regulated by the allosteric activator AMP, which increases in the cell as ATP is used for muscular contraction (Fig. 9.8). Thus, a rapid increase in the rate of glycogen degradation to glucose 1-phosphate is achieved when an increase of AMP signals that more fuel is needed for ATP generation in the glycolytic pathway.

Glycogen phosphorylase also can be activated through phosphorylation by glycogen phosphorylase kinase. Either phosphorylation or AMP binding can change the enzyme to the same fully active conformation. The phosphate is removed by protein phosphatase-1. Glycogen phosphorylase kinase links the activation of muscle glycogen phosphorylase to changes in the level of the hormone adrenaline in the blood. It is regulated through phosphorylation by protein kinase A and by activation of Ca^{2+}-calmodulin (a modulator protein) during contraction.

3. PROTEIN KINASE A

Some protein kinases, called dedicated protein kinases, are tightly bound to a single protein and regulate only the protein to which they are tightly bound. However,

When **Ann O'Rexia** begins to jog, adenosine monophosphate (AMP) activates her muscle glycogen phosphorylase, which degrades glycogen to glucose 1-phosphate. This compound is converted to glucose 6-phosphate, which feeds into the glycolytic pathway to generate ATP for muscle contraction. As she continues to jog, her adrenaline (epinephrine) levels rise, producing the signal that activates glycogen phosphorylase kinase. This enzyme phosphorylates glycogen phosphorylase, causing it to become even more active than with AMP alone (see Fig. 9.8).

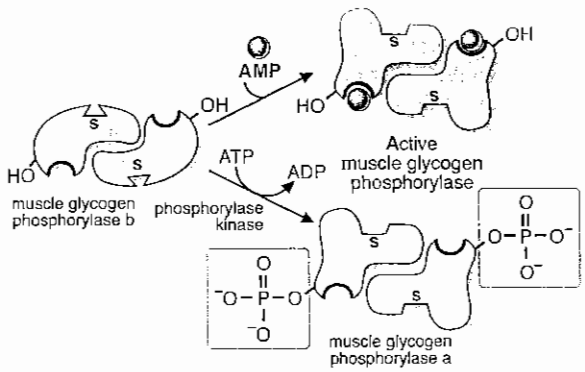


FIG. 9.8. Activation of muscle glycogen phosphorylase by AMP and by phosphorylation. Muscle glycogen phosphorylase is composed of two identical subunits. The substrate-binding sites in the active catalytic site are denoted by *S*. AMP binds to the allosteric site, a site separate from the active catalytic site. Glycogen phosphorylase kinase can transfer a phosphate from ATP to one serine residue in each subunit. Either phosphorylation or binding of AMP causes a change in the active site that increases the activity of the enzyme. The first event at one subunit facilitates the subsequent events that convert the enzyme to the fully active form.

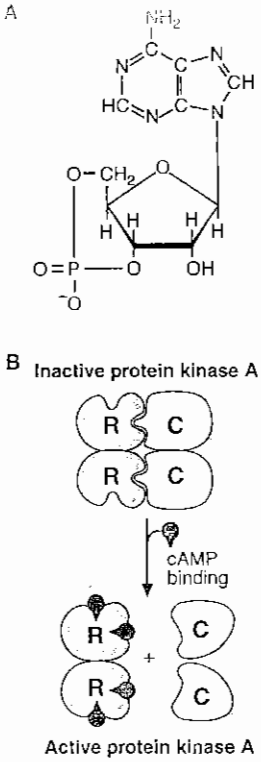


FIG. 9.9. **A.** Structure of cAMP (3′,5′-cyclic AMP). The phosphate group is attached to hydroxyl groups on both the third (3′) and fifth (5′) carbons of ribose, forming a cyclic molecule. **B.** Protein kinase A. When the regulatory subunits (*R*) of protein kinase A bind the allosteric activator, cAMP, they dissociate from the enzyme, thereby releasing active catalytic subunits (*C*).

other protein kinases and protein phosphatases simultaneously regulate several rate-limiting enzymes in a cell to achieve a coordinated response. For example, protein kinase A, a serine/threonine protein kinase, phosphorylates several enzymes that regulate different metabolic pathways. One of these enzymes is glycogen phosphorylase kinase (see Fig. 9.8).

Protein kinase A provides a means for hormones to control metabolic pathways. Adrenaline and many other hormones increase the intracellular concentration of the allosteric regulator 3′,5′-cyclic AMP (cAMP), which is referred to as a *hormonal second messenger* (Fig. 9.9A). cAMP binds to regulatory subunits of protein kinase A, which dissociate and release the activated catalytic subunits (see Fig. 9.9B). Dissociation of inhibitory regulatory subunits is a common theme in enzyme regulation. The active catalytic subunits phosphorylate glycogen phosphorylase and other enzymes at serine residues.

In the example shown in Figure 9.8, adrenaline (epinephrine) indirectly increases cAMP, which activates protein kinase A, which phosphorylates and activates glycogen phosphorylase kinase, which phosphorylates and activates glycogen phosphorylase. The sequence of events in which one kinase phosphorylates another kinase is called a *phosphorylation cascade*. Because each stage of the phosphorylation cascade is associated with one enzyme molecule activating many enzyme molecules, the initial activating event is greatly amplified.

4. OTHER COVALENT MODIFICATIONS

Several proteins are modified covalently by the addition of groups such as acetyl, ADP-ribose, or lipid moieties (see Chapter 6). These modifications may activate or inhibit the enzyme directly. However, they also may modify the ability of the enzyme to interact with other proteins or to reach its correct location in the cell.

C. Conformational Changes from Protein–Protein Interactions

Changes in the conformation of the active site also can be regulated by direct protein–protein interaction. This type of regulation is illustrated by Ca^{2+}-calmodulin and small (monomeric) G proteins.

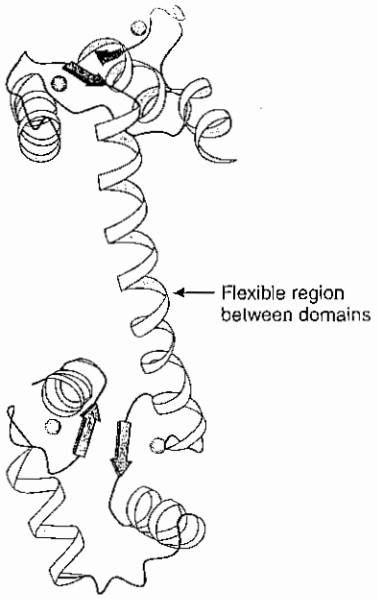


FIG. 9.10. Calcium-calmodulin has four binding sites for calcium (shown in green). Each calcium forms a multiligand coordination sphere by simultaneously binding several amino acid residues on calmodulin. Thus, calmodulin can create large conformational changes in proteins to which it is bound when calcium binds. Calmodulin has a flexible region in the middle connecting the two domains.

1. THE CALCIUM-CALMODULIN FAMILY OF MODULATOR PROTEINS

Modulator proteins bind to other proteins and regulate their activity by causin conformational change at the catalytic site or by blocking the catalytic site (ste hindrance). They are protein allosteric effectors that can either activate or inh the enzyme or protein to which they bind.

Ca^{2+}-calmodulin is an example of a dissociable modulator protein that binds several different proteins and regulates their function. It also exists in the cytc and functions as a Ca^{2+}-binding protein (Fig. 9.10). The center of the symme molecule is a hinge region that bends as Ca^{2+}-calmodulin folds over the protei is regulating.

One of the enzymes activated by Ca^{2+}-calmodulin is muscle glycogen ph phorylase kinase (see Fig. 9.8), which is also activated by protein kinase A. Whe neural impulse triggers Ca^{2+} release from the sarcoplasmic reticulum, Ca^{2+} bind the calmodulin subunit of muscle glycogen phosphorylase kinase, which underg a conformational change. This conformational change leads to the activation of g cogen phosphorylase kinase, which then phosphorylates glycogen phosphoryl ultimately increasing the generation of ATP to supply energy for muscle cont tion. Simultaneously, Ca^{2+} binds to troponin C, a member of the Ca^{2+}-calmodu superfamily that serves as a nondissociable regulatory subunit of troponin, a re lator of muscle contraction. Calcium binding to troponin prepares the muscle contraction. Thus, the supply of energy for contraction is activated simultaneou with the contraction machinery.

2. SMALL (MONOMERIC) G PROTEINS REGULATE THROUGH CONFORMATIONAL CHANGES

The masters of regulation through reversible protein association in the cell the monomeric G proteins, small single-subunit proteins that bind and hydrol guanosine triphosphate (GTP). GTP is a purine nucleotide that, like ATP, cont high-energy phosphoanhydride bonds that release energy when hydrolyzed. W G proteins bind GTP, their conformation changes so that they can bind to a ta protein, which is then either activated or inhibited in carrying out its func (Fig. 9.11, step 1).

G proteins are said to possess an internal clock because they are GTPases slowly hydrolyze their own bound GTP to GDP and phosphate. As they hydro GTP, their conformation changes and the complex they have formed with the get protein disassembles (see Fig. 9.11, step 2). The bound GDP on the inac G protein is eventually replaced by GTP, and the process can begin again Fig. 9.11, step 3).

The activity of many G proteins is regulated by accessory proteins (G [*G*TPase-*a*ctivating *p*roteins], GEFs [guanine nucleotide *e*xchange *f*actors], GDIs [*G*DP *d*issociation *i*nhibitors]), which may, in turn, be regulated by allost effectors. GAPs increase the rate of GTP hydrolysis by the G protein, and th fore the rate of dissociation of the G protein–target protein complex (see Fig. 9 step 2). When a GEF protein binds to a G protein, it increases the rate of C exchange for a bound GDP, and therefore activates the G protein (see Fig. 9 step 3). GDI proteins bind to the GDP–G protein complex and inhibit dissocia of GDP, thereby keeping the G protein inactive.

The Ras superfamily of small G proteins is divided into five families: Ras, I Arf, Rab, and Ran. These monomeric G proteins play major roles in the regula of cell growth, morphogenesis, cell motility, axonal guidance, cytokinesis, and ficking through the Golgi, nucleus, and endosomes. They are generally bound lipid membrane through a lipid anchor such as a myristoyl group or farnesyl gr and regulate the assembly and activity of protein complexes at these sites. The f tions of some of these G proteins will be discussed further in Chapters 10 and

D. Proteolytic Cleavage

Although many enzymes undergo some cleavage during synthesis, others enter lysosomes, secretory vesicles, or are secreted as proenzymes, which are precursor proteins that must undergo proteolytic cleavage to become fully functional. Unlike most other forms of regulation, proteolytic cleavage is irreversible.

The precursor proteins of proteases (enzymes that cleave specific peptide bonds) are called *zymogens*. To denote the inactive zymogen form of an enzyme, the name is modified by addition of the suffix "-ogen" or the prefix "pro-." The synthesis of zymogens as inactive precursors prevents them from cleaving proteins prematurely at their sites of synthesis or secretion. Chymotrypsinogen, for example, is stored in vesicles within pancreatic cells until it is secreted into ducts leading to the intestinal lumen. In the digestive tract, chymotrypsinogen is converted to chymotrypsin by the proteolytic enzyme trypsin, which cleaves off a small peptide from the N-terminal region (and two internal peptides). This cleavage activates chymotrypsin by causing a conformational change in the spacing of amino acid residues around the binding site for the denatured protein substrate and around the catalytic site.

Most of the proteases involved in blood clotting are zymogens, such as fibrinogen and prothrombin, which circulate in blood in the inactive form. They are cleaved to the active form (fibrin and thrombin, respectively) by other proteases, which have been activated by their attachment to the site of injury in a blood vessel wall. Thus, clots form at the site of injury and not randomly in the circulation.

IV. REGULATION THROUGH CHANGES IN AMOUNT OF ENZYME

Tissues continuously adjust the rate at which different proteins are synthesized to vary the amount of different enzymes present. The expression for V_{max} in the Michaelis-Menten equation incorporates the concept that the rate of a reaction is proportional to the amount of enzyme present. Thus, the maximal capacity of a tissue can change with increased protein synthesis or with increased protein degradation.

A. Regulated Enzyme Synthesis

Protein synthesis begins with the process of gene transcription, transcribing the genetic code for that protein from DNA into messenger RNA. The code in messenger RNA is then translated into the primary amino acid sequence of the protein. Generally, the rate of enzyme synthesis is regulated by increasing or decreasing the rate of gene transcription, processes that are generally referred to as *induction* (increase) and *repression* (decrease). However, the rate of enzyme synthesis is sometimes regulated through stabilization of the messenger RNA. (These processes are covered in Section III of the text). Compared with the more immediate types of regulation discussed earlier, regulation by means of induction/repression of enzyme synthesis is usually slow in humans, occurring over hours to days.

B. Regulated Protein Degradation

The content of an enzyme in the cell can be altered through selective regulated degradation as well as through regulated synthesis. Although all proteins in the cell can be degraded with a characteristic half-life within lysosomes, protein degradation via two specialized systems, proteosomes and caspases, is highly selective and regulated. During fasting or infective stress, protein degradation in skeletal muscle is activated to increase the supply of amino acids in the blood for gluconeogenesis, or for the synthesis of antibodies and other components of the immune response. Under these conditions, synthesis of ubiquitin, a protein that targets proteins for degradation in proteosomes, is increased by the steroid hormone cortisol. Protein degradation is discussed in more detail in Chapter 37.

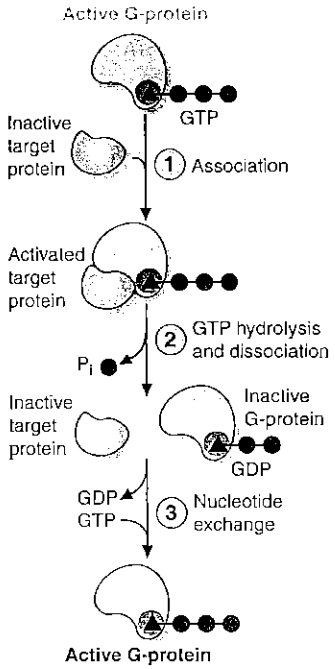


FIG. 9.11. Monomeric G proteins. Step 1: When GTP is bound, the conformation of the G protein allows it to bind target proteins, which are then activated (as shown) or inhibited. Step 2: The G protein hydrolyzes a phosphate from GTP to form guanosine diphosphate (GDP), which changes the G protein conformation and causes it to dissociate from the target protein. Step 3: GDP is exchanged for GTP, which reactivates the G protein.

The maximal capacity of MEOS (cytochrome P450-2E1) is increased in the liver with continued ingestion of ethanol through a mechanism involving induction of gene transcription. Thus, **Al Martini** has a higher capacity to oxidize ethanol to acetaldehyde than a naive drinker (a person not previously subjected to alcohol). Nevertheless, the persistence of his elevated blood alcohol level shows that he has saturated his capacity for ethanol oxidation (V-maxed out). Once his enzymes are operating near V_{max}, any additional ethanol he drinks will not appreciably increase the rate of ethanol clearance from his blood.

V. REGULATION OF METABOLIC PATHWAYS

The different means of regulating enzyme activity we have described are used tc control metabolic pathways, cellular events, and physiologic processes to match the body's requirements. Although many metabolic pathways are present in the body a few common themes or principles are involved in their regulation. Of course, the overriding principle is that regulation of a pathway matches its *function*.

A. Principles of Pathway Regulation

Metabolic pathways are a series of sequential reactions in which the product of one reaction is the substrate of the next reaction (Fig. 9.12). Each step or reaction is usually catalyzed by a separate enzyme. The enzymes of a pathway have a common function—conversion of substrate to the final end products of the pathway. A pathway also may have a branch point at which an intermediate becomes the precurso for another pathway.

1. REGULATION OCCURS AT THE RATE-LIMITING STEP

Pathways are principally regulated at one key enzyme, the regulatory enzyme which catalyzes the rate-limiting step in the pathway. This is the slowest step and is usually not readily reversible. Thus, changes in the rate-limiting step can influence flux through the rest of the pathway (see Fig. 9.1). The rate-limiting step is usually the first committed step in a pathway, or a reaction that is related to, o influenced by, the first committed step. Additional regulated enzymes occur after each metabolic branch point to direct flow into the branch (e.g., in Fig. 9.12, feedback inhibition of enzyme 2 results in accumulation of B, which enzyme 5 then uses for synthesis of compound G). Inhibition of the rate-limiting enzyme in a pathway usually leads to accumulation of the pathway precursor.

2. FEEDBACK REGULATION

Feedback regulation refers to a situation in which the end product of a pathway controls its own rate of synthesis (see Fig. 9.12). Feedback regulation usually involves allosteric regulation of the rate-limiting enzyme by the end product of a pathway (o a compound that reflects changes in the concentration of the end product). The end product of a pathway may also control its own synthesis by inducing or repressing the gene for transcription of the rate-limiting enzyme in the pathway. This type of regulation is much slower to respond to changing conditions than allosteric regulation.

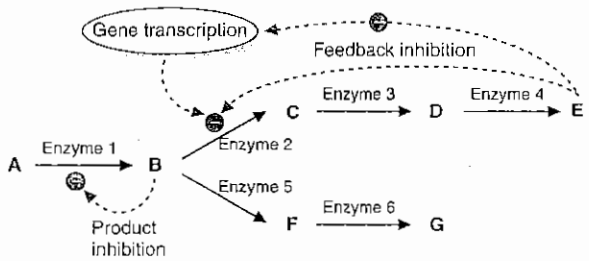


FIG. 9.12. A common pattern for feedback inhibition of metabolic pathways. The letter represent compounds formed from different enzymes in the reaction pathway. *Compound B* i at a metabolic branch point: It can go down one pathway to *E* or down an alternate pathwa to *G*. The end product of the pathway, *E*, might control its own synthesis by allostericall inhibiting *enzyme 2*, the first committed step of the pathway, or by inhibiting transcription c the gene for *enzyme 2*. As a result of the feedback inhibition, *B* accumulates and more *B* enter the pathway for conversion to *G*, which could be a storage pathway or a disposal pathway. I this hypothetical pathway, *B* is a product inhibitor of *enzyme 1*, competitive with respect to *A Precursor A* might induce the synthesis of *enzyme 1*, which would allow more *A* to go to *G*

3. FEED-FORWARD REGULATION

Certain pathways, such as those involved in the disposal of toxic compounds, are feed-forward–regulated. Feed-forward regulation may occur through an increased supply of substrate to an enzyme with a high K_m, allosteric activation of a rate-limiting enzyme through a compound related to substrate supply, substrate-related induction of gene transcription (e.g., induction of cytochrome P450-2E1 by ethanol), or increased concentration of a hormone that stimulates a storage pathway by controlling enzyme phosphorylation state.

4. TISSUE ISOZYMES OF REGULATORY PROTEINS

The human body is composed of several different cell types that perform specific functions unique to that cell type and synthesize only the proteins consistent with their functions. Because regulation matches function, regulatory enzymes of pathways usually exist as tissue-specific isozymes, with somewhat different regulatory properties unique to their function in different cell types. For example, hexokinase and glucokinase are tissue-specific isozymes with different kinetic properties. These different isozymes arose through gene duplication. Glucokinase, the low-affinity enzyme found in liver, is a single polypeptide chain with a molecular weight of 55 kDa that contains one active catalytic site. The hexokinases found in erythrocytes, skeletal muscles, and most other tissues are 110 kDa and are essentially two mutated glucokinase molecules synthesized as one polypeptide chain. However, only one catalytic site is functional. All of the tissue-specific hexokinases except glucokinase have a K_m for glucose that is <0.2 mM.

5. COUNTERREGULATION OF OPPOSING PATHWAYS

A pathway for the synthesis of a compound usually has one or more enzymatic steps that differ from the pathway for degradation of that compound. A biosynthetic pathway can therefore have a different regulatory enzyme than the opposing degradative pathway, and one pathway can be activated while the other is inhibited (e.g., glycogen synthesis is activated while glycogen degradation is inhibited).

6. SUBSTRATE CHANNELING THROUGH COMPARTMENTATION

In the cell, compartmentation of enzymes into multienzyme complexes or organelles provides a means of regulation, either because the compartment provides unique conditions or because it limits or channels access of the enzymes to substrates. Enzymes of pathways with a common function are often assembled into organelles. For example, enzymes of the TCA cycle are all located within the mitochondrion. The enzymes catalyze sequential reactions, and the product of one reaction is the substrate for the next reaction. The concentration of the pathway intermediates remains much higher within the mitochondrion than in the surrounding cellular cytoplasm.

Another type of compartmentation involves the assembly of enzymes that catalyze sequential reactions into multienzyme complexes so that intermediates of the pathway can be transferred directly from the active site on one enzyme to the active site on another enzyme, thereby preventing loss of energy and information. One example of this is the MEOS, which is composed of two different subunits with different enzyme activities. One subunit transfers electrons from NADPH to a cytochrome Fe-heme group on the second subunit, which then transfers the electrons to O_2.

7. LEVELS OF COMPLEXITY

You may have noticed by now that regulation of metabolic pathways in humans is exceedingly complex; this might be called the second principle of metabolic regulation. As you study different pathways in subsequent chapters, it may help to develop diagrams such as Figure 9.12 to keep track of the function and rationale behind different regulatory interactions.

When **Ann O'Rexia** jogs, the increased use of ATP for muscle contraction results in an increase of AMP, which allosterically activates both the allosteric enzyme phosphofructokinase-1, the rate-limiting enzyme of glycolysis, and glycogen phosphorylase, the rate-limiting enzyme of glycogenolysis. This is an example of feedback regulation by the ATP/AMP ratio. Unfortunately, her low caloric consumption has not allowed feed-forward activation of the rate-limiting enzymes in her fuel storage pathways, and she has very low glycogen stores. Consequently, she has inadequate fuel stores to supply the increased energy demands of exercise.

The hormone epinephrine (released during stress and exercise) and glucagon (released during fasting) activate the synthesis of cyclic-adenosine monophosphate (cAMP) in several tissues. cAMP activates protein kinase A. Because protein kinase A is able to phosphorylate key regulatory enzymes in many pathways, these pathways can be regulated coordinately. In muscle, for example, glycogen degradation is activated while glycogen synthesis is inhibited. At the same time, fatty-acid release from adipose tissue is activated to provide more fuel for muscle. The regulation of glycolysis, glycogen metabolism, and other pathways of metabolism is much more complex than we have illustrated here and is discussed in many subsequent chapters of this text.

CLINICAL COMMENTS

Al Martini. In the emergency room, **Al Martini** was evaluated for he injuries. From the physical examination and blood alcohol levels, it w determined that his mental state resulted from his alcohol consumptio Although his chronic ethanol consumption had increased his level of microsom ethanol oxidizing system (MEOS) (and therefore the rate of ethanol oxidation in h liver), his excessive drinking resulted in a blood alcohol level higher than the leg limit of 80 mg/dL. He suffered bruises and contusions but was otherwise uninjure He left in the custody of the police officer.

Ann O'Rexia. Ann O'Rexia's physician explained that she h inadequate fuel stores for her exercise program. To jog, her muscl require an increased rate of fuel oxidation to generate the adenosine t phosphate (ATP) for muscle contraction. The fuels used by muscles for exerci include glucose from muscle glycogen, fatty acids from adipose-tissue triacy glycerols, and blood glucose supplied by liver glycogen. These fuel stores we depleted during her prolonged bout of starvation. In addition, starvation result in the loss of muscle mass as muscle protein was degraded to supply amino aci for other processes, including gluconeogenesis (the synthesis of glucose fro amino acids and other noncarbohydrate precursors). Therefore, Ann will need increase her caloric consumption to rebuild her fuel stores. Her physician helpe her calculate the additional amount of calories her jogging program will nee and they discussed which foods she would eat to meet these increased calor requirements. He also helped her visualize the increase of weight as an increa in strength.

BIOCHEMICAL COMMENTS

The Lineweaver-Burk Transformation. The K_m and V_{max} f an enzyme can be visually determined from a plot of $1/v_i$ versus $1/$ called a Lineweaver-Burk or a double-reciprocal plot. The reciproc of both sides of the Michaelis-Menten equation generates an equation that h the form of a straight line, $y = mx + b$ (Fig. 9.13). K_m and V_{max} are equal to t reciprocals of the intercepts on the abscissa and ordinate, respectively. Althou double-reciprocal plots are often used to illustrate certain features of enzym

$$\frac{1}{v} = \frac{K_m}{V_{max}}\left(\frac{1}{[S]}\right) + \frac{1}{V_{max}}$$

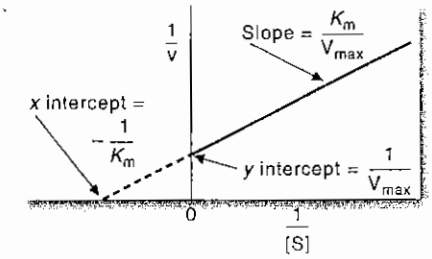


FIG. 9.13. The Lineweaver-Burk transformation (shown in the *green box*) for th Michaelis-Menten equation converts it to a straight line of the form $y = mx + b$. When [S is infinite, $1/[S] = 0$, and the line crosses the ordinate (y axis) at $1/v = 1/V_{max}$. The slope c the line is K_m/V_{max}. Where the line intersects the abscissa (x axis), $1/[S] = -1/K_m$.

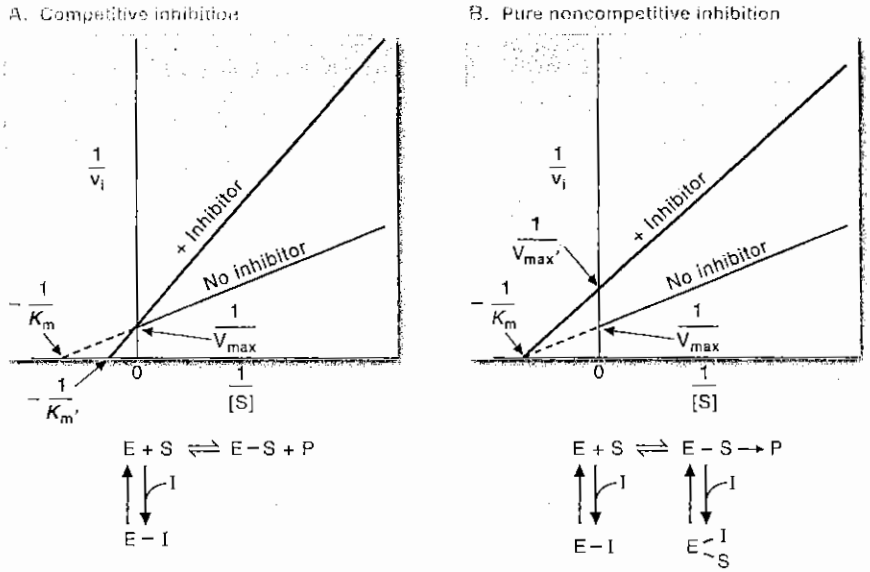


FIG. 9.14. Lineweaver-Burk plots of competitive and pure noncompetitive inhibition. **A.** $1/v_i$ versus $1/[S]$ in the presence of a competitive inhibitor. The competitive inhibitor alters the intersection on the abscissa. The new intersection is $1/K_{m,app}$ (also called $1/K_m'$). A competitive inhibitor does not affect V_{max}. **B.** $1/v_i$ versus $1/[S]$ in the presence of a pure noncompetitive inhibitor. The noncompetitive inhibitor alters the intersection on the ordinate, $1/V_{max,app}$ or $1/V_{max}'$, but does not affect $1/K_m$. A pure noncompetitive inhibitor binds to E and ES with the same affinity. If the inhibitor has different affinities for E and ES, the lines will intersect above or below the abscissa, and the noncompetitive inhibitor will change both the K_m' and the V_m'.

reactions, they are not used directly for the determination of K_m and V_{max} values by researchers.

Lineweaver-Burk plots provide a good illustration of competitive inhibition and pure noncompetitive inhibition (Fig. 9.14). In competitive inhibition, plots of 1/v versus 1/[S] at a series of inhibitor concentrations intersect on the ordinate. Thus, at infinite substrate concentration, or 1/[S] = 0, there is no effect of the inhibitor. In pure noncompetitive inhibition, the inhibitor decreases the velocity even when [S] has been extrapolated to an infinite concentration. However, if the inhibitor has no effect on the binding of the substrate, the K_m is the same for every concentration of inhibitor, and the lines intersect on the abscissa.

Key Concepts

- Enzyme activity is regulated to reflect the physiologic state of the organism.
- The rate of an enzyme-catalyzed reaction depends on substrate concentration and can be represented mathematically by the Michaelis-Menten equation.
- Allosteric activators or inhibitors are compounds that bind at sites other than the active catalytic site and regulate the enzyme through conformational changes that affect the catalytic site.
- Several different mechanisms are available to regulate enzyme activity. These include the following:
  - Feedback inhibition, which often occurs at the first committed step of a metabolic pathway
  - Covalent modification of an amino acid residue (or residues) within the protein
  - Interactions with modulator proteins, which, when bound to the enzyme, alter the conformation of the enzyme and hence activity
  - Altering the primary structure of the protein via proteolysis
  - Increasing or decreasing the amount of enzyme available in the cell via alterations in the rate or synthesis of degradation of the enzyme
- Metabolic pathways are frequently regulated at the slowest, or rate-limiting, step of the pathway.
- Diseases discussed in this chapter are summarized in Table 9.1.

Table 9.1 Diseases Discussed in Chapter 9

Disorder or Condition	Genetic or Environmental	Comments
Alcoholism	Both	Both alcohol dehydrogenase and the microsomal ethanol oxidizing system (MEOS) are active in detoxifying ethanol. High NADH can inhibit alcohol dehydrogenase, allowing toxic metabolites to accumulate.
Anorexia	Both	Effects of malnutrition on energy production was discussed.
Maturity onset diabetes of the young (MODY)	Genetic	Mutations in various proteins can lead to this form of diabetes, which is manifest by hyperglycemia, without, however, other complications associated with either type 1 or 2 diabetes. Specifically, mutations in pancreatic glucokinase were discussed.

REVIEW QUESTIONS—CHAPTER 9

1. Which of the following describes a characteristic feature of an enzyme that obeys Michaelis-Menten kinetics?
   A. The enzyme velocity is at one-half the maximal rate when 100% of the enzyme molecules contain bound substrate.
   B. The enzyme velocity is at one-half the maximal rate when 50% of the enzyme molecules contain bound substrate.
   C. The enzyme velocity is at its maximal rate when 50% of the enzyme molecules contain bound substrate.
   D. The enzyme velocity is at its maximal rate when all of the substrate molecules in solution are bound by the enzyme.
   E. The velocity of the reaction is independent of the concentration of enzyme.

2. The pancreatic glucokinase of a patient with MODY had a mutation replacing a leucine with a proline. The result was that the K_m for glucose was decreased from a normal value of 6 mM to a value of 2.2 mM, and the V_{max} was changed from 93 U/mg protein to 0.2 U/mg protein. Which of the following best describes the patient's glucokinase compared with the normal enzyme?
   A. The patient's enzyme requires a lower concentration of glucose to reach $\frac{1}{2}V_{max}$.
   B. The patient's enzyme is faster than the normal enzyme at concentrations of glucose <2.2 mM.
   C. The patient's enzyme is faster than the normal enzyme at concentrations of glucose >2.2 mM.
   D. At near-saturating glucose concentration, the patient would need 90 to 100 times more enzyme than normal to achieve normal rates of glucose phosphorylation.
   E. As blood glucose levels increase after a meal from a fasting value of 5 mM to 10 mM, the rate of the patient's enzyme will increase more than the rate of the normal enzyme.

3. Methanol (CH_3OH) is converted by alcohol dehydrogenases to formaldehyde (CH_2O), a compound that is highly toxic to humans. Patients who have ingested toxic levels of methanol are sometimes treated with ethanol (CH_3CH_2OH) to inhibit methanol oxidation by alcohol dehydrogenase. Which of the following statements provides the best rationale for this treatment?
   A. Ethanol is a structural analog of methanol and might therefore be an effective noncompetitive inhibitor.
   B. Ethanol is a structural analog of methanol that can be expected to compete with methanol for its binding site on the enzyme.
   C. Ethanol can be expected to alter the V_{max} of alcohol dehydrogenase for the oxidation of methanol to formaldehyde.
   D. Ethanol is an effective inhibitor of methanol oxidation regardless of the concentration of methanol.
   E. Ethanol can be expected to inhibit the enzyme by binding to the formaldehyde-binding site on the enzyme, even though it cannot bind at the substrate-binding site for methanol.

4. An allosteric enzyme has the following kinetic properties: a V_{max} of 25 U/mg enzyme, and a $K_{0.5\,app}$ of 1.0 mM. These

kinetic parameters were then measured in the presence of an allosteric activator. Which one of the following would best describe the findings of that experiment?

A. A V_{max} of 25 U/mg enzyme, and a $K_{m,app}$ of 0.2 mM.
B. A V_{max} of 15 U/mg enzyme, with a $K_{m,app}$ of 2.0 mM.
C. A V_{max} of 25 U/mg enzyme, with a $K_{m,app}$ of 2.0 mM.
D. A V_{max} of 50 U/mg enzyme, with a $K_{m,app}$ of 5.0 mM.
E. A V_{max} of 50 U/mg enzyme, with a $K_{m,app}$ of 10.0 mM.

5. A rate-limiting enzyme catalyzes the first step in the conversion of a toxic metabolite to a urinary excretion product. Which of the following mechanisms for regulating this enzyme would provide the most protection to the body?

A. The product of the pathway should be an allosteric inhihitor of the rate-limiting enzyme.
B. The product of the pathway should act through gene transcription to decrease synthesis of the enzyme.
C. The toxin should act through gene transcription to increase synthesis of the enzyme.
D. The enzyme should have a high K_m for the toxin.
E. The toxin allosterically activates the last enzyme in the pathway.

10 Relationship between Cell Biology and Biochemistry

The basic unit of a living organism is the cell. In humans, each tissue is composed of a variety of cell types, which differ from those cell types in other tissues. The diversity of cell types serves the function of the tissue and organs in which they reside, and each cell type has unique structural features that reflect its role. In spite of their diversity in structure, human cell types have certain architectural features in common, such as the plasma membrane, membranes around the nucleus and organelles, and a cytoskeleton (Fig. 10.1). In this chapter, we review some of the chemical characteristics of these common features, the functions of organelles, and the transport systems for compounds into cells and between organelles.

Plasma Membrane. *The cell membrane consists of a* **lipid bilayer** *that serves as a selective barrier; it restricts the entry and exit of compounds. Within the plasma membrane, different* **integral membrane proteins** *facilitate the* **transport** *of compounds by* **energy-requiring active transport, facilitated diffusion,** *or by forming* **pores** *or* **gated channels**. *The plasma membrane is supported by a* **membrane skeleton** *composed of proteins.*

Organelles and Cytoplasmic Membrane Systems. *Most organelles within the cell are compartments surrounded by a membrane system that restricts exchange of compounds and information with other compartments (see Fig. 10.1). In general, each organelle has unique functions that are served by the enzymes and other compounds it contains, or the environment it maintains.* **Lysosomes** *contain* **hydrolytic enzymes** *that degrade proteins and other large molecules. The* **nucleus** *contains the genetic material and carries out* **gene replication** *and* **transcription** *of* **DNA**, *the first step of protein synthesis. The last phase of protein synthesis occurs on ribosomes. For certain proteins, the ribosomes become attached to the complex membrane system called the* **endoplasmic reticulum** *(ER); for other proteins, synthesis is completed on ribosomes that remain in the cytoplasm. The* **ER** *is also involved in lipid synthesis and transport of molecules to the Golgi. The* **Golgi** *forms vesicles for transport of molecules to the plasma membrane and other membrane systems and for secretion.* **Mitochondria** *are organelles committed to* **fuel oxidation** *and* **adenosine triphosphate** *(ATP)* **generation**. **Peroxisomes** *contain many enzymes that use or produce* **hydrogen peroxide**. *The* **cytosol** *is the intracellular compartment free of organelles and membrane systems.*

Cytoskeleton. *The* **cytoskeleton** *is a flexible fibrous protein support system that maintains the geometry of the cell, fixes the position of organelles, and moves compounds within the cell. The cytoskeleton also facilitates movement of the cell itself. It is composed primarily of* **actin microfilaments, intermediate filaments** *(IFs),* **tubulin microtubules**, *and their attached proteins.*

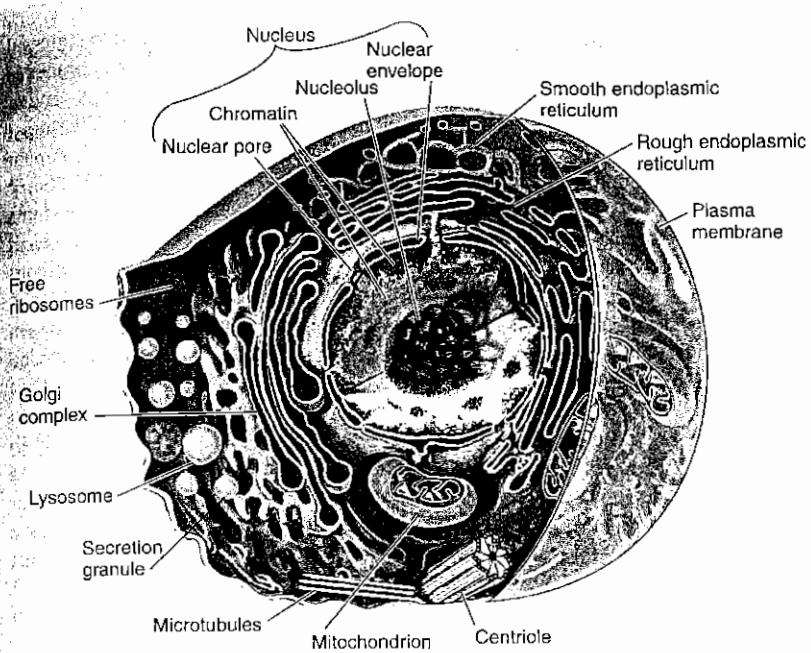


FIG. 10.1. Common components of human cells.

THE WAITING ROOM

Al Martini had been drinking heavily when he drove his car off the road and was taken to the hospital emergency room (see Chapters 8 and 9). Although he suffered only minor injuries, his driving license was suspended.

Two years after Dennis "the Menace" Veere recovered from his malathion poisoning, he visited his grandfather, **Percy Veere**. Mr. Veere took Dennis with him to a picnic at the shore, where they ate steamed crabs. Later that night, Dennis experienced episodes of vomiting and watery diarrhea, and Mr. Veere rushed him to the hospital emergency room. Dennis's hands and feet were cold; he appeared severely dehydrated, and he was approaching hypovolemic shock (a severe drop in blood pressure related to fluid losses). He was diagnosed with cholera, caused by the bacteria *Vibrio cholerae*.

Before **Lotta Topaigne** was treated with allopurinol (see Chapter 8), her physician administered colchicine (acetyltrimethylcolchicinic acid) for the acute attack of gout affecting her great toe. After taking two doses of colchicine divided over 1 hour (1.2 mg for the first dose, followed 1 hour later by 0.6 mg), the throbbing pain in her toe had abated significantly. The redness and swelling also seemed to have lessened slightly.

I. COMPARTMENTATION IN CELLS

Membranes are lipid structures that separate the contents of the compartment they surround from its environment. An outer plasma membrane separates the cell from

Vibrio cholerae epidemics are associated with unsanitary conditions affecting the drinking water supply and are rare in the United States. However, these bacteria grow well under the alkaline conditions found in seawater and attach to chitin in shellfish. Thus, sporadic cases occur in the southeast United States associated with the ingestion of contaminated shellfish.

Uric acid levels in blood or urine can be determined enzymatically through the use of the enzyme uricase, which converts uric acid (plus oxygen) to allantoin and hydrogen peroxide. Uricase is found in lower primates but not in humans. Uric acid has a strong light absorbance at 293 nm, whereas allantoin does not. Therefore, measurement of the decrease in absorbance at 293 nm after treating an unknown sample with uricase can allow determination of uric acid levels. Because the presence of proteins can reduce the sensitivity of this method, an alternative method is to determine the amount of hydrogen peroxide formed during the course of the reaction. In most cases, the enzymes peroxidase or catalase is used, and the enzymatic products are coupled to a chemical indicator reaction (a color change). One can determine the extent of color change during the reaction, which is proportional to the hydrogen peroxide concentration, which is the same as the concentration of uric acid in the sample.

Bacteria are single cells surrounded by a cell membrane and a cell wall exterior to the membrane. They are prokaryotes, which do not contain nuclei or other organelles (i.e., membrane-surrounded subcellular structures) found in eukaryotic cells. Nonetheless, bacteria carry out many similar metabolic pathways, with the enzymes located in either the intracellular compartment or the cell membrane.

The *Vibrio cholerae* responsible for **Dennis Veere's** cholera are gram-negative bacteria. Their plasma membrane is surrounded by a thin cell wall composed of a protein–polysaccharide structure called *peptidoglycan* and an outer membrane. In contrast, gram-positive bacteria have a plasma membrane and a thick peptidoglycan cell wall that retains the Gram stain. *V. cholerae* grow best not only under aerobic conditions, but also under low-oxygen conditions. They possess enzymes similar to those in human cells for glycolysis, the tricarboxylic acid (TCA) cycle, and oxidative phosphorylation. They have a low tolerance for acid, which partially accounts for their presence in slightly basic seawater and shellfish.

the external environment. Organelles (such as the nucleus, mitochondria, lysosomes and peroxisomes) are also surrounded by membrane systems that separate the interna compartment of the organelle from the cytosol. The function of these membranes i to allow the organelle to collect or concentrate enzymes and other molecules servin a common function into a compartment within a localized environment. The trans porters and receptors in each membrane system control this localized environmer and facilitate communication of the cell or organelle with the surrounding milieu.

The following sections describe the various organelles and membrane system found in most human cells and outline the relationship between their properties an function. Each organelle contains different enzymes and carries out different gen eral functions. For example, the nucleus contains the enzymes for DNA and RN synthesis. The cells of humans and other animals are eukaryotes (*eu*, good; *karyon* nucleus) because the genetic material is organized into a membrane-enclose nucleus. In contrast, bacteria are prokaryotes (*pro*, before; *karyon*, nucleus); the do not contain nuclei or other organelles found in eukaryotic cells.

Not all cells in the human are alike. Different cell types differ quantitatively i their organelle content, and their organelles may contain vastly different amount of a particular enzyme, *consistent with the function of the cell*. For example, live mitochondria contain a key enzyme for synthesizing ketone bodies, but they lack key enzyme required for their use. The reverse is true in muscle mitochondria. Thus the enzyme content of the organelles varies somewhat from cell type to cell type.

II. PLASMA MEMBRANE

A. Structure of the Plasma Membrane

All mammalian cells are enclosed by a plasma membrane composed of a lipic bilayer (two layers) containing embedded proteins (Fig. 10.2). The membranes are continuous and sealed so that the hydrophobic lipid bilayer selectively restricts the exchange of polar compounds between the external mileau and the intracellular compartment. The membrane is sometimes referred to as a *fluid mosaic* because it consists of a mosaic of proteins and lipid molecules that can, for the most part, move laterally in the plane of the membrane. The proteins are classified as *integral proteins*, which span the cell membrane, or *peripheral proteins*, which are attached to the membrane surface through electrostatic bonds to lipids or integral proteins.

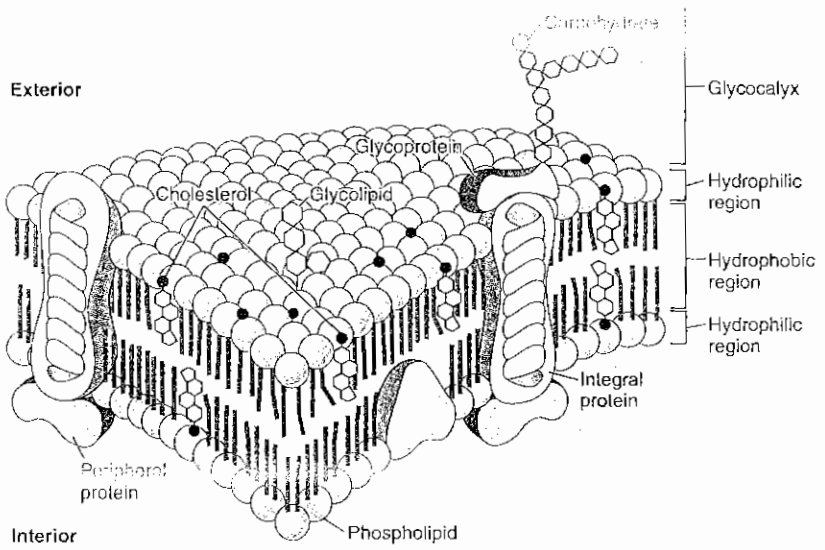


FIG. 10.2. Basic structure of a mammalian cell membrane.

Many of the proteins and lipids on the external leaflet of the plasma membrane contain covalently bound carbohydrate chains and therefore are glycoproteins and glycolipids. This layer of carbohydrate on the outer surface of the cell is called the *glycocalyx*. The variable carbohydrate components of the glycolipids on the cell surface function, in part, as cell recognition markers for small molecules or other cells.

1. LIPIDS IN THE PLASMA MEMBRANE

Each layer of the plasma membrane lipid bilayer is formed primarily by phospholipids, which are arranged with their hydrophilic head groups facing the aqueous medium and their fatty acyl tails forming a hydrophobic membrane core (see Fig. 10.2). The principal phospholipids in the membrane are the glycerol lipids phosphatidylcholine, phosphatidylethanolamine, and phosphatidylserine, and the sphingosine-based sphingomyelin (Fig. 10.3). Sphingosine also forms the base for the *glycosphingolipids*, which are membrane-anchored lipids with carbohydrates attached. The lipid composition varies among different cell types, with phosphatidylcholine being the major plasma membrane phospholipid in most cell types and glycosphingolipids being the most variable.

The lipid composition of the bilayer is asymmetric, with a higher content of phosphatidylcholine and sphingomyelin in the outer leaflet and a higher content of phosphatidylserine and phosphatidylethanolamine in the inner leaflet. Phosphatidylinositol, which can function in the transfer of information from hormones and neurotransmitters across the cell membrane (see Chapter 11), is also

One of the bacterial toxins secreted by *Clostridium perfringens*, the bacteria that cause gas gangrene, is a lipase that hydrolyzes phosphocholine from phosphatidylcholine and from sphingomyelin. The resulting lysis (breakage) of the cell membrane releases intracellular contents that provide the bacteria with nutrients for rapid growth. These bacteria are strict anaerobes and grow only in the absence of oxygen. As their toxins lyse membranes in the endothelial cells of blood vessels, the capillaries are destroyed, and the bacteria are protected from oxygen transported by the red blood cells. They are also protected from antibiotics and components of the immune system carried in the blood.

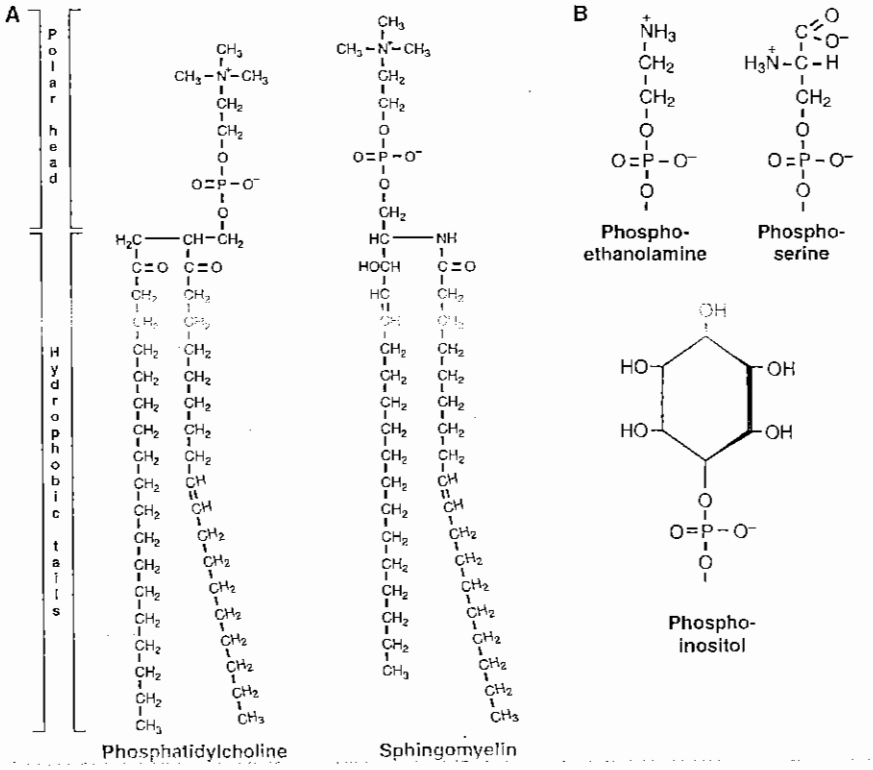


FIG. 10.3. Common phospholipids in the mammalian cell membrane. **A.** Phosphatidylcholine (a glycerol-based lipid) and sphingomyelin (a sphingosine-based lipid). Note the similarity in structures. **B.** Different head groups for the phospholipids. These head groups replace the choline in phosphatidylcholine and form, respectively, phosphatidylethanolamine, phosphatidylserine, and phosphatidylinositol.

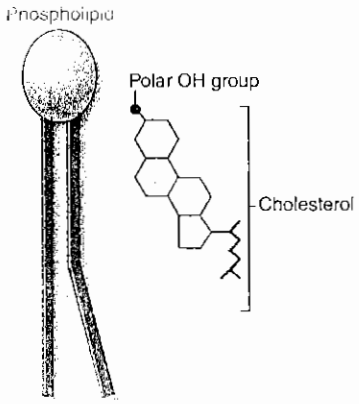


FIG. 10.4. Cholesterol in the plasma membrane. The polar hydroxyl group of cholesterol is oriented toward the surface. The hydrocarbon tail and the steroid nucleus (*orange*) lie in the hydrophobic core. A *cis* double bond in the fatty acyl chain of a phospholipid bends the chain to create a hydrophobic binding site for cholesterol.

Al Martini is suffering from both short-term and long-term effects of ethanol on his central nervous system. Data support the theory that the short-term effects of ethanol on the brain arise partly from an increase in membrane fluidity caused when ethanol intercalates between the membrane lipids. The changes in membrane fluidity may affect proteins that span the membrane (integral proteins), such as ion channels and receptors for neurotransmitters involved in conducting the nerve impulse.

only found in the inner leaflet. Phosphatidylserine contains a net negative charge that contributes to the membrane potential and may be important for binding positively charged molecules within the cell.

Cholesterol, which is interspersed between the phospholipids, maintains membrane fluidity. In the glycerol-based phospholipids, unsaturated fatty acid chains bent into the *cis* conformation form a pocket for cholesterol, which binds with its hydroxyl group in the external hydrophilic region of the membrane and its hydrophobic steroid nucleus in the hydrophobic membrane core (Fig. 10.4). The presence of cholesterol and the *cis* unsaturated fatty acids in the membrane prevent the hydrophobic chains from packing too closely together. As a consequence, lipid and protein molecules that are not bound to external or internal structural proteins can rotate and move laterally in the plane of the leaflet. This movement enables the plasma membrane to partition between daughter cells during cell division, to deform as cells pass through capillaries, and to form and fuse with vesicle membranes. The fluidity of the membrane is partially determined by the unsaturated fatty acid content of the diet.

The composition of the membrane is dynamic. Sections of membrane form buds that pinch off into vesicles, and membrane vesicles formed in the Golgi and elsewhere bring new and recycled components back to the membrane. Individual fatty acyl chains turn over as they are hydrolyzed from the lipids and replaced, and enzymes called *flipases* transfer lipids between leaflets.

2. PROTEINS IN THE PLASMA MEMBRANE

The integral membrane proteins contain transmembrane domains with hydrophobic amino acid side chains that interact with the hydrophobic portions of the lipids to seal the membrane (see Fig. 10.2). Hydrophilic regions of the proteins protrude into either the extracellular milieu or the cytoplasm. These proteins function primarily as channels or transporters for the movement of compounds across the membrane, as receptors for the binding of hormones and neurotransmitters, or as structural proteins (Fig. 10.5).

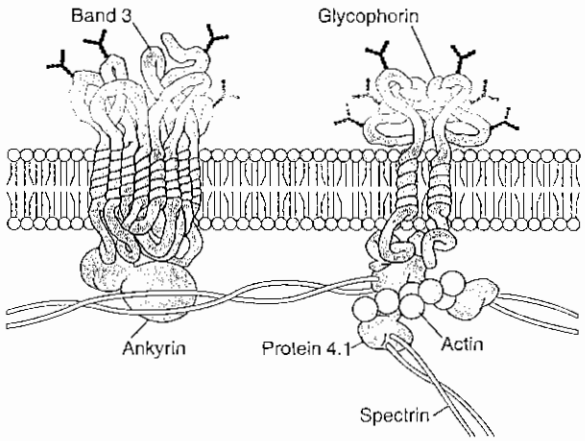


FIG. 10.5. Proteins in the red blood cell membrane. The proteins named *Band 3* (the bicarbonate–chloride exchange transporter) and glycophorin (provides an external negative charge that repels other cells) both contain nonpolar α helical segments spanning the lipid bilayer. These proteins contain a large number of polar and charged hydrophilic amino acids in the intracellular and extracellular domains. On the inside of the cell, they are attached to peripheral proteins constituting the inner membrane skeleton. Band 3 is connected to spectrin filaments via the protein ankyrin. Glycophorin is connected to short actin filaments and spectrin via protein 4.1.

Peripheral membrane proteins, which were originally defined as those proteins that can be released from the membrane by ionic solvents, are bound through weak electrostatic interactions with the polar head groups of lipids or with integral proteins. One of the best characterized classes of peripheral proteins is the spectrin family of proteins, which are bound to the intracellular membrane surface and provide mechanical support for the membrane. Spectrin is bound to actin, which together form a structure that is called the *inner membrane skeleton* or the *cortical skeleton* (see Fig. 10.5).

A third classification of membrane proteins consists of lipid-anchored proteins bound to the inner or outer surface of the membrane. The *glycophosphatidylinositol glycan* (GPI) anchor is a covalently attached lipid that anchors proteins to the external surface of the membrane (Fig. 10.6). Several proteins involved in hormonal regulation are anchored to the internal surface of the membrane through palmityl (C16) or myristyl (C14) fatty acyl groups or through geranylgeranyl (C20) or farnesyl (C15) isoprenyl groups (see Fig. 6.13). However, many integral proteins also contain attached lipid groups to increase their stability in the membrane.

3. THE GLYCOCALYX OF THE PLASMA MEMBRANE

Some of the proteins and lipids on the external surface of the membrane contain short chains of carbohydrates (oligosaccharides) that extend into the aqueous medium. Carbohydrates constitute 2% to 10% of the weight of plasma membranes. This hydrophilic carbohydrate layer, called the *glycocalyx*, protects the cell from digestion and restricts the uptake of hydrophobic compounds.

The glycoproteins generally contain branched oligosaccharide chains of approximately 15 sugar residues that are attached through *N*-glycosidic bonds to the amide nitrogen of an asparagine side chain (*N*-glycosidic linkage) or through a glycosidic bond to the oxygen of serine (*O*-glycoproteins). The membrane glycolipids are usually galactosides or cerebrosides. Specific carbohydrate chains on the glycolipids serve as cell-recognition molecules (see Chapter 5 for structures of these compounds).

B. Transport of Molecules across the Plasma Membrane

Membranes form hydrophobic barriers around cells to control the internal environment by restricting the entry and exit of molecules. As a consequence, cells require transport systems to permit entry of small polar compounds that they need (e.g., glucose) to concentrate compounds inside the cell (e.g., K^+) and to expel other compounds (e.g., Ca^{++} and Na^+). The transport systems for small organic molecules and inorganic ions fall into four categories: simple diffusion through the lipid bilayer, facilitative diffusion, gated channels, and active transport pumps (Fig. 10.7). These transport mechanisms are classified as passive if energy is not required or active if energy is required. The energy is often provided by the hydrolysis of adenosine triphosphate (ATP).

In addition to these mechanisms for the transport of small individual molecules, cells engage in a process called *endocytosis*. The plasma membrane extends or invaginates to surround a particle, a foreign cell, or extracellular fluid, which then closes into a vesicle that is released into the cytoplasm (see Fig. 10.7).

1. SIMPLE DIFFUSION

Gases such as oxygen and carbon dioxide and lipid-soluble substances (such as steroid hormones) can cross membranes by simple diffusion (see Fig. 10.7). In simple diffusion (free diffusion), molecules move by engaging in random collisions with other like molecules. There is a net movement from a region of high concentration to a region of low concentration because molecules keep bumping into each other where their concentration is highest. Energy is not required for diffusion, and compounds that are uncharged eventually reach the same concentrations on both sides of the membrane.

All cells contain an inner membrane skeleton of spectrin-like proteins. Red blood cell spectrin was the first member of the spectrin family to be described. The protein *dystrophin*, present in skeletal muscle cells, is a member of the spectrin family. Genetic defects in the dystrophin gene are responsible for Duchenne and Becker muscular dystrophies.

The prion protein, present in neuronal membranes, provides an example of a protein attached to the membrane through a GPI anchor. This is the protein that develops an altered pathogenic conformation in both mad cow disease and Creutzfeldt-Jakob disease (see Section IX.B.3, Chapter 7).

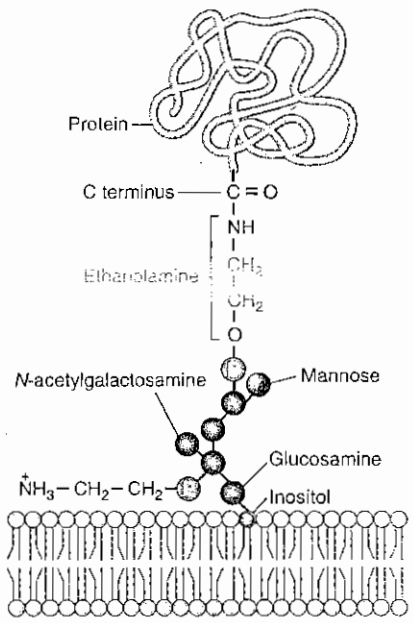


FIG. 10.6. An example of a GPI anchor. The carboxy terminus of the protein is attached to phosphoethanolamine, which is bound to a branched oligosaccharide that is attached to the inositol portion of phosphatidylinositol. The hydrophobic fatty acyl chains of the phosphatidylinositol portion are bound in the hydrophobic core of the membrane.

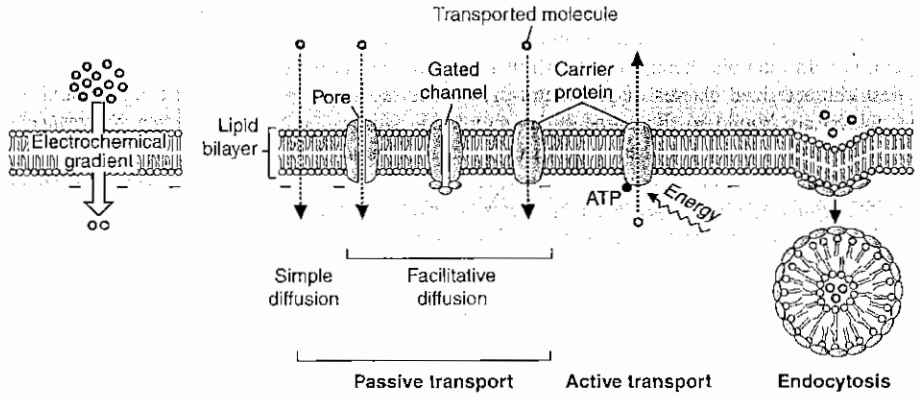


FIG. 10.7. Common types of transport mechanisms for human cells. The electrochemical gradient consists of the concentration gradient of the compound and the distribution of charge on the membrane, which affects the transport of charged ions such as Cl^-. Both protein amino acid residues and lipid polar head groups contribute to the net negative charge on the inside of the membrane. Generally, the diffusion of uncharged molecules (passive transport) is net movement from a region of high concentration to a region of low concentration, and active transport (energy requiring) is net movement from a region of low concentration to one of high concentration.

The A, B, and O blood groups are determined by the carbohydrate composition of the glycolipids on the cell surface. These glycolipids may also serve as binding sites for viruses and bacterial toxins before penetrating the cell. For example, the cholera AB toxin (which is affecting **Dennis Veere**) binds to GM_1-gangliosides on the surface of the intestinal epithelial cells. The toxin is then endocytosed in caveolae (invaginations or "caves" that can form in specific regions of the membrane). Once inside the cell, the toxin will alter normal cellular metabolism.

Dennis Veere has become dehydrated because he has lost so much water through vomiting and diarrhea (see Chapter 4). Cholera toxin increases the efflux of sodium and chloride ions from his intestinal mucosal cells into the intestinal lumen. The increase of water in his stools results from the passive transfer of water from inside the cell and body fluids, where it is in high concentration (i.e., intracellular Na^+ and Cl^- concentrations are low), to the intestinal lumen and bowel, where water is in lower concentration (relative to high Na^+ and Cl^-). The watery diarrhea is also high in K^+ ions and bicarbonate. All of the signs and symptoms of cholera generally derive from this fluid loss.

Water is considered to diffuse through membranes by nonspecific movement through ion channels, pores, or around proteins embedded in the lipids. Certain cells (e.g., renal tubule cells) also contain large protein pores, called *aquaporins*, which permit a high rate of water flow from a region of a high water concentration (low solute concentration) to one of low water concentration (high solute concentration).

2. FACILITATIVE DIFFUSION THROUGH BINDING TO TRANSPORTER PROTEINS

Facilitative diffusion requires that the transported molecule bind to a specific carrier or transport protein in the membrane (Fig. 10.8A). The transporter protein then undergoes a conformational change that allows the transported molecule to be released on the other side of the membrane. Although the transported molecules are bound to proteins, the transport process is still classified as diffusion because energy is not required, and the compound equilibrates (achieves a balance of concentration and charge) on both sides of the membrane.

Transporter proteins, like enzymes, exhibit saturation kinetics; when all the binding sites on the transporter proteins in the membrane are occupied, the system is saturated and the rate of transport reaches a plateau (the maximum velocity). By analogy to enzymes, the concentration of a transported compound required to reach half the maximum velocity is often called the K_m (see Fig. 10.8B). Facilitative transporters are similar to enzymes with respect to two additional features: They are relatively specific for the compounds they bind, and they can be inhibited by compounds that block their binding sites or change their conformation.

3. GATED CHANNELS IN PLASMA MEMBRANES

In the case of gated channels, transmembrane proteins form a pore for ions that is either opened or closed in response to a stimulus. These stimuli can be voltage changes across the membrane (voltage-gated channels), the binding of a compound (ligand-gated channels), or a regulatory change in the intracellular domain (phosphorylation-gated and pressure-gated channels). For example, the conduction of a nerve impulse along an axon depends on the passive flux of Na^+ ions through a voltage-gated channel that is opened by depolarization of the membrane.

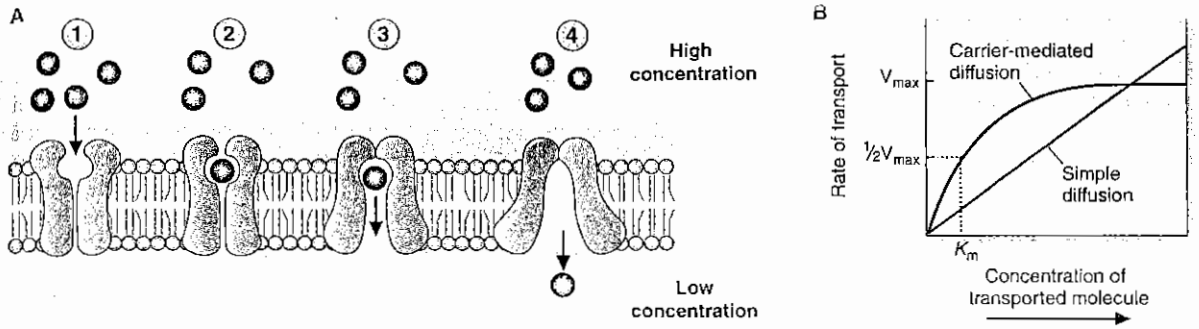


FIG. 10.8. **A.** Facilitative transport. Although the molecule being transported must bind to the protein transporter, the mechanism is passive diffusion, and the molecule moves from a region of high concentration to one of low concentration. *Passive* refers to the lack of an energy requirement for the transport. **B.** Saturation kinetics of transporter proteins. When a compound must bind to a protein to be transported across a membrane, the velocity of transport depends on the amount of compound bound. It reaches a maximum rate when the compound's concentration is raised so high that all of the transporter-binding sites are occupied. The curve is a rectangular hyperbola that approaches V_{max} at infinite substrate concentration, identical to that of Michaelis-Menten enzymes. The K_m of transport is the concentration of compound required for $1/2V_{max}$. In contrast, simple diffusion of a compound does not require its binding to a protein, and the rate of transport increases linearly with increasing concentration of the compound.

Cystic fibrosis transmembrane conductance regulator (CFTR) is a chloride channel that provides an example of a ligand-gated channel regulated through phosphorylation (phosphorylation-gated channels) (Fig. 10.9). CFTR is a member of the *ABC* (adenine nucleotide–binding cassette or ATP-binding cassette) superfamily of transport proteins. It has two transmembrane domains that form a closed channel, each connected to an ATP-binding site, and a regulatory domain that sits in front of the channel. When the regulatory domain is phosphorylated by a kinase, its conformation changes and it moves away from the ATP-binding domains. As ATP binds and is hydrolyzed, the transmembrane domains change conformation and open the channel, and chloride ions diffuse through. As the conformation reverts back to its original form, the channel closes.

Transport through a ligand-gated channel can be considered simple diffusion, although ATP is involved, because only a few ATP molecules are being used to open and close the channel through which many chloride ions diffuse. However, the distinction between ligand-gated channels and facilitative transporters is not always as clear. Many gated channels show saturation kinetics at very high concentrations of the compounds being transported, which is why Figure 10.7 characterizes ligand-gated channels as facilitative transport.

All of the cells in the body have facilitative glucose transporters that transport glucose across the plasma membrane down an electrochemical (concentration) gradient as it is rapidly metabolized in the cell. In muscle and adipose tissue, insulin increases the content of facilitative glucose transporters in the cell membrane, thus increasing the ability of these tissues to take up glucose. Patients with type 1 diabetes mellitus, who do not produce insulin (e.g., **Di Abietes**, see Chapter 7), have a decreased ability to transport glucose into these tissues, thereby contributing to hyperglycemia (high blood glucose).

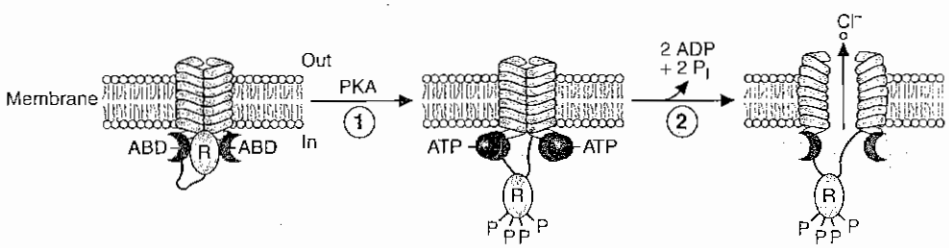


FIG. 10.9. CFTR, a ligand-gated channel controlled by phosphorylation. Two intracellular binding domains control opening of the channel, an adenine nucleotide-binding domain (*ABD*) and a regulatory domain (*R*). (*1*) Phosphorylation of the regulatory subunit by protein kinase A (*PKA*) causes a conformational change that allows *ATP* to bind to the *ABD*. (*2*) Hydrolysis of bound *ATP* opens the channel so that chloride ions can diffuse through.

The CFTR was named for its role in cystic fibrosis. A mutation in the gene encoding its transmembrane subunits results in dried mucus accumulation in the airways and pancreatic ducts. The CFTR is also involved in the dehydration experienced by cholera patients such as **Dennis Veere**. In intestinal mucosal cells, the cholera A-subunit indirectly promotes phosphorylation of the regulatory domain of CFTR by protein kinase A (PKA). Thus, the channel stays open and Cl^- and H_2O flow from the cell into the intestinal lumen, resulting in dehydration.

The dehydration of cholera is often treated with an oral rehydration solution containing sodium, potassium, and glucose or a diet of rice (which contains glucose and amino acids). Glucose is absorbed from the intestinal lumen via the Na^+-dependent glucose cotransporters, which cotransport Na^+ into the cells together with glucose. Many amino acids are also absorbed by Na^+-dependent cotransport. With the return of Na^+ to the cytoplasm, the release of water from the cell into the intestinal lumen decreases.

4. ACTIVE TRANSPORT REQUIRES ENERGY AND TRANSPORTER PROTEINS

Both active transport and facilitative transport are mediated by protein transporters (carriers) in the membrane. However, in facilitative transport, the compound is transported down an electrochemical gradient (the balance of concentration and charge across a membrane), usually from a high concentration to a low concentration, to equilibrate between the two sides of the membrane. In active transport, energy is used to concentrate the compound on one side of the membrane. If energy is applied directly to the transporter (e.g., ATP hydrolysis by Na^+,K^+-ATPase), the transport is called *primary active transport*; if energy is used to establish an ion gradient (e.g., the Na^+ gradient), and the gradient is used to concentrate another compound, the transport is called *secondary active transport*. Protein-mediated transport systems, whether facilitative or active, are classified as antiports if they specifically exchange compounds of similar charge across a membrane; they are called *symports* or *cotransporters* if they simultaneously transport two molecules across the membrane in the same direction. Band 3 in the red blood cell membrane, which exchanges chloride ion for bicarbonate, provides an example of an antiport.

The Na^+,K^+-ATPase spans the plasma membrane, much like a gated pore, with a binding site for three Na^+ ions open to the intracellular side (Fig. 10.10). Energy from ATP hydrolysis is used to phosphorylate an internal domain and change the transporters' conformation so that bound Na^+ ions are released to the outside, and two external K^+ ions bind. K^+ binding triggers hydrolysis of the bound phosphate group and a return to the original conformation, accompanied by release of K^+ ions inside the cell. As a consequence, cells are able to maintain a much lower intracellular Na^+ concentration and a much higher intracellular K^+ ion concentration than are present in the external fluid.

The Na^+ gradient, which is maintained by primary active transport, is used to power the transport of glucose, amino acids, and many other compounds into the cell through secondary active transport. An example is provided by the transport of glucose into cells of the intestinal epithelium in conjunction with Na^+ ions (Fig. 10.11). These cells create a gradient in Na^+ and then use this gradient to drive the transport of glucose from the intestinal lumen into the cell against its concentration gradient.

The Ca^{2+}-ATPase, a calcium pump, uses a mechanism similar to that of Na^+,K^+-ATPase to maintain intracellular Ca^{2+} concentration below 10^{-7} M in spite of the high extracellular concentration of 10^{-3} M. This transporter is inhibited by

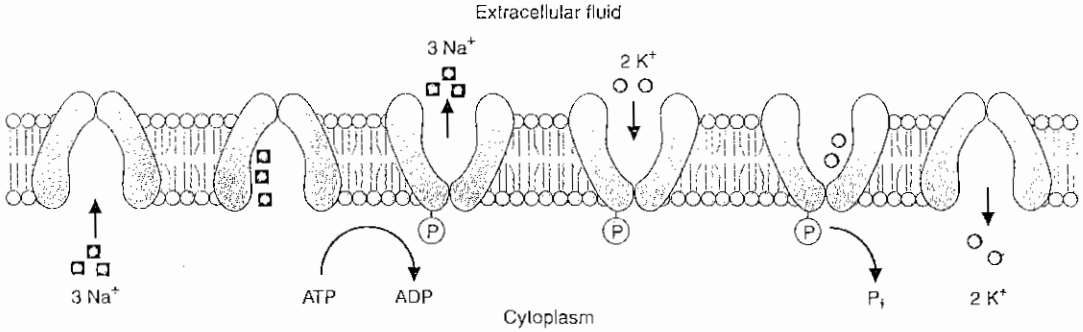


FIG. 10.10 Active transport by Na^+,K^+-ATPase. Three sodium ions bind to the transporter protein on the cytoplasmic side of the membrane. When ATP is hydrolyzed to adenosine diphosphate (ADP), the carrier protein is phosphorylated and undergoes a change in conformation that causes the sodium ions to be released into the extracellular fluid. Two potassium ions then bind on the extracellular side. Dephosphorylation of the carrier protein produces another conformational change, and the potassium ions are released on the inside of the cell membrane. The transporter protein then resumes its original conformation, ready to bind more sodium ions.

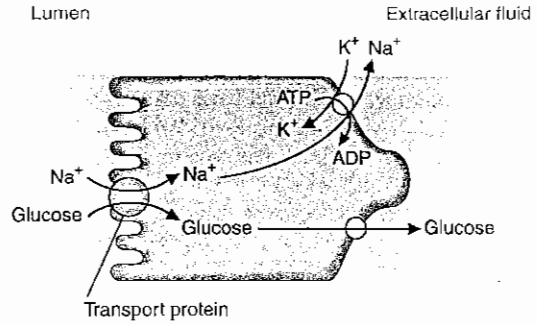


FIG. 10.11. Secondary active transport of glucose by the Na^+-glucose cotransporter. One sodium ion binds to the carrier protein in the luminal membrane, stimulating the binding of glucose. After a conformational change, the protein releases Na^+ and glucose into the cell and returns to its original conformation. Na^+,K^+-ATPase in the basolateral membrane pumps Na^+ against its concentration gradient into the extracellular fluid. Thus, the Na^+ concentration in the cell is low, and Na^+ moves from the lumen down its concentration gradient into the cell and is pumped against its gradient into the extracellular fluid. Glucose, consequently, moves against its concentration gradient from the lumen into the cell by traveling on the same carrier as Na^+. Glucose then passes down its concentration gradient into the extracellular fluid on a passive transporter protein.

binding of the regulatory protein calmodulin. When the intracellular Ca^{2+} concentration increases, Ca^{2+} binds to calmodulin, which dissociates from the transporter, thereby activating it to pump Ca^{2+} out of the cell (see Fig. 9.10 for the structure of calmodulin). High levels of intracellular Ca^{2+} are associated with irreversible progression from cell injury to cell death.

C. Vesicular Transport across the Plasma Membrane

Vesicular transport occurs when a membrane completely surrounds a compound, particle, or cell and encloses it into a vesicle, which buds from the membrane. When the released vesicle fuses with another membrane system, the entrapped compounds are released. *Endocytosis* refers to vesicular transport into the cell, and *exocytosis* refers to transport out of the cell. Endocytosis is further classified as phagocytosis if the vesicle forms around particulate matter (such as whole bacterial cells or metals and dyes from a tattoo) and pinocytosis if the vesicle forms around fluid containing dispersed molecules. *Receptor-mediated endocytosis* is the name given to the formation of clathrin-coated vesicles that mediate the internalization of membrane-bound receptors in vesicles coated on the intracellular side with subunits of the protein clathrin. Cholesterol uptake, as mediated by the low-density lipoprotein (LDL) receptor, occurs via this mechanism. *Potocytosis* is the name given to endocytosis that occurs via caveolae (small invaginations or "caves"), which are regions of the cell membrane with a unique lipid and protein composition (including the protein caveolin-1). The transport of the vitamin folate occurs via potocytosis.

III. LYSOSOMES

Lysosomes are the intracellular organelles of digestion and are enclosed by a single membrane to prevent the release of the lysosome's digestive enzymes into the cytosol. Lysosomes are central to a wide variety of body functions that involve eliminating unwanted material, including destruction of infectious bacteria and yeast, recovery from injury, tissue remodeling, involution of tissues during development, and normal turnover of cells and organelles. The destroyed components are typically recycled for additional use by the cell.

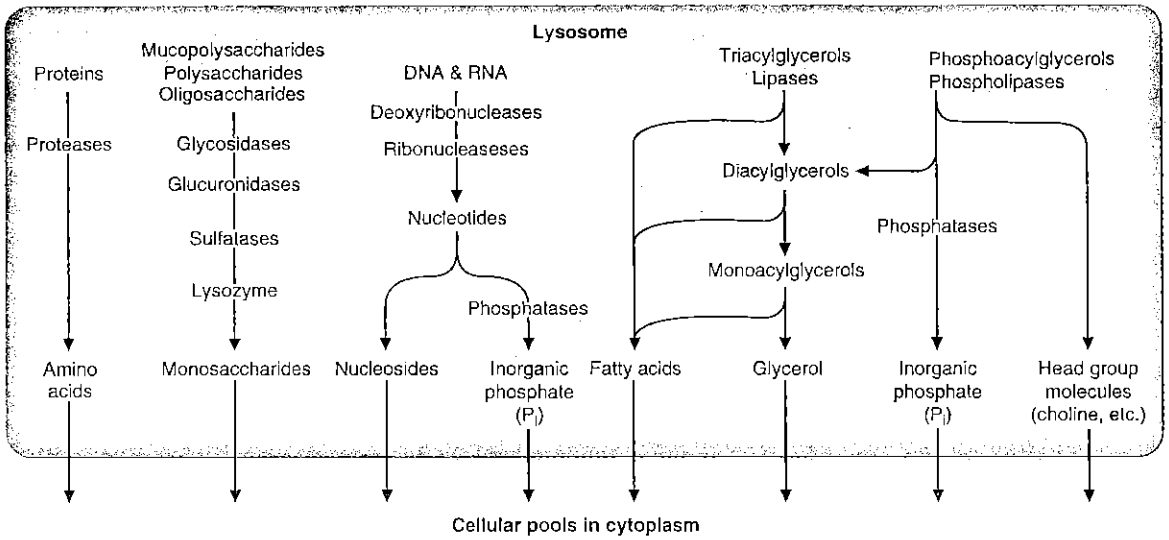


FIG. 10.12. Lysosomal reactions. Most lysosomal enzymes are hydrolases, which cleave peptide, ester, and glycosidic bonds by adding the components of water across the bond. These enzymes are active at the acidic pH of the lysosome and inactive if accidentally released into the cytosol.

Genetic defects in lysosomal enzymes, or in proteins such as the mannose 6-phosphate receptors required for targeting the enzymes to the lysosome, lead to an abnormal accumulation of undigested material in lysosomes that may be converted to residual bodies. The accumulation may be so extensive that normal cellular function is compromised, particularly in neuronal cells. Genetic diseases such as the Tay-Sachs disease (an accumulation of partially digested [illegible] in lysosomes) and Pompe disease (an accumulation of glycogen particles in lysosomes) are caused by the absence or deficiency of specific lysosomal enzymes. Such diseases, in which a lysosomal function is compromised, are known as *lysosomal storage diseases.*

A. Lysosomal Hydrolases

The lysosomal digestive enzymes include nucleases, phosphatases, glycosidases, esterases, and proteases (Fig. 10.12). These enzymes are all hydrolases, enzymes that cleave amide, ester, and other bonds through the addition of water. Many of the products of lysosomal digestion, such as the amino acids, return to the cytosol. Lysosomes are therefore involved in recycling compounds. The proteases are classified as serine, cysteine, or aspartyl proteases, depending on the amino acid residue at the active site of the enzyme involved in the hydrolytic reaction. The cysteine proteases are also known as *cathepsins*.

Most of these lysosomal hydrolases have their highest activity near a pH of approximately 5.5 (the optimal pH for hydrolysis). The intralysosomal pH is maintained near 5.5 principally by v-ATPases (vesicular ATPases), which actively pump protons into the lysosome. The cytosol and other cellular compartments have a pH nearer 7.2 and are therefore protected from escaped lysosomal hydrolases.

B. Endocytosis, Phagocytosis, and Autophagy

Lysosomes are formed from digestive vesicles called *endosomes*, which are involved in receptor-mediated endocytosis. They also participate in digestion of foreign cells acquired through phagocytosis and the digestion of internal contents in the process of autophagocytosis.

1. RECEPTOR-MEDIATED ENDOCYTOSIS

Lysosomes are involved in the digestion of compounds brought into the cells in endocytotic clathrin-coated vesicles formed by the plasma membrane. These vesicles fuse to form multivesicular bodies called *early endosomes*. The early endosomes can either recycle back to the cell surface or mature into late endosomes as they recycle clathrin, lipids, and other membrane components back to the plasma membrane in vesicles called *recycling endosomes*. The late endosomes mature into lysosomes as they progressively accumulate newly synthesized acid hydrolases and vesicular proton pumps brought to them in clathrin-coated vesicles from the Golgi. Thus, lysosomes do not acquire their full digestive power until after sorting of membrane lipids and proteins for recycling.

Within the Golgi, enzymes are targeted for endosomes (and eventually lysosomes) by addition of mannose 6-phosphate residues that bind to mannose 6-phosphate receptor proteins in the Golgi membrane. The mannose 6-phosphate receptors together with the associated bound acid hydrolases are incorporated into the clathrin-coated Golgi transport vesicles and the vesicles are released. The transport vesicles lose their clathrin coat and then fuse with the late endosomal membrane. The acidity of the endosome releases the acid hydrolases from the receptors into the vesicle lumen. The receptors are eventually recycled back to the Golgi.

2. PHAGOCYTOSIS AND AUTOPHAGY

One of the major roles of lysosomes is phagocytosis. Neutrophils and macrophages, the major phagocytic cells, devour pathogenic microorganisms and clean up wound debris and dead cells, thus aiding in repair. As bacteria or other particles are enclosed into clathrin-coated pits in the plasma membrane, these vesicles bud off to form intracellular phagosomes. The phagosomes fuse with lysosomes, where the acidity and digestive enzymes destroy the contents. Pinocytotic vesicles also may fuse with lysosomes.

In autophagy (self-eating), intracellular components such as organelles or glycogen particles are surrounded by a membrane derived from endoplasmic reticulum (ER) vesicles, forming an autophagosome. The autophagosome fuses with a lysosome, and lysosomal enzymes digest the contents of the phagolysosome. Organelles usually turn over much more rapidly than the cells in which they reside (e.g., approximately four mitochondria in each liver cell are degraded per hour). Cells that are damaged, but still viable, recover, in part, by using autophagy to eliminate damaged components.

If a significant amount of undigestible material remains within the lysosome after the digestion process is completed, the lysosome is called a *residual body*. Depending on the cell type, residual bodies may be expelled (exocytosis) or remain indefinitely in the cell as lipofuscin granules that accumulate with age.

IV. MITOCHONDRIA

Mitochondria contain most of the enzymes for the pathways of fuel oxidation and oxidative phosphorylation and thus generate most of the ATP required by mammalian cells. Each mitochondrion is surrounded by two membranes, an outer membrane and an inner membrane, separating the mitochondrial matrix from the cytosol (Fig. 10.13). The inner membrane forms invaginations known as *cristae* containing the electron-transport chain and ATP synthase. Most of the enzymes for the tricarboxylic acid (TCA) cycle and other pathways for oxidation are located in the mitochondrial matrix, the compartment enclosed by the inner mitochondrial membrane. (The TCA cycle and the electron-transport chain are described in more detail in Chapters 20 and 21.)

The inner mitochondrial membrane is highly impermeable, and the proton gradient that is built up across this membrane during oxidative phosphorylation is essential for ATP generation from adenosine diphosphate (ADP) and phosphate. The transport of ions occurs principally through facilitative transporters in a type of secondary active transport powered by the proton gradient established by the electron-transport chain. The outer membrane contains pores made from proteins called *porins* and is permeable to molecules with a molecular weight up to about 1,000 g/mol.

Mitochondria can replicate by division; however, most of their proteins must be imported from the cytosol. Mitochondria contain a small amount of DNA, which encodes for only 13 different subunits of proteins involved in oxidative phosphorylation. Most of the enzymes and proteins in mitochondria are encoded by nuclear DNA and synthesized on cytoplasmic ribosomes. They are imported through membrane pores by a receptor-mediated process involving members of the

The elevated level of uric acid in **Lotta Topaigne's** blood led to the deposition of monosodium urate crystals in the joint space (synovial fluid) of her right great toe, resulting in podagra (painful great toe). Neutrophils, the mediators of the acute inflammation that followed, attempted to phagocytose the urate crystals. The engulfed urate crystals were deposited in the late endosomes and lysosomes of the neutrophil. Because urate crystals are particles that cannot be degraded by any of the lysosomal acid hydolases, their accumulation caused lysis of the lysosomal membranes, followed by cell lysis and release of lysosomal enzymes into the joint space. The urate crystals also resulted in release of chemical mediators of inflammation that recruited other cells into the area. This further amplified the acute inflammatory reaction in the tissues of the joint capsule (synovitis), leading to the extremely painful swelling of acute gouty arthritis.

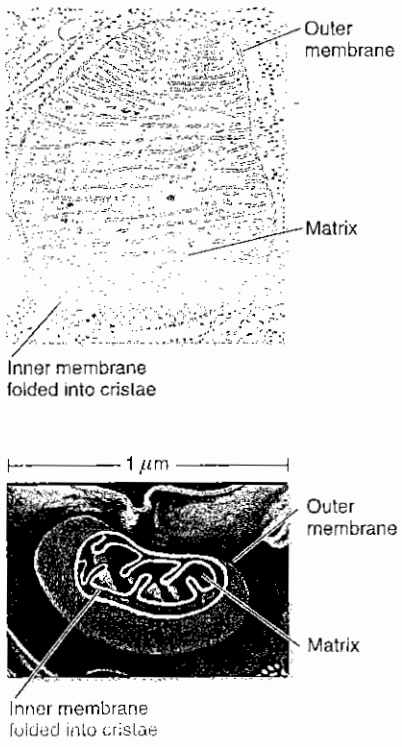


FIG. 10.13. Mitochondrion. Electron micrograph (**top**); three-dimensional drawing (**bottom**).

Q: Pyruvate transfer across the inner mitochondrial membrane is dependent on the proton gradient, and the carrier for pyruvate transports both pyruvate and a proton into the matrix of the mitochondria. Such transport allows for the accumulation of pyruvate within the organelle at concentrations greater than in the cytosol. This is an example of which type of transport?

A: Several diseases are associated with peroxisomes. Peroxisomal diseases are caused by mutations that affect either the synthesis of functional peroxisomal enzymes or their incorporation into peroxisomes. For example, adrenoleukodystrophy involves a mutation that decreases the content of a fatty acid transporter in the peroxisomal membrane. Zellweger syndrome is caused by the failure to complete the synthesis of peroxisomes.

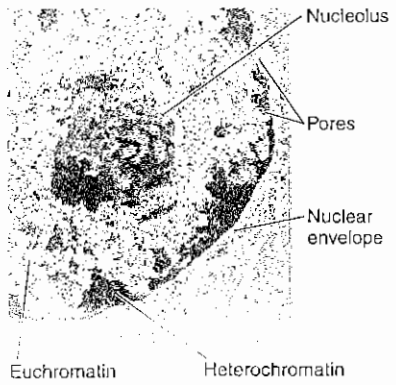


FIG. 10.14. Nucleus. Electron micrograph (**top**); three-dimensional drawing (**bottom**).

heat-shock family of proteins. Inherited mutations within mitochondrial DNA resu in several genetic diseases that affect skeletal muscle, neuronal tissue, and ren tissue. Spontaneous mutations within mitochondrial DNA have been implicate with aging.

V. PEROXISOMES

Peroxisomes are cytoplasmic organelles, similar in size to lysosomes that ar involved in oxidative reactions using molecular oxygen. These reactions produc the toxic chemical hydrogen peroxide (H_2O_2), which is subsequently used c degraded within the peroxisome by catalase and other enzymes. Peroxisomes func tion in the oxidation of very long-chain fatty acids (containing 20 or more carbon to shorter chain fatty acids, the conversion of cholesterol to bile acids, and the syr thesis of ether lipids called *plasmalogens*. They are bounded by a single membran

Like mitochondria, peroxisomes can replicate by division. However, they ar dependent on the import of proteins to function. They contain no DNA.

VI. NUCLEUS

The largest of the subcellular organelles of animal cells is the nucleus (Fig. 10.14 Most of the genetic material of the cell is located in the nucleus in the form c chromosomes, which are composed of DNA, an equal weight of small, positivel charged proteins called *histones*, and a variable amount of other proteins. Thi nucleoprotein complex is called *chromatin*. The *nucleolus*, a substructure of th nucleus, is the site of rRNA transcription and processing and of ribosome assembl Replication, transcription, translation, and the regulation of these processes are th major focus of the molecular biology section of this text (see Section III).

The nucleus is separated from the rest of the cell (the cytoplasm) by the nuclea envelope, which consists of two membranes joined at nuclear pores. The oute nuclear membrane is continuous with the rough endoplasmic reticulum (RER). T convert the genetic code of the DNA into the primary sequence of a protein, DN is transcribed into RNA, which is modified and edited into mRNA. The mRN travels through the nuclear pores into the cytoplasm, where it is translated into th primary sequence of a protein on ribosomes. Ribosomes, which are generated i the nucleolus, also must travel through nuclear pores to the cytoplasm. Conversel proteins required for replication, transcription, and other processes enter into th nucleus through these pores. These proteins contain a specific sequence of amin acids known as a *nuclear localization signal* (NLS). Thus, transport through th pore is specific for the molecule and the direction of transport.

VII. ENDOPLASMIC RETICULUM

The ER is a network of membranous tubules within the cell consisting of smoot endoplasmic reticulum (SER), which lacks ribosomes, and RER, which is studde with ribosomes (Fig. 10.15). The SER has several functions. It contains enzyme for the synthesis of many lipids, such as triacylglycerols and phospholipids. It als contains the cytochrome P450 oxidative enzymes involved in metabolism of drug and toxic chemicals such as ethanol and the synthesis of hydrophobic molecule such as steroid hormones. Glycogen is stored in regions of liver cells that are ric in SER.

The RER is involved in the synthesis of certain proteins. Ribosomes attache to the membranes of the RER give them their "rough" appearance. Proteins pro duced on these ribosomes enter the lumen of the RER, travel to the Golgi comple in vesicles, and are subsequently either secreted from the cell, sequestered withi membrane-enclosed organelles such as lysosomes, or embedded in the plasm membrane. Posttranslational modifications of these proteins, such as the initia tion of *N*-linked glycosylation and the addition of GPI anchors, occur in the RER

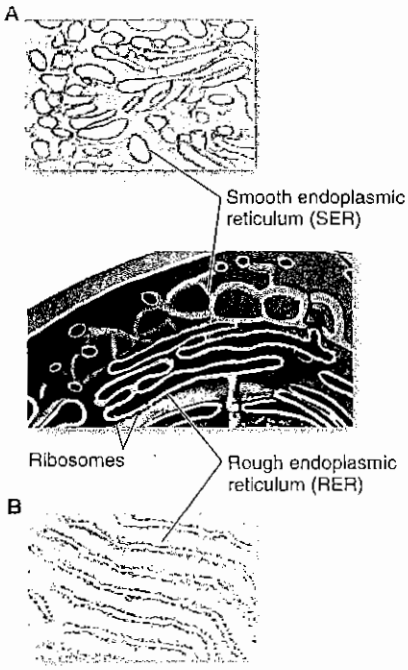


FIG. 10.15. A. Smooth endoplasmic reticulum. B. Rough endoplasmic reticulum. A and B are electron micrographs. A three-dimensional drawing is in the **middle**.

In contrast, proteins encoded by the nucleus and found in the cytosol, peroxisomes, or mitochondria are synthesized on free ribosomes in the cytosol and are seldom modified by the attachment of oligosaccharides.

VIII. GOLGI COMPLEX

The Golgi complex is involved in modifying proteins produced in the RER and in sorting and distributing these proteins to the lysosomes, secretory vesicles, or the plasma membrane. It consists of a curved stack of flattened vesicles in the cytoplasm that is generally divided into three compartments: the *cis* Golgi network, which is often convex and faces the nucleus; the *medial* Golgi stacks; and the *trans* Golgi network, which often faces the plasma membrane. Vesicles transport material to and from the Golgi. The Golgi complex also participates in posttranslational modification of proteins, such as complex branched-chain oligosaccharide addition, sulfation, and phosphorylation.

IX. CYTOSKELETON

The structure of the cell, the shape of the cell surface, and the arrangement of subcellular organelles is organized by three major protein components: microtubules composed of tubulin, which move and position organelles and vesicles; thin filaments composed of actin, which form a cytoskeleton; and intermediate filaments (IFs) composed of different fibrous proteins. *Actin* and *tubulin*, which are also involved in cell movement, are dynamic structures composed of continuously associating and dissociating globular subunits. IFs, which play a structural role, are composed of stable fibrous proteins that turn over more slowly.

A. Microtubules

Microtubules, cylindrical tubes composed of tubulin subunits, are present in all nucleated cells and the platelets in blood (Fig. 10.16). They are responsible for

Cotransport of pyruvate and a proton, down the proton's electrochemical gradient, is an example of secondary active transport. Energy has been expended, during the transfer of electrons through the electron transfer chain, to generate the proton gradient across the inner mitochondrial membrane. The energy of the proton gradient is then used by the carrier to carry protons down their electrochemical gradient (favorable), bringing pyruvate along with the protons. This will allow pyruvate active transport, secondary to favorable proton entry into the mitochondrial matrix.

Chronic ingestion of ethanol has increased the content of MEOS, the microsomal ethanol oxidizing system, in **Al Martini's** liver. MEOS is a cytochrome P450 enzyme that catalyzes the conversion of ethanol, NADPH, and O_2 to acetaldehyde, $NADP^+$, and 2 H_2O (see Chapter 9). The adjective *microsomal* is a term derived from experimental cell biology that is sometimes used for processes that occur in the ER. When cells are lysed in the laboratory, the ER is fragmented into vesicles called *microsomes*, which can be isolated by centrifugation. Microsomes, as such, are not actually present in cells.

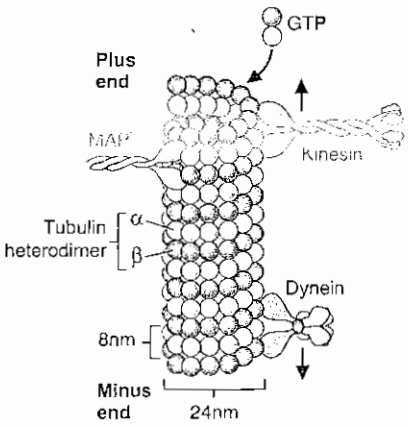


FIG. 10.16. Microtubules composed of $\alpha\beta$-tubulin heterodimers. *MAP*, microtubule-associated protein. These proteins project outward to attach the microtubules to other cellular components. The microtubule grows by the addition of $\alpha\beta$-dimers containing bound guanosine triphosphate (GTP) to the plus end of the polymer. Kinesin and dyneins are motor proteins that transport cargo (e.g., vesicles) along the microtubule.

The monomeric G protein Arf (a member of the Ras superfamily of regulatory proteins, see Section III.C.2 in Chapter 9) was named for its contribution to the pathogenesis of cholera and not for its normal function in the assembly of intracellular vesicles. In the case of cholera, it is required for the transport of *Vibrio cholerae A* toxin subunit into the cell. The cholera toxin is endocytosed in caveolae vesicles that subsequently merge with lysosomes (or are transformed into lysosomes), where the acidic pH contributes to activation of the toxin. As the toxin is transported through the Golgi and ER, it is further processed and activated. Arf forms a complex with the A-subunit that promotes its travel between compartments. The A-subunit is actually an ADP-ribosylase (an enzyme that cleaves NAD and attaches the ADP portion to a protein) (see Chapter 6, Fig. 6.14), and hence, Arf became known as the *ADP-ribosylating factor*. The ADP-ribosylation of proteins regulating the CFTR chloride channel led to **Dennis Veere's** dehydration and diarrhea.

Lotta Topaigne was given colchicine, a drug that is frequently used to treat gout. One of its actions is to prevent phagocytic activity by binding to dimers of the α- and β-subunits of tubulin. When the tubulin dimer–colchicine complexes bind to microtubules, further polymerization of the microtubules is inhibited, depolymerization predominates, and the microtubules disassemble. Microtubules are necessary for vesicular movement of urate crystals during phagocytosis and release of mediators that activate the inflammatory response. Thus, colchicine diminishes the inflammatory response, swelling, and pain caused by formation of urate crystals.

the positioning of organelles in the cell cytoplasm and the movement of vesicles, including phagocytic vesicles, exocytotic vesicles, and the transport vesicles between the ER, Golgi, and endosomes. They also form the spindle apparatus for cell division. The microtubule network (the minus end) begins in the nucleus at the centriole and extends outward to the plasma membrane (usually the plus end). Microtubule-associated proteins (MAPs) attach microtubules to other cellular components and can determine cell shape and polarity.

Motor proteins called *kinesins* and *cytoplasmic dyneins* use ATP energy to move cargo along the microtubules. Kinesins move molecules, vesicles, and organelles toward the plus end of the microtubule, usually toward the plasma membrane. Cytoplasmic dyneins are huge proteins that move vesicles and organelles to the minus end, generally toward the nucleus. They are also involved in the positioning of the Golgi complex and the movement of chromosomes during mitosis.

Microtubules consist of polymerized arrays of α- and β-tubulin dimers that form 13 protofilaments organized around a hollow core (see Fig. 10.16). Three different tubulin polypeptides (α, β, and γ) of similar amino acid composition are encoded by related genes; α- and β-dimers polymerize to form most microtubules, and γ-tubulin is found only in the centrosomes and spindle pole bodies. Two other forms of tubulin, δ and ε, have been found in centrioles. Tubulin dimers composed of one α- and one β-subunit bind guanosine triphosphate (GTP), which creates a conformational change in the dimer that favors addition of dimers to the tubulin polymer. The dimers can add to and dissociate from both ends of the tubulin, but the end to which they add more rapidly (the plus end) has a net rate of growth, and the end to which they add more slowly (the minus end) has a net rate of loss. As GTP is hydrolyzed to GDP, the binding of tubulin subunits is weakened, resulting in their dissociation (dynamic instability). Thus, the net rate and direction of growth is dictated by the fastest growing end of the microtubule.

B. Actin Filaments

Actin filaments form a critical network that controls the shape of the cell and movement of the cell surface, thereby allowing cells to move, divide, engulf particles, and contract. Actin is present in all living cells. The actin polymer, called *F-actin*, is composed of a helical arrangement of globular G-actin subunits (Fig. 10.17). Within the polymer, each G-actin subunit contains a bound ATP or ADP that holds the actin fold into a closed conformation (see Chapter 7). The actin polymer is dynamic. New subunits of G-actin containing ATP continuously combine with the assembled F-actin polymer at the plus end. As F-actin elongates, bound ATP is hydrolyzed to ADP, so that most of the polymer contains G-actin–ADP subunits. The conformation of ADP-actin favors dissociation from the minus end of the polymer; thus, the polymer is capable of lengthening from the plus end. This directional growth can account for certain types of cell movement and shape changes: the formation of pseudopodia that surround other cells during phagocytosis, the migration of cells in the developing embryo, or the movement of white blood cells through tissues.

Actin polymers form the thin filaments (also called *microfilaments*) in the cells that are organized into compact-ordered bundles or loose network arrays by cross-linking proteins. Short actin filaments bind to the cross-linking protein spectrin to form the cortical actin skeleton network (see Fig. 10.5). In muscle cells, long actin filaments combine with thick filaments, composed of the protein myosin, to produce muscle contraction. The assembly of G-actin subunits into polymers, bundling of fibers, and attachments of actin to spectrin and to the plasma membrane proteins and organelles are mediated by several actin-binding proteins and G proteins from the Rho family.

C. Intermediate Filaments

IF are composed of fibrous protein polymers that provide structural support to membranes of the cells and scaffolding for attachment of other cellular components. Each IF subunit is composed of a long, rodlike α-helical core containing globular spacing domains and globular N- and C-terminal domains. The α-helical segments of two subunits coil around each other to form a coiled coil, and then combine with another dimer coil to form a tetramer. Depending on the type of filament, the dimers may be either heterodimers or homodimers. The tetramers join end to end to form protofilaments, and approximately eight protofilaments combine to form filaments (Fig. 10.18). Filament assembly is partially controlled through phosphorylation.

In contrast to actin thin filaments, the 50 or so different types of IFs are each composed of a different protein having the same general structure described previously. Some of the IFs, such as the nuclear lamins, are common to all cell types. These filaments provide a latticelike support network attached to the inner nuclear membrane. Other IFs are specific for types of cells (e.g., epithelial cells have cytokeratins, and neurons have neurofilaments). These provide an internal network that helps to support the shape and resilience of the cell.

CLINICAL COMMENTS

Al Martini. **Al Martini** has been drinking for 5 years and has begun to exhibit mental and systemic effects of chronic alcohol consumption. In his brain, ethanol has altered the fluidity of neuronal lipids, causing changes in their response to neurotransmitters released from exocytotic vesicles. In his liver, increased levels of the microsomal ethanol oxidizing system (MEOS, CYP2E1) located in the smooth endoplasmic reticulum (SER) have increased his rate of ethanol oxidation to *acetaldehyde*, a compound that is toxic to the cell. His liver also continues to oxidize ethanol to acetaldehyde through a cytosolic enzyme, liver alcohol dehydrogenase.

One of the toxic effects of acetaldehyde is inhibition of tubulin polymerization. Tubulin is used in the liver for secretion of very low-density lipoprotein (VLDL) particles containing newly synthesized triacylglycerols. As a result, these triacylglycerols accumulate in the liver, and he has begun to develop a fatty liver. Acetaldehyde may also damage protein components of the inner mitochondrial membrane and affect its ability to pump protons to the cytosol.

Lotta Topaigne. **Lotta Topaigne** had a rapid and gratifying clinical response to the hourly administration of colchicine. This drug diminishes phagocytosis and the subsequent release of the lysosomal enzymes that initiate the inflammatory response in synovial tissue.

The inflammatory response that causes the symptoms of an acute gout attack begins when neutrophils and macrophages ingest urate crystals. In neutrophils, urate activates the conversion of the polyunsaturated fatty acid arachidonic acid (present in membrane phospholipids) to leukotriene B_4. The release of this messenger contributes to the pain. Colchicine, through its effect on tubulin, inhibits phagocytosis, leukotriene B_4 release, and recruitment and cell division of additional cells involved in inflammation. Colchicine also inhibits the tubulin-dependent release of histamine from mast cells. As a result, there was a rapid improvement in the pain and swelling in Lotta's great toe.

After the gout attack subsided, Ms. Topaigne was placed on daily *allopurinol*, a drug that inhibits urate production (see Chapter 8). During the next 6 months of allopurinol therapy, Ms. Topaigne's blood urate levels decreased. She did not have another gout attack during this time.

Colchicine, an initial drug used to treat **Lotta Topaigne**, has a narrow therapeutic index (i.e., the amount of drug that produces the desirable therapeutic effect is not much lower than the amount that produces an adverse effect). Its therapeutic effect depends on inhibiting tubulin synthesis in neutrophils, but it can also prevent tubulin synthesis (and thus, cell division and other cellular processes) in other cells. Fortunately, neutrophils concentrate colchicine, so they are affected at lower intakes than other cell types. Neutrophils lack the transport protein P-glycoprotein, a member of the *ABC* cassette family (which includes the CFTR channel). In most other cell types, P-glycoprotein exports chemicals such as colchicine, thus preventing their accumulation.

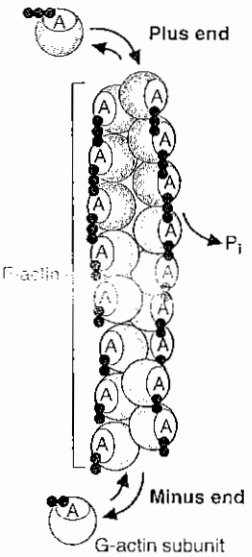


FIG. 10.17. Actin filaments. The polymer F-actin is assembled from G-actin subunits containing bound ATP. While bound, the ATP is slowly hydrolyzed to ADP. The conformational change shifts the equilibrium so that dissociation of the G-actin subunits is favorable at the minus end of the polymer. Once they are dissociated, the actin subunits exchange ADP for ATP, which may again associate with the actin polymer. At the plus end of the molecule, association is favored over dissociation.

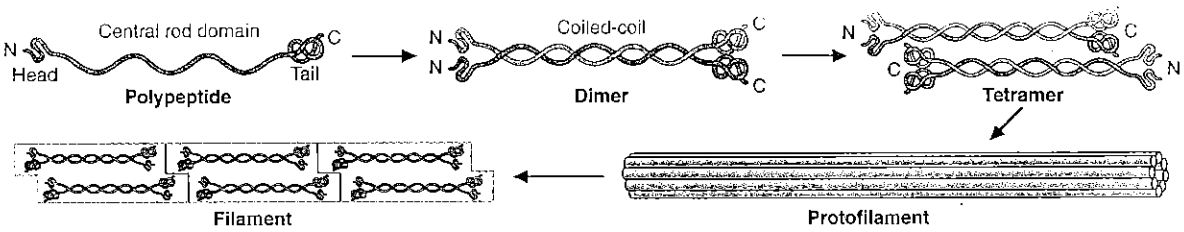


FIG. 10.18. Formation of a cytokeratin filament. The central rod of the keratin monomer is principally an α-helical structure. A specific acidi keratin monomer combines with a specific basic keratin monomer to form a heterodimer coil (a coiled-coil structure). Two dimers combin in antiparallel fashion to form a tetramer, and the tetramers combine head to tail to form protofilaments. Approximately eight protofilament combine to form a filament. The filament is thicker than actin filaments (called *thin filaments* or *microfilaments*) and thinner than microtubule (thick tubes) and is therefore called an *intermediate filament* (IF).

Dennis Veere. Dennis Veere was diagnosed with cholera. He wa placed on intravenous rehydration therapy, followed by oral rehydratio therapy with high glucose- and Na^+-containing fluids (to be continued i Chapter 11).

Vibrio cholerae secrete a toxin consisting of one A- and multiple B-subunit The B-subunits allowing binding to the intestinal epithelia, and the A-subun is processed and transported into the cell, where it associates with the monc meric G protein Arf (ADP-ribosylation factor). The cholera toxin A-subunit ADI ribosylates the Gα-subunit of the heterotrimeric G protein $G\alpha_s$ (a process discusse in Chapter 11). The net result is activation of protein kinase A (PKA), which the phosphorylates the cystic fibrosis transmembrane conductance regulator (CFTR chloride channel so that it remains permanently open. The subsequent efflux c chloride, sodium, and water into the bowel lumen is responsible for **Dennis Veere'** diarrhea and subsequent dehydration.

BIOCHEMICAL COMMENTS

Differences between Bacteria and Human Cells. Differe species of bacteria have some common structural features that distinguis them from animal cells. They are single-cell organisms that are classifie as prokaryotes ("before nucleus"). Their genetic material (DNA) is concentrated the central region of the cell called a *nucleoid* rather than a nucleus because it is n separated from the rest of the cellular contents by a membrane. Likewise, bacter contain no cytoplasmic organelles defined by membranes. They do have a plasm membrane that encloses the cytoplasm. External to the plasma membrane is a pept doglycan cell wall composed of extensively cross-linked polysaccharides that for a protective shield on the surface of the cell.

Bacterial cells obtain nutrients from the medium on which they grow. Mar of their metabolic pathways for fuel oxidation are similar to those in eukaryot and generate reduced nicotinamide adenine dinucleotide (NADH) and adenosi triphosphate (ATP); however, individual steps in these pathways may use differe coenzymes or very different enzymes for catalysis than do human cells. Like huma cells, bacteria use intermediates of glycolysis and other basic degradative pathwa to serve as precursors for biosynthetic pathways, and energy acquired from cat bolic pathways is used in anabolic pathways. Aerobic bacteria, such as *Escherich coli*, contain enzymes of the tricarboxylic acid (TCA) cycle and the componen of the electron-transport chain, which are located in the cell membrane. Howeve many bacteria are anaerobes and can function in the absence of oxygen.

Many of the metabolic differences between human cells and bacteria are relat to their interactions with their environment. Some bacteria, such as *E. coli*, c

adapt to adverse or changing conditions (high vs. low O_2 tension, or a single supply of nutrients from which to synthesize all required components) by dramatic shifts in the genes that are transcribed. Other bacteria find a unique environmental niche where they do not have to compete with other bacteria for nutrients (e.g., *Lactobacilli* in yogurt are adapted to an acidic pH). In contrast, the human cells are adapted to interacting with blood and interstitial fluid, which provides a well-controlled pH, a constant nutrient supply, and a medium for communication between very distant cells. As a consequence of their constant environment, adult human cells seldom need to adapt (or can adapt) to widely fluctuating conditions through large variations in the genes transcribed. As a consequence of being organized into a multicellular organism, human cell types have been able to specialize in function, structure, and enzyme content.

Key Concepts

- The cell is the basic unit of living organisms.
- Unique features of each cell type define tissue specificity and function.
- Despite the variety of cell types, there are many common features that all cells share, such as the presence of a plasma membrane.
  - The plasma membrane is composed primarily of lipids and proteins (both integral and peripheral).
  - Specific transport proteins are required to allow compounds to cross membranes, either by facilitative diffusion, gated channels, or active transport.
  - Eukaryotes contain intracellular organelles, whereas prokaryotic cells do not.
- In eukaryotes, the intracellular organelles consist of lysosomes, the nucleus, ribosomes, the endoplasmic reticulum (ER), the Golgi apparatus, mitochondria, peroxisomes, and the cytoplasm. Each organelle contributes a different function to the cell. Some cells may lack one or more of these internal organelles.
  - Lysosomes are the intracellular organelles of digestion.
  - The nucleus contains the genetic material of the cell and is the site of RNA synthesis.
  - Ribosomes are intracellular organelles on which protein synthesis occurs.

Table 10.1 Diseases Discussed in Chapter 10

Disorder or Condition	Genetic or Environmental	Comments
Cholera	Environmental	Watery diarrhea leading to dehydration caused by cholera toxin ADP ribosylating a class of G proteins, altering their function and affecting water and salt transport across the intestinal mucosa.
Gout	Both	Treat by inhibiting xanthine oxidase, thereby reducing the production of uric acid, using the analog allopurinol. Prior to allopurinol, the patient is treated with colchicine, which blocks microtubule formation and the migration of neutrophils to the affected area.
Gas gangrene	Environmental	Bacterial infection that secretes a toxin, which is a lipase, leading to cell membrane destruction. This leads to capillary destruction, and impaired blood flow to the affected area.
Alcoholism	Both	Ethanol poisoning caused by increased production of acetaldehyde, because of the combined actions of alcohol dehydrogenase and the induction of the microsomal ethanol oxidizing system (MEOS). MEOS is induced because of the high levels of ethanol in the patient's diet.

- The ER contains enzymes for the synthesis of many lipids, for drug and toxic chemical metabolism, and for posttranslational modification of proteins.
- The Golgi complex modifies proteins produced in the ER and sorts and distributes them to other organelles.
- The mitochondria are the cells' power plants, synthesizing adenosine triphosphate (ATP).
- The peroxisomes sequester various oxidative reactions that have the potential to generate dangerous radical species.
- The cytoskeleton aids in defining the structure of the cell, the shape of the cell surface, and the arrangement of subcellular organelles within the cytoplasm.

- The diseases discussed in this chapter are summarized in Table 10.1.

REVIEW QUESTIONS—CHAPTER 10

1. Which of the following is a characteristic of the plasma membrane?
   A. It is composed principally of triacylglycerols and cholesterol.
   B. It contains principally nonpolar lipids.
   C. It contains phospholipids with their acyl groups extending into the cytosol.
   D. It contains more phosphatidylserine in the inner than the outer leaflet.
   E. It contains oligosaccharides sandwiched between the inner and outer leaflets.

2. Transmembrane proteins can best be described by which one of the following?
   A. They can usually be dissociated from membranes without disrupting the lipid bilayer.
   B. They are classified as peripheral membrane proteins.
   C. They contain hydrophobic amino acid residues at their carboxy terminus.
   D. They contain hydrophilic amino acid residues extending into the lipid bilayer.
   E. They contain membrane-spanning regions that are α-helices.

3. A patient had a sudden heart attack caused by inadequate blood flow through the vessels of the heart. As a consequence, there was an inadequate supply of oxygen to generate ATP in his cardiomyocytes. The compartment of the cardiomyocyte most directly involved in ATP generation is which one of the following?
   A. Mitochondrion
   B. Peroxisome
   C. Lysosome
   D. Nucleus
   E. Golgi

4. The release of insulin from the β-cells of the pancreas requires Ca^{2+} influx through a channel that is activated by a change in the membrane potential across the plasma membrane. The movement of calcium across the membrane is an example of which one of the following?
   A. Voltage-gated channel
   B. Passive diffusion
   C. Active transport
   D. Ligand-gated channel
   E. Phosphorylation-gated channel

5. ATP is required for the appropriate functioning of the lysosome because of which one of the following?
   A. Maintaining an acidic environment in the lysosom
   B. Maintaining a basic environment in the lysosome
   C. Regulation of enzyme activity
   D. Activation of lysosomal zymogens
   E. As a cofactor for lysosomal hydrolases