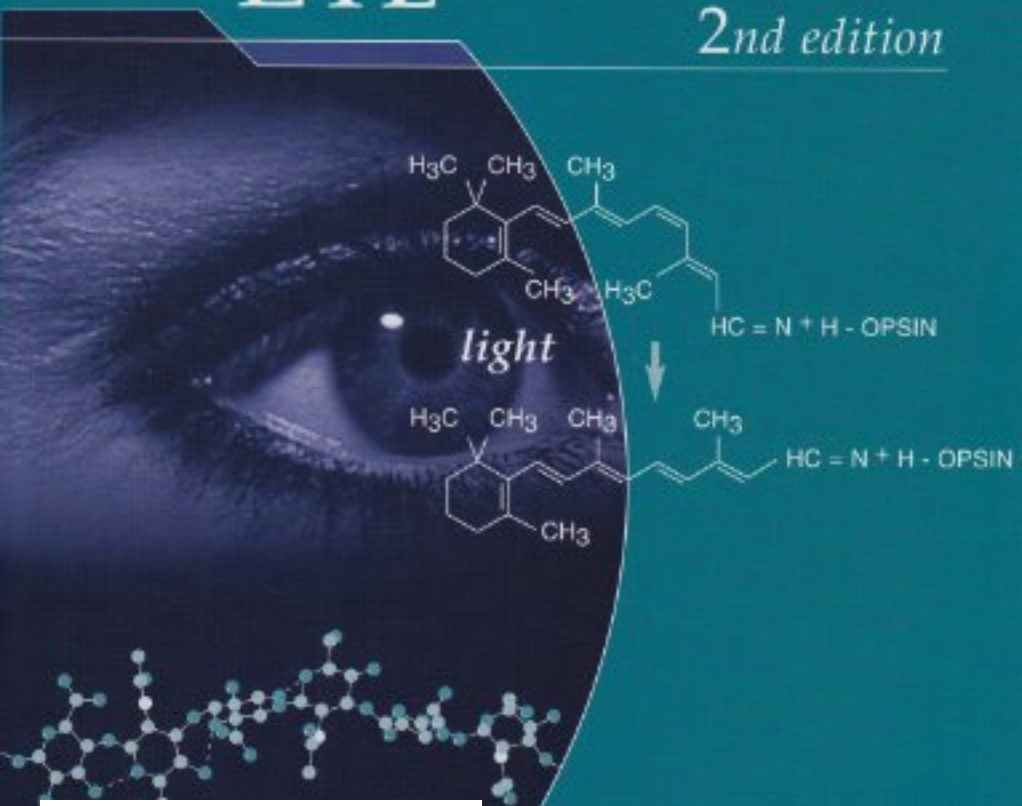
David R. Whikehart

Biochemistry of the Eye

2nd edition



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Butterworth Heinemann

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Preface to the 2nd edition

It is a modest statement to say that much has happened in this area since the 1st edition appeared. A perusal through the literature will show that great strides have occurred in the understanding of many heretofore difficult sections of ocular biochemistry including: diabetes, visual transduction, chemical tissue degradation, immunochemistry, cataractogenesis, and so on.

This new edition continues the tradition of the former edition in perusing the basic parts of biochemistry first and then continuing on to describe those characteristics of biochemistry that are peculiar and characteristic to the eye. It is my philosophy that illustrations are of immense help in understanding difficult concepts and I have liberally included them throughout the book. Two new chapters on aqueous/ocular fluids and tissue degradation have been added. In the older chapters much updating and revision should be evident to those familiar with the first edition.

As always, the objective in putting together such a work is to increase the student's and reader's awareness of those biochemical structures and molecular events that influence the normal and pathological performance of the eye as an organ of vision. The complexity of all that is involved in vision and vision care can hardly be understood without some knowledge of the molecular events that go on in the eye.

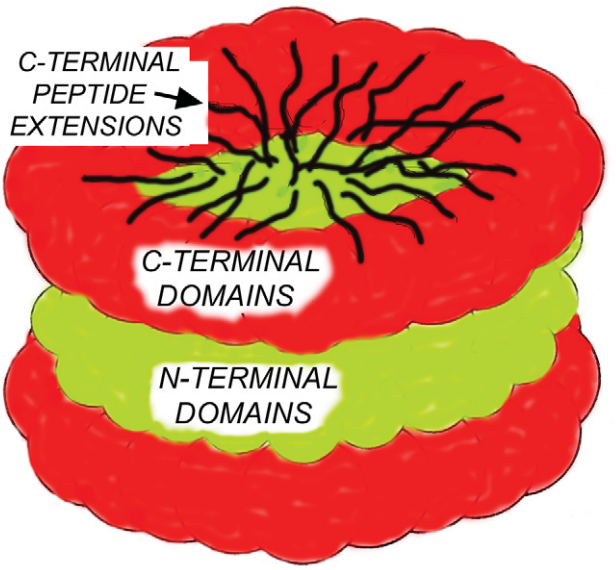
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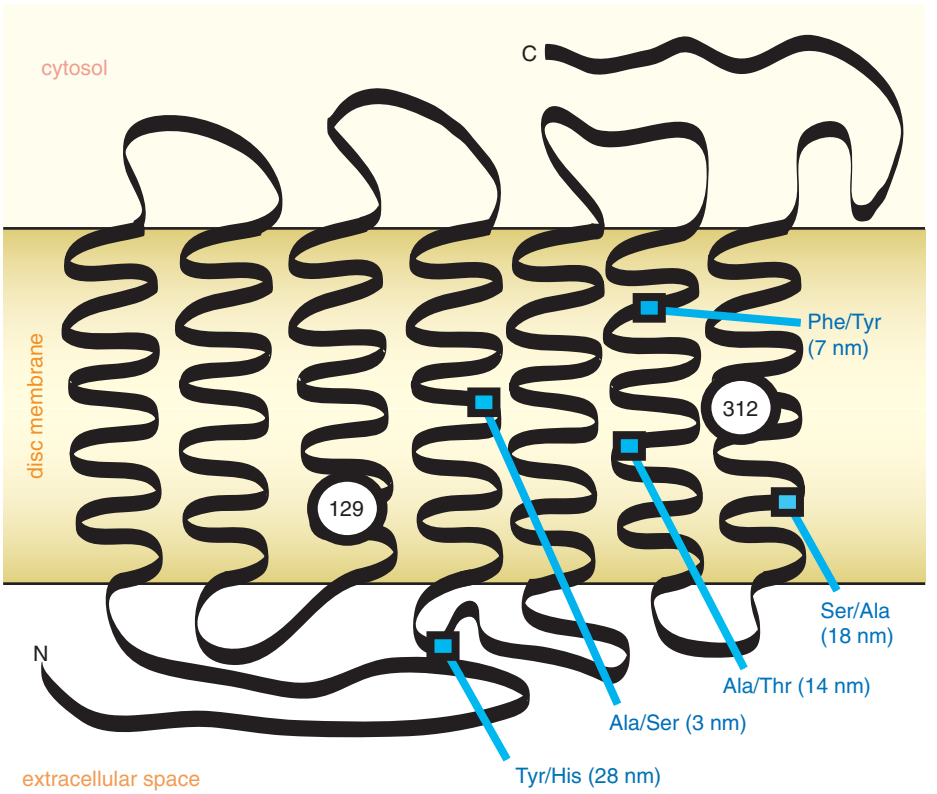
I am indebted to those students who have made suggestions about improving the book prior to publication. Gratitude is also expressed to my daughter, Erin Whikehart, who designed the cover, and to Brigette Bailey, who allowed me to photograph her eye for the cover. Finally, I am especially thankful to Christie Hart, the managing editor of Elsevier Science (Butterworth-Heinemann Division) and to Karen Oberheim, formerly of Butterworth-Heinemann, for their long standing patience as I wrote this text.

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Color Plate 1
One of several proposed structures for α-crystallin. ➤ The model shows bound rings of crystallin subunits laid on top of each other. The large subunit spheres are hydrophilic C-terminal domains while the small subunit spheres are hydrophobic N-terminal domains. Extending from the C-terminal domains are C-terminal peptide extensions that cover the central cavity where chaperone activity may take place. (Redrawn from Carver JA, Aquilina JA, and Truscott RJW: A possible chaperone-like quaternary structure for a-crystallin. *Exp Eye Res* 59: 231–234, 1994.)

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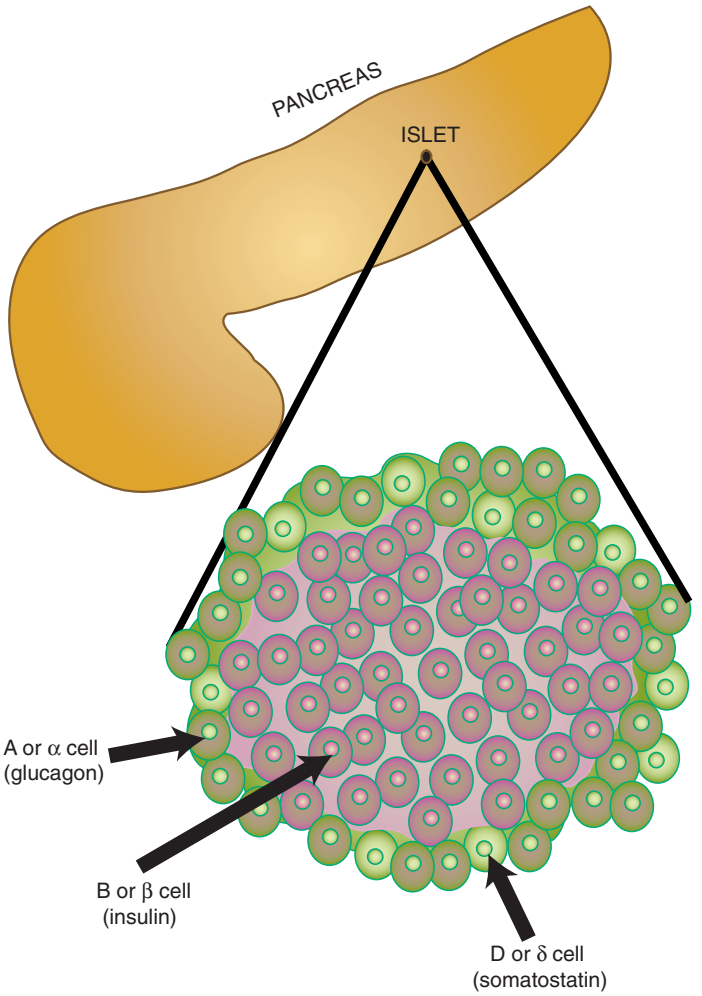


129 Glu #129, the site of attachment for the Schiff base counterion

312 Lys #312, the site of covalent attachment for 11-cis retinal

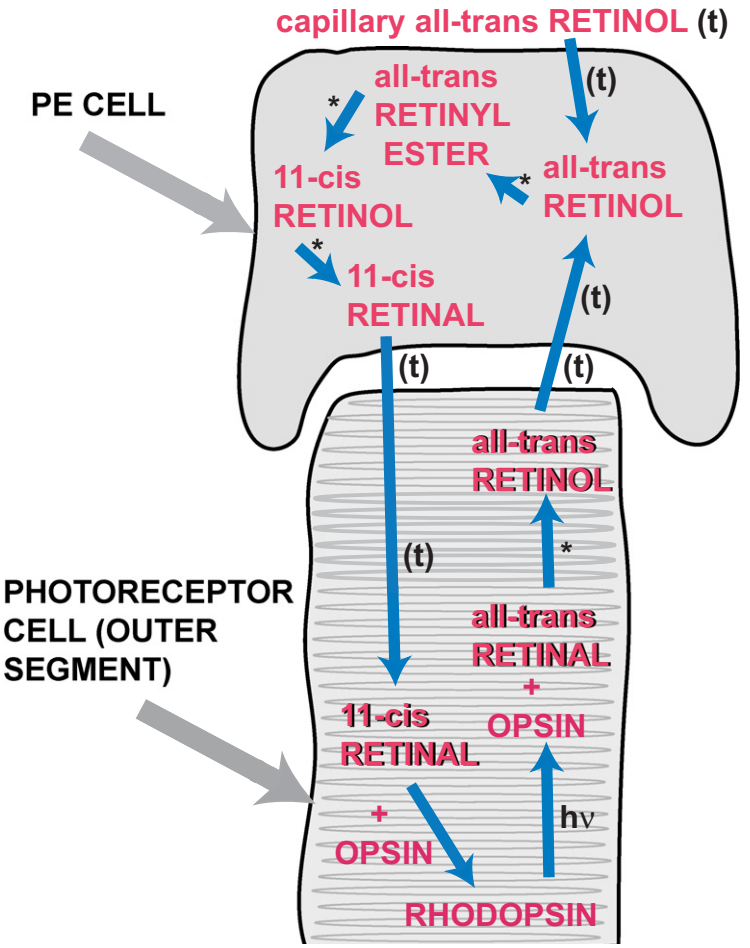
Color Plate 2
Diagram of a red or green cone pigment protein inserted into a cone membrane showing the site of attachment of the Schiff base counter ion and covalent attachment for the 11-*cis* retinal. ➤ Also illustrated are shifts in color vision produced by a substitution of certain amino acids (e.g., Thr for Ala to produce a shift of 18 nm). (The figure was redrawn from Nathans J: The evolution and physiology of human color vision: insights from molecular genetic studies of visual pigments, *Neuron* 24:299–312, 1999.)

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Color Plate 3
Diagram of islet cells in the human pancreas. ➤ Essentially, three types of cells produce insulin (β cells), glucagon (α cells), and somatostatin (δ cells). A fourth type of cell (F) produces pancreatic polypeptide. All cell types secrete hormones that deal with carbohydrate (and other intermediate) metabolic control. (Based on a drawing in Williams G, Pickup JC: *Handbook of diabetes,* ed 2, Oxford, 1999, Blackwell Science; 27.)

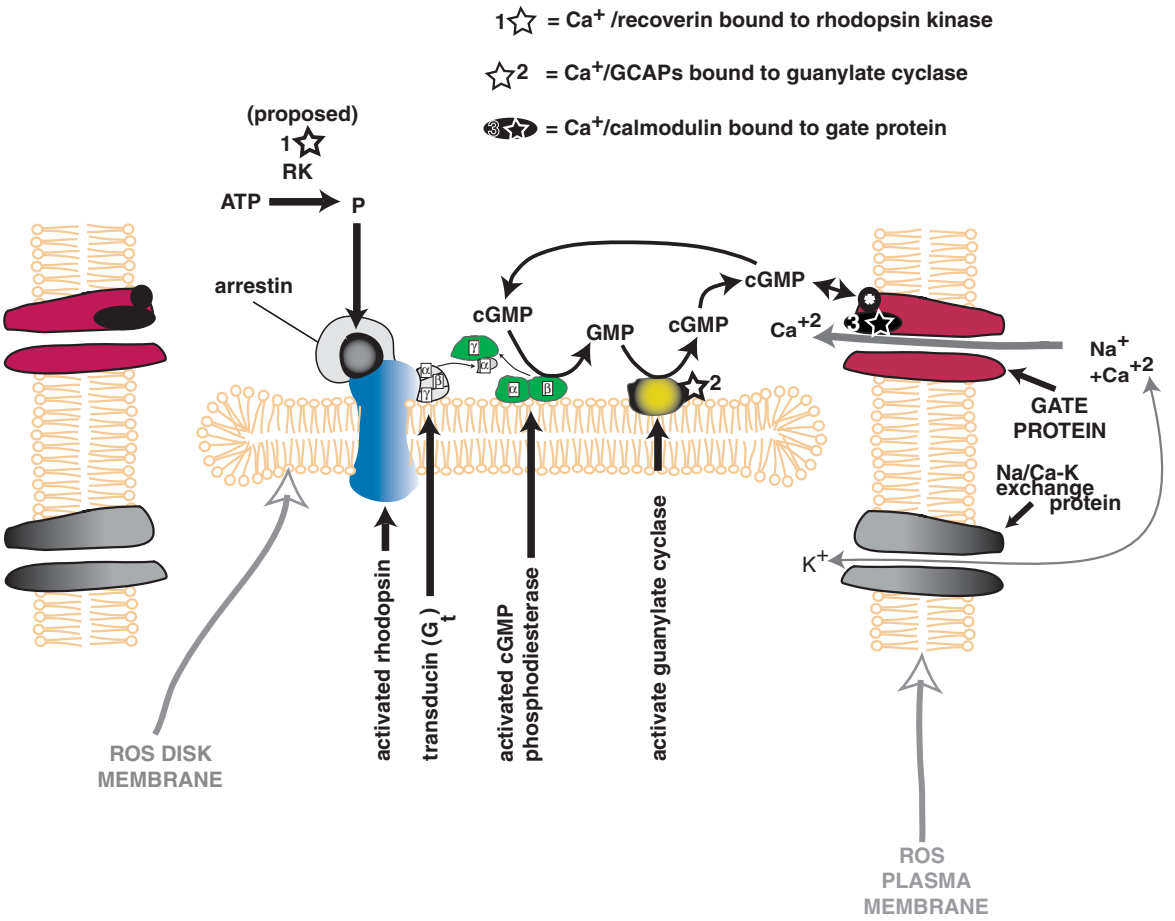
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Color Plate 4

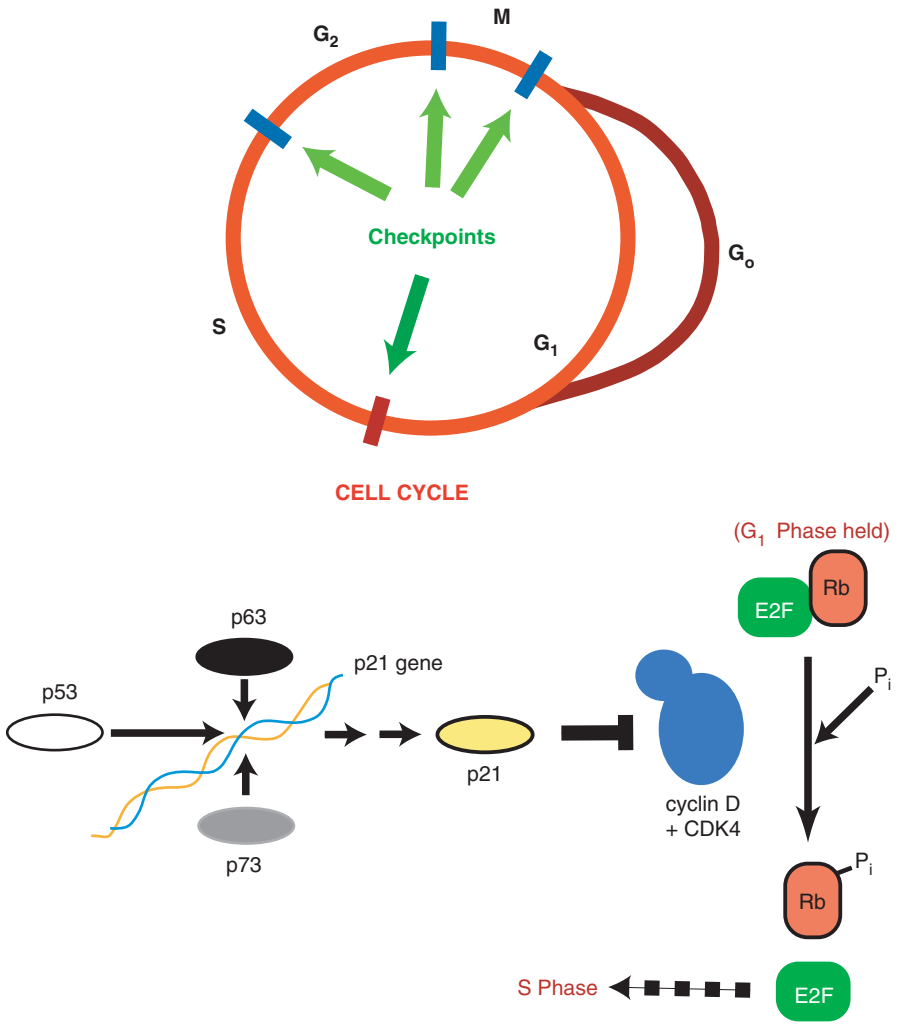
The processing and transport of vitamin A at PE (pigment epithelial) cells and photoreceptor outer segments. ➤ At PE cells retinol may be stored as an ester or transported to the outer segment. The transport between and within PE cells and photoreceptor outer segments of 11-*cis* retinal as well as between photoreceptor cells and PE cells of all-*trans* retinol makes use of other transport binding proteins (t), an interstitial retinol binding protein (IRBP) as well as an intracellular retinal binding protein (CRBP) as described by Hollyfield et al (1985). Metabolic transformations of the different vitamin A forms are enzymatically catalyzed (*) in both the PE and photoreceptor cells. See text for further explanations.

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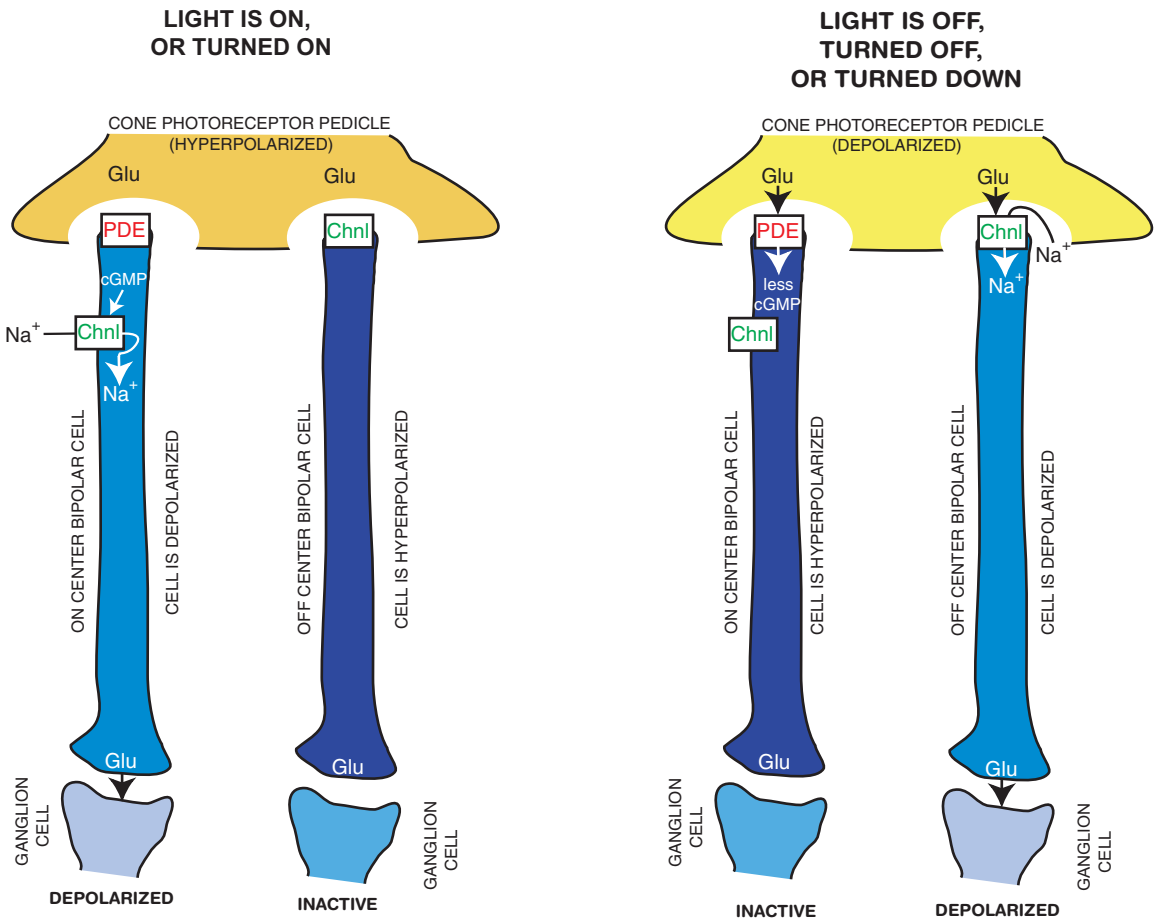
Color Plate 5

Diagram of visual transduction showing roles proposed to be played by Ca^{+2} and cGMP in visual transduction. ➤ Cyclic GMP levels are maintained by guanylate cyclase during the dark current. Cyclic GMP levels are reduced by cGMP phosphodiesterase during visual transduction under the influence of light/ activated rhodopsin/G_t. Ca^{+2} ions bind to recoverin to inactivate rhodopsin kinase preventing inactivation of activated rhodopsin by phosphorylation at 1*. Ca^{+2} ions bind to GCAPS proteins to inactivate guanylate cyclase. Ca^{+2} ions bind to calmodulin in order to negatively modulate ion flow through the gate proteins. In essence, Ca^{+2} ions are self-modulating at higher concentrations by at least three known mechanisms. See text for further explanation.



Color Plate 6

The cell cycle and some of the many biochemical pathways that influence it. ➤ A diagram of the cell cycle is shown in the upper part of the figure. In the resting stage, G_1 or gap one, no synthetic processes leading to cell division occur. A subdivision of the G_1 phase, known as G_o also occurs in which the cell cannot enter into a synthetic process leading to cell division. Cells may be in G_o, for example, when there is insufficient nourishment to begin DNA synthesis. In the S or synthesis phase, synthesis of new DNA (replication) occurs prior to cell division. The G_2 phase (gap two), is an interphase prior to mitosis or cell division. In this phase there are four sets of chromosomes present. In mitosis (the M phase), the complex process of cell division occurs (see, for example, Lodish et al, 2000). The movement or commitment of a cell into its next phase occurs by passage through so called "checkpoints" or "restriction points," which are determined by a variety of biochemical mechanisms. The lower part of the Figure illustrates one phase commitment mechanism for passage though the checkpoint from the G_1 to the S phase. Such a mechanism involves the proteins *E2F* and *Rb* (*right*). When E2F and Rb are bound together, the cell is held at the G_1 phase. When the Rb protein is phosphorylated by cyclin dependent kinase 4 (when cyclin D is bound to the kinase) the Rb and E2F proteins are released from each other and both promote checkpoint passage by the stimulation of mRNAs to make proteins necessary for the S phase of the cell cycle. Obviously any influence on the activity of the cyclin D/CDK4 complex will influence the ability of the cell to divide (i.e., to enter the S phase). Three proteins (of many such as KIP [*k*inase *i*nhibitor *p*roteins]) that are known to influence this complex are p53, p63, and p73. These proteins cause the synthesis of mRNA for p21 a protein that inhibits the cyclin D/CDK4 enzyme complex. Any defect in p53, for example, may allow the cyclinD/CDK4 enzyme complex to carry out unchecked catalysis to separate E2F and Rb at a high rate and allow uncontrolled cell division. This, in fact, occurs in some cancer cells in which p53 occurs in mutated and useless forms.

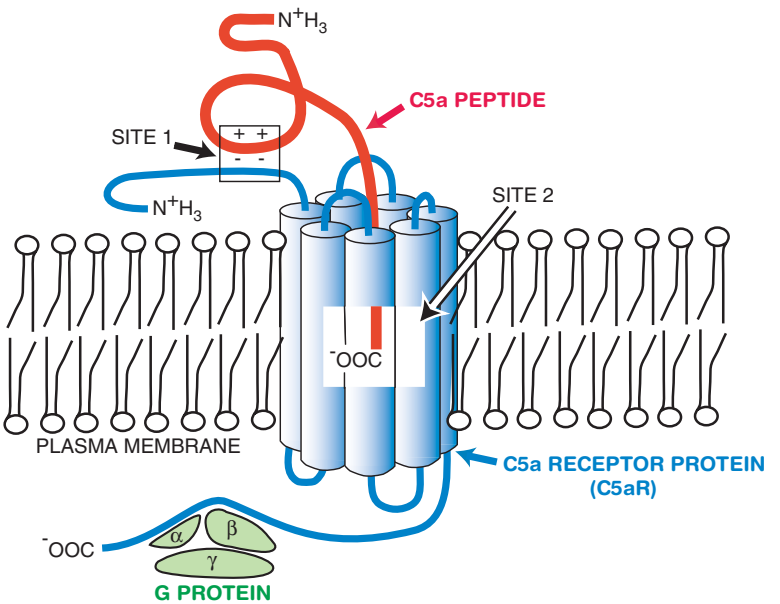


Color Plate 7

Biochemical and physiological diagram of neurotransmission differences between "on" and "off" mechanisms when light is turned on or off. ➤ Note, in particular, how the release (light off) of Glu affects the two classes of bipolar cells differently and, likewise, how the nonrelease (or decreased release) of Glu differentially affects the two classes of bipolar cells. This is largely due to whether the Na^+ channel protein is present as a NT receptor for Glu or whether the channel protein function is mediated by cGMP binding (i.e., phosphodiesterase, PDE, functions as a receptor for Glu).

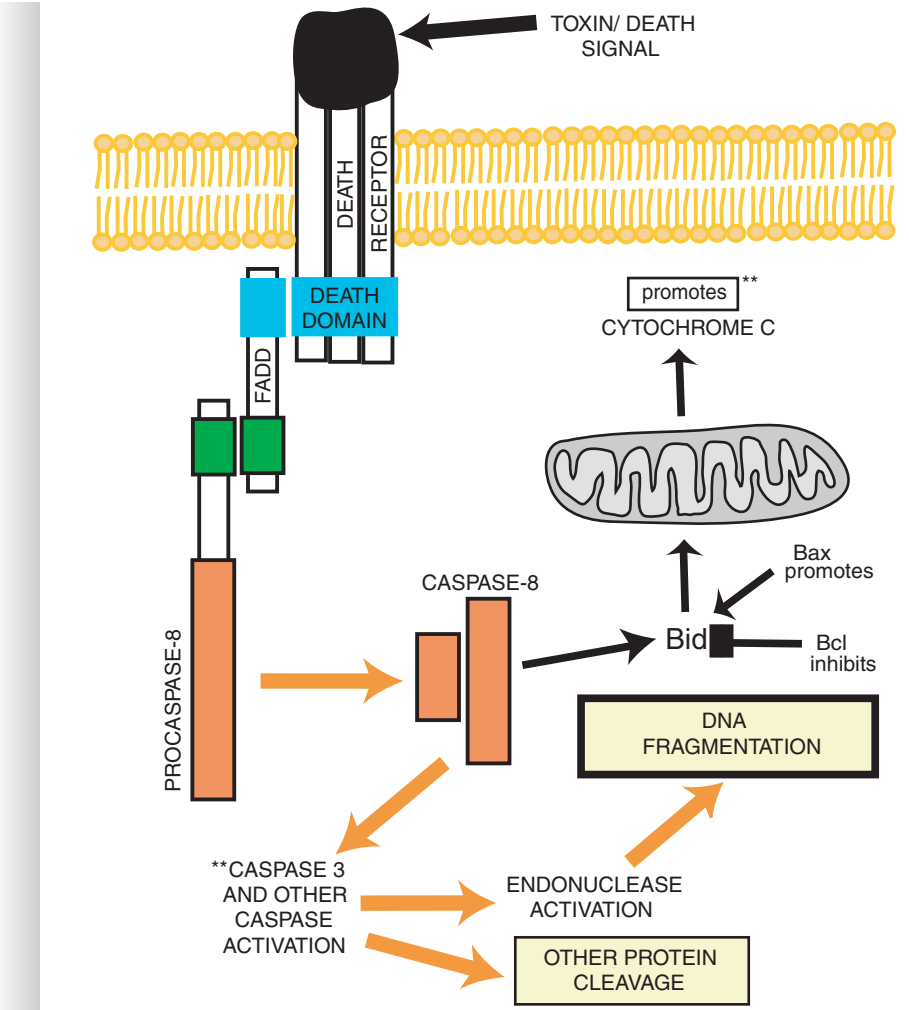
Color Plate 8

Peptide binding complex C5a bound to its receptor protein C5aR. ➤ The figure shows the complement protein fragment, C5a, bound to its receptor protein (C5aR) and associated G_i protein on the plasma membrane of a mast cell. The figure demonstrates two significant binding sites: one ionic (site 1) and the other hydrophobic (site 2).



Color Plate 9

The Fas receptor mechanism for apoptosis. ➤ This represents a typical and the most simple mechanism for programmed cell death. A protein, such as FasL (ligand) binds to the Fas receptor protein (labeled in the figure as the "death receptor"). On the inside of the plasma membrane, the portion of the peptide known as the *death domain* (or DD) binds to an intermediate or adapter molecule as a result (in this case: FADD or *Fas-associated death domain protein*). In turn, at least two units of procaspase 8 bind to FADD where they are activated by autoproteolysis to caspase 8. The active caspase 8 begins a cascade of activation/ proteolysis of other caspases (such as caspase 3) and a member of the *B-cell lymphoma protein* known as Bid. The other caspases cause the significant breakdown of important cell-function proteins such as fodrin, a protein that holds the plasma membrane to the cell cytoskeleton. In addition, these caspases activate the endonuclease known as CAD (caspase-activated dexoxyribonuclease) bringing about the truncation of the cell's vital genome. The activated form of Bid binds to the surface of cellular mitochondria affecting the release of cytochrome C which augments caspase activation. (Adapted from Zimmerman KC, Bonzon C, Green DR: The machinery of programmed cell death, *Pharm Therap* 92:57–70, 2001.)



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CHAPTER 1

Water and Ocular Fluids

PHYSICAL CHEMISTRY OF OCULAR FLUIDS

Many biochemistry texts begin with a discussion of water since it is rather certain that all life began in the sea (Voet, Voet, 1995) and land-based creatures carry their sea with them in the form of blood, cerebrospinal fluid, aqueous fluid, and other body liquids. In this chapter, we will examine the physical-chemical nature of water and, in particular, biological water in the form of blood, aqueous fluid, and the ocular vitreous. Of particular interest will be how these biological fluids interact with dissolved solutes (proteins) and solid tissues (e.g., cell and tissue boundaries).

Water

Water has peculiar physical-chemical properties that allow it to be *partially charged* in specific areas of its molecule (Lehninger, Nelson, Cox, 1993). As Figure 1–1 shows, the electron withdrawing nature of the oxygen atom in the molecule tends to pull electrons more toward the oxygen atom and away from its two hydrogen atoms. This imparts a

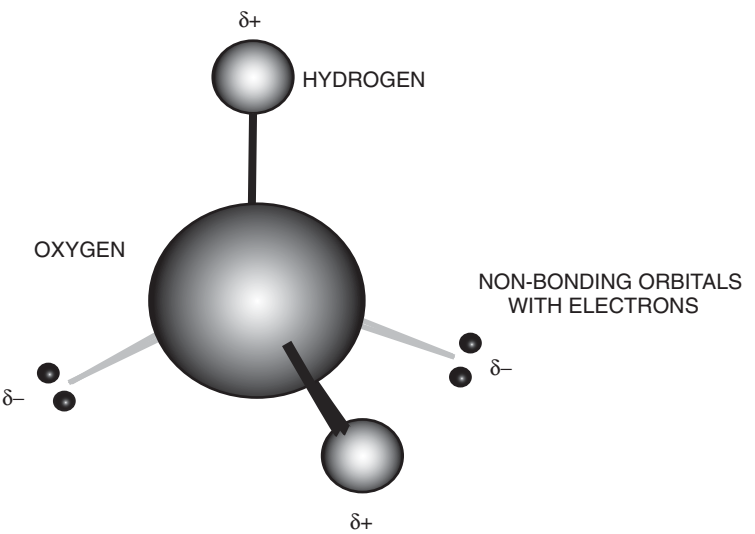


Figure 1–1
The water molecule. ➤ Electrons are pulled more toward the oxygen atom (O) than toward the two hydrogen atoms (H). This imparts a partial negative charge on O while each H has a partial negative charge. The molecule is described as being *polar* in which regions (atoms) of the molecule have either a positive or negative partial charge.

polar nature to the molecule and makes it attractive to oppositely charged ions on the appropriate sides of the water molecule. Other polar molecules, such as ethanol (ethyl alcohol), are similarly attracted toward water molecules on the appropriate oppositely partially charged sides. In biological fluids, such as cell cytoplasm, this attractive property of water helps large molecules (e.g., proteins) to maintain both their presence (soluble state) and functional conformation (shape). Furthermore, water acts as a containment medium for the many smaller molecules (e.g., ions and sugars) whose presence is also required for biological functions.

Solubility of small and large molecules in water and biological fluids is, therefore, explained partly in terms of the ability of water to associate with charged atoms by the use of its partially charged regions (*polar interactions*). This form of solvation is shown in Figure 1–2 for sodium chloride (common table salt). The partially, positively charged hydrogen atoms of water tend to surround and pull negatively charged chloride ions away from salt crystals while the partially, negatively charged oxygen atoms of water tend to surround and pull positively charged sodium ions away from the same crystals. A second form of solvation is also explained in terms of the ability of water to associate with other polar molecules by means of *hydrogen bonding*.

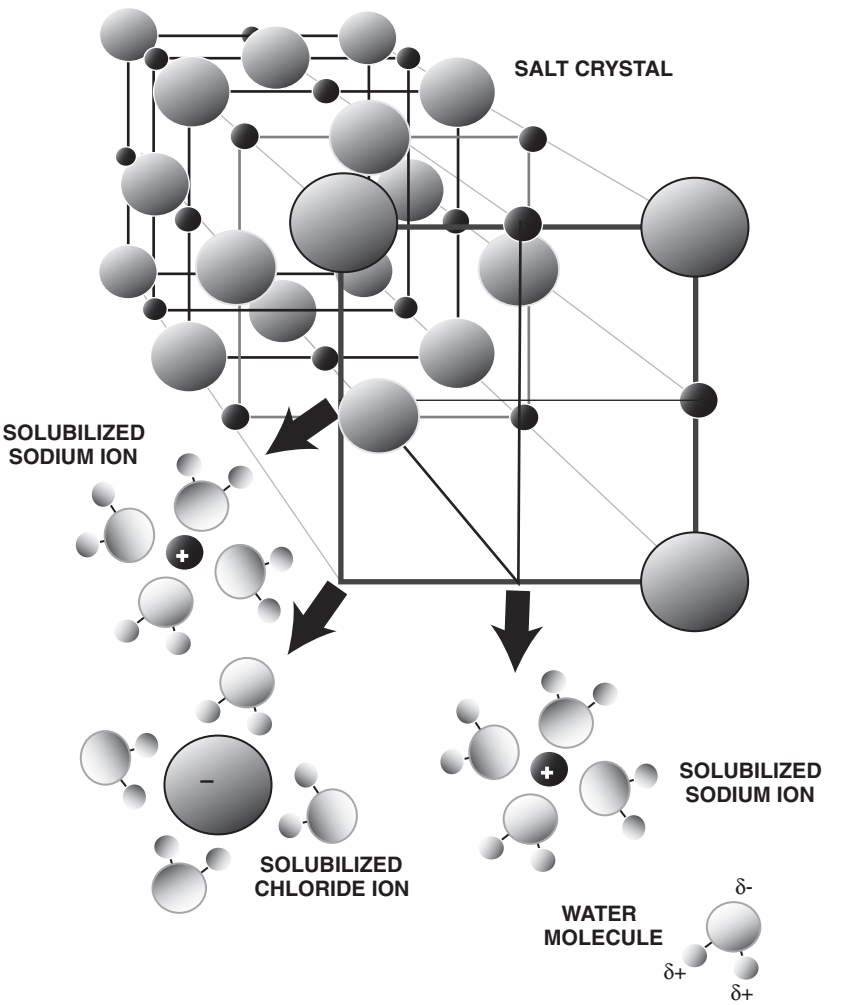


Figure 1–2
Water solvation of a salt crystal. ➤ Salts like sodium chloride (NaCl) are electrostatically bound together by their full positive and negative charges. When introduced into water, each sodium and chloride ion is dissociated away from the crystal by appropriately oppositely, partially charged regions of polar water molecules. The salt ions are described as being hydrated by the water molecules. In this manner the salt "dissolves and goes into solution."

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HYDROGEN BONDS

These are weak bonds that form between a hydrogen (whose electrons have been delocalized or pulled away by an electronegative element such as oxygen) and a second electronegative atom (commonly oxygen or nitrogen). Hydrogen bonds are not unique to water, but water constantly forms hydrogen bonds with its nearest neighbor water molecules both in the liquid and solid states (Figure 1–3). During solvation, water may also form hydrogen bonds with polar portions of proteins as seen in Figure 1–4. As shown, a water molecule forms a hydrogen bond with an amino group ($R\text{-}NH_2$) on a protein. Hydrogen bonds are individually quite weak (approximately 14 kJ/mol) (Table 1–1), but their aggregate strength, especially in proteins may be very strong. This explains how water can be a very powerful solvent for large molecules.

Weak Electrolytes and Buffers in Water

Cells and tissues (with some notable exceptions such as cells that line the stomach) are bathed in aqueous solutions that must maintain a consistent near neutral concentration of hydrogen ions. The hydrogen ion concentration in these cells is usually at $1 \times 10^{-7.4}$ M and is necessary to sustain protein structure and function (i.e., life itself). This concentration is conveniently expressed as its negative logarithm value of 7.4 and is the

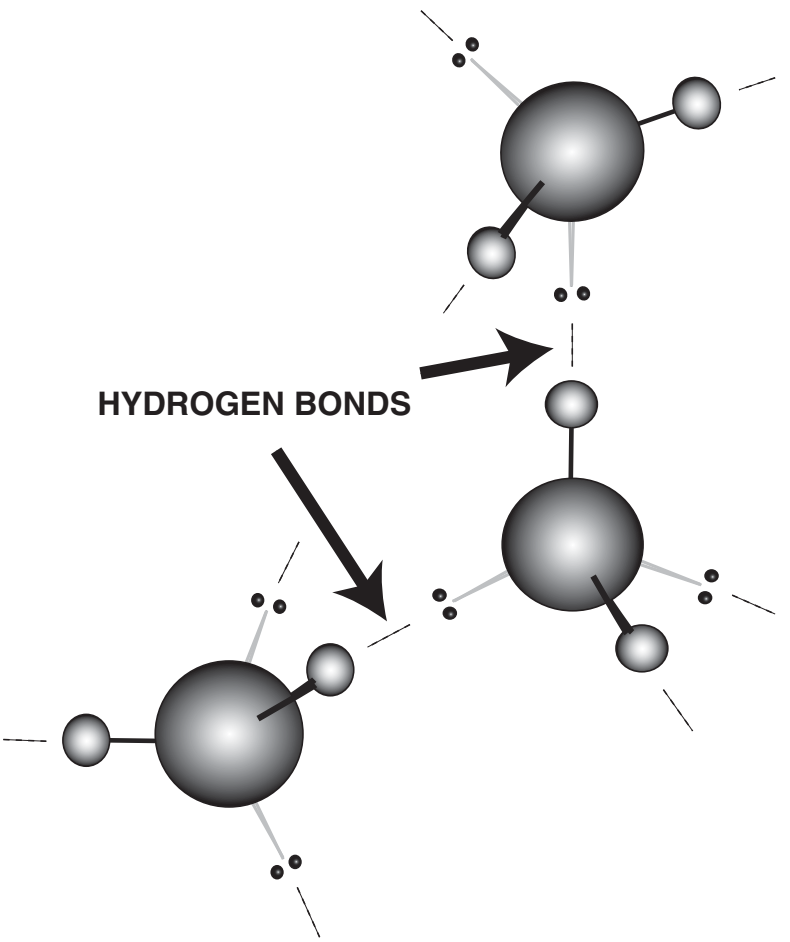


Figure 1–3
Hydrogen bonds between water molecules. ➤ Water molecules form "temporary" hydrogen bonds, that is, bonds that are constantly made and broken. When water freezes, the bonds become permanent giving water a more ordered structure and it expands.

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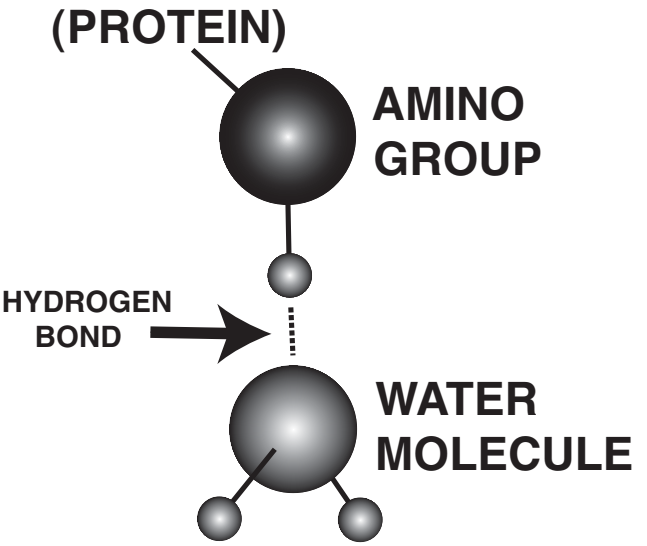


Figure 1–4
A hydrogen bond between a water molecule and an amino group (NH_3^+) on a protein. ➤ These bonds are weak and often break, but quickly reform. The total number of hydrogen bonds formed by proteins (and water, for example) can be very high and help to keep a protein in solution.

TABLE 1–1 ➤ STABILIZATION ENERGIES FOR WEAK AND STRONG MOLECULAR BONDS

	Example	Stabilization Energy (kJ/mol)[1]
Weak interactions		
Hydrogen bonds	C=O---H-O	14
Ionic interactions	Na^+ Cl^-	42
van der Waals interactions	Atoms in close proximity	4
Strong interactions		
Carbon-carbon bonds	C-C	350
Carbon-hydrogen bonds	C-H	410
Oxygen-hydrogen bonds	O-H	460

[1]A kilojoule (kJ) is 1000 × the amount of energy necessary to accelerate a 1 kg mass through a distance of 1 meter per sec^2. One kJ is equal to .239 kilocalories. The values are given as the amounts necessary per molecule (mol) to break one bond. See Appendix.
(Data from Weast, 1986; Daniels, Alberty, 1961; Lehninger, Nelson, Cox, 1993.)

most common pH value for biological fluids both inside and outside of cells. The term "pH" was first explained and used by the Scandinavian biochemist Sørensen in 1909 (Voet, Voet, 1995). The pH is controlled primarily by weak electrolytes present in both ocular and nonocular biological, aqueous solutions. These weak electrolytes are acids that only partially ionize (form ions) in solution. *Due to their partial ionization, they can either take up or donate hydrogen ions to control solution pH.* An example of a weak electrolyte is acetic acid, the acid found in household vinegar.

In order to understand how buffers function, it is necessary to grasp the concept of how weak electrolyte ionization can be determined. This is done by comparing the concentrations of the ionized to the nonionized forms to obtain a ratio known as the *dissociation constant*. Therefore, one uses the expression:

$$K_a = [H^+] [A^-] / [HA]$$

where K_a is the dissociation constant; $[H^+]$ is the molar hydrogen ion concentration; $[A^-]$ is the molar anion concentration; and [HA] is the molar concentration of the undissociated (nonionized) acid. Using the example of acetic acid, it has been found that the dissociation constant (K_a) for this acid is: 1.74×10^{-5}. The negative logarithm or pK_a of that value = 4.76. Using the negative logarithm of the K_a is convenient to determine the pH of a solution of this acid.

Now, if you are getting a little bored with this discussion, go open a bottle of vinegar and sniff it. The characteristic odor is acetate anion from the acetic acid and the sour taste (take a little taste) is from the hydrogen ions dissociated in the vinegar. Although the amount dissociated is only 0.000009 M from the total of 0.5 M present in the vinegar, it is potent to one's senses! It is easily realized then that not much is needed to produce a pH effect in biological systems including the eye.

Let us return to the pK_a value for acetic acid (4.76). This value is equivalent to the pH value of a solution of acetic acid when it is 50% ionized. Its pH can be changed, but only with difficulty, by adding acid or base to the weak electrolyte (i.e., the acetic acid/acetate mixture). This is a desirable property since, by resisting pH changes, the weak electrolyte acts as a *buffer* to pH change and does so by ionizing to a greater extent whenever base enters the solution and by ionizing to a lesser extent whenever acid enters the solution (Figure 1–5). The *capacity* of the buffer is the extent to which it will absorb acid or base and depends on its concentration in solution. The *range* of the buffer is the pH limit (increased or decreased) that will be buffered and this depends on the pK_a of the buffer to act as the mid-range value. The range will extend approximately 1 pH unit above and below the pK_a of the buffer as shown in Figure 1–6. Buffering is a very useful and necessary property of all biological fluids both ocular and nonocular.

THE HENDERSON-HASSELBALCH EQUATION

This equation is useful in the determination of pH, pK_a, and the relative concentrations of ionized and nonionized components of a buffer. It is important in the preparation of buffers to be used in clinical and research laboratories and in the formulation of drugs and drug vehicles that require buffers on topical installation of a drug (such as ocular drugs, which are placed on the corneal surface). The equation is:

$$pH = pK_a + \log [salt] / [acid]$$

where pH is the negative logarithm of the hydrogen ion concentration, pK_a is the negative logarithm of the dissociation constant of the weak electrolyte, and log $[A^-]/[HA]$ is the logarithm of the *ratio* of the ionized anion to the nonionized acid of the weak electrolyte. The derivation of the equation may be found in a number of contemporary biochemical texts (Lehninger, Nelson, Cox, 1993). Examples of problems involving the Henderson-Hasselbalch equation are found at the end of this chapter.

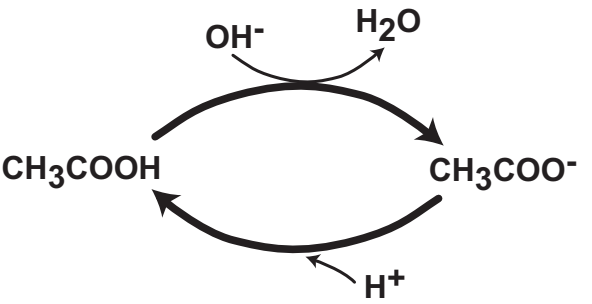


Figure 1–5
The interaction between a buffer, hydrogen ions and water. ➤ The buffer, in this case acetic acid/ acetate ion, can either place a hydrogen ion (or proton) in solution in order to retain acidity or it can bind to a hydrogen ion in order to retain alkalinity. Water acts to ionize or take up protons also, but its range is very limited. A buffer has exceeded its range when it can no longer accept or give up protons. Usually some other component in the solution acts to disrupt the pH by contributing protons or alkali to the solution.

BIOLOGICAL BUFFERS IN OCULAR AND OTHER BODY FLUIDS AND TISSUES

There are three common kinds of biological buffers: phosphate, bicarbonate, and protein. *Phosphate buffer* consists of the system:

$$\left[\; H_2PO_4^- \longleftrightarrow HPO_4^{-2} + H^+ \;\right]$$

Here, an already ionized electrolyte (shown on the left of the equation) is able to ionize even further by the release of a proton (as shown on the right). The arrows indicate that the buffer form can be driven in either direction depending on local H^+ changes. Phosphate buffer has a $pK_a = 6.86$ with a buffering range of 5.86 to 7.86. *It is a common buffer that is present within cells.*

Bicarbonate buffer consists of the system

$$\left[\; H_2O + CO_2 \longleftrightarrow H_2CO_3 \longleftrightarrow HCO_3^- + H^+ \;\right]$$

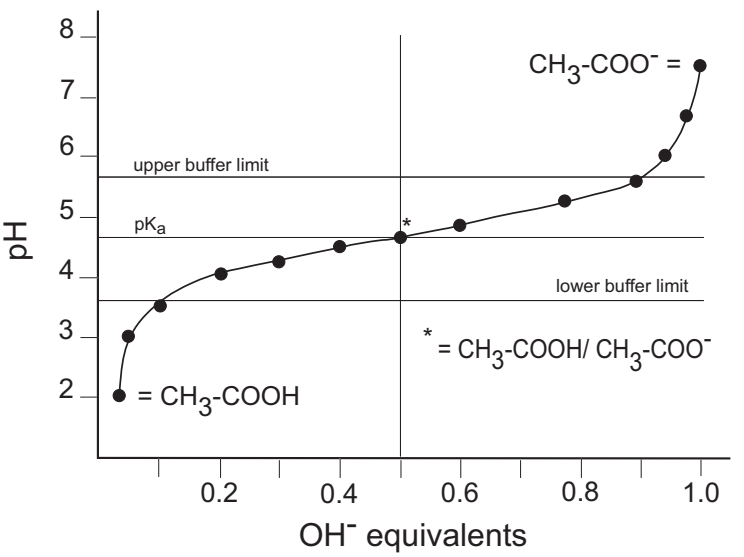
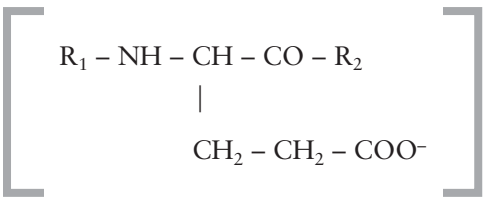


Figure 1–6
Graphical representation of the acetic acid/ acetate ion buffer system. ➤ At the pK of the buffer, both buffer components are present at a one to one ratio (50%). This is indicated by the vertical line at 0.5 OH^- equivalents. At this point, the buffer holds maximal potential to control pH in either direction. At approximately 1 pH unit above or below the pK, buffer power is lost. The pK is the pH at which the concentration of the two components are equal.

This system is more complex in that the carbon dioxide (CO_2) can be removed with the expired air and, therefore, one's breathing rate can influence the HCO_3^-/H_2CO_3 ratio and, conclusively, *the extracellular pH of blood and all ocular fluids*. As a consequence of this system, an elevated breathing rate would tend to raise the pH by shifting both arrows to the left as more CO_2 is removed from the body while the converse would hold true. Removing CO_2 converts HCO_3^- to H_2CO_3 and then to CO_2. The H^+ is incorporated into water as CO_2 is formed. One can produce respiratory alkalosis just by hyperventilating (forced rapid breathing). The pH in one's own blood may be made to rise to as high as 7.9!

Protein buffers, it turns out, are the *most important buffers both inside and outside of cells* (Mathews, van Holde, 1990). This is due to the presence of the myriad number of acidic and basic groups, which are present on protein amino acid components, and the fact that there are so many proteins present both inside and outside of cells. An example of an ionizable group on a protein is the glutamate ion:



Its acidic properties are balanced by basic groups such as that found on the amino acid lysine on the same protein. (See Chapter 2 for further information on the amino acid components of proteins.) In this manner, the pH buffering from a single protein is quite sophisticated. Moreover, the ionizable groups on proteins have altered pK values such that prediction of the exact buffering tendencies and capacities of proteins are impossible without experimental evidence.

Ocular Fluids

Ocular fluids that are found in common with nonocular regions of the body are *cellular cytoplasm, interstitial fluid, and blood*. Ocular fluids that are exclusive to the eye are *aqueous fluid, the vitreous, and precorneal tears*. All of these fluids make use of the particular physical-chemical properties of water to carry out their functions. No further mention will be made of cellular cytoplasm and the interstitial fluids as these subjects are covered in basic texts in cell biology and biochemistry. Their roles are identical in the eye.

BLOOD IN THE OCULAR GLOBE

Blood is a complex mixture of cellular and biochemical components that can be separated by centrifugation. The ocular physiological functions of blood include the nourishment and ridding of waste components of ocular cells; a source for the generation of the intraocular pressure of the eye; and a source for the formation of aqueous and vitreous fluids as well as the homeostasis of retinal functions.

The usual pH of blood is 7.4, but it can vary from 7.33 to 7.45 under normal circumstances (Tietz, 1976). Gases dissolved or carried in the blood include oxygen, nitrogen, and carbon dioxide. The partial

pressure of O_2 in the arterial blood is approximately 83 to 108 mm Hg while that of CO_2 in venous blood is approximately 38 to 50 mm Hg. These pressures become lower in ocular vessels such that the pO_2 in ocular capillary beds is only about 50 mm Hg (Sebag, 1992). A partial listing of biochemical and chemical components of blood is shown in Table 1–2. Blood may be considered both a solution and a suspension of insoluble compounds (e.g., lipids) and cells (red blood cells and white blood cells). In the eye, it has the same functions as mentioned above. In addition, it is a source for the formation of aqueous fluid (an ultrafiltrate found behind the cornea) and vitreous (a partial gel sandwiched between the lens and the retina).

TABLE 1–2 ➤ SOME NORMAL COMPONENTS OF BLOOD[1]

Component	Range	Remarks
Albumin	3.8–5 gm/100 ml	A protein that carries water-insoluble blood components. Discussed in Chapter 6.
Calcium	4.2–5.4 mg/100 ml	A soluble ion with a myriad of functions including: blood clotting, enzyme activation, hormone activity, and muscle contraction. Discussed in Chapter 6.
Cholesterol	140–250 mg/100 ml	A well-known form of lipid that is carried in lipoprotein bodies. It is not soluble in blood, but exists as a suspension. Discussed in Chapter 5.
Globulin	2.3–3.5 gm/100 ml	One of several related water-soluble proteins some of which are involved in immunological functions. Discussed in Chapter 9.
Glucose	70–105 mg/100 ml	A water-soluble sugar that has great importance as a nutrient. Discussed in Chapter 4.
Hemoglobin	13–16 g/100 ml	A protein that carries O_2 to cells.
Phosphate	3–4.5 mg/100 ml	Part of the components of phosphate buffer (mentioned previously). Phosphate is also a source of protein function and cellular energy. It is water soluble. Discussed in Chapter 4.
Potassium	~105 mmol/liter in red blood cells	The principal cation of intracellular fluid. It is very important for enzyme function. Discussed in Chapter 3.
Triacylglycerols (Triglycerides)	35–140 mg/100 ml	A lipid class that is also carried in lipoprotein bodies. Like cholesterol, it is not soluble in blood, but exists as a suspension. Discussed in Chapter 5.

[1]Modified from Tietz, 1976.

AQUEOUS FLUID

The aqueous fluid is a carefully controlled filtrate of blood produced by the ciliary body. It involves the processes of diffusion, ultrafiltration, and active transport (Caprioli, 1992). These processes will be discussed later in Chapter 3. The aqueous fluid nourishes the cells of the posterior cornea, the iris, and the lens (Figure 1–7). It is the sole source of nourishment for cells of the corneal endothelium, stromal keratocytes, most of the corneal epithelial cells, and the entire lens. It is considered to be a source of antioxidants for the lens and, perhaps, the corneal endothelium. The aqueous fluid may be considered to be analogous to interstitial fluid in that no red blood cells are present in it, yet it carries released O_2 and nutrients to the cells it serves. In addition, the hydrostatic pressure (intraocular pressure or IOP) exerted by the aqueous fluid maintains the shape of the ocular globe and protects it to some degree from physical shock. The generation of the IOP is discussed further in Chapter 3. The components of the aqueous when compared with those of normal blood are shown in Table 1–3.

An examination of this table reveals three characteristics of the aqueous humor compared to blood: some components are unchanged (pH, sodium); some components are decreased (proteins); and at least one component is increased in concentration (ascorbate or vitamin C). These changes reveal that the aqueous is a filtrate of blood. Since it has lost its cellular components, it should not be surprising that there is a decrease in protein and potassium levels. What is not so obvious is that the absence of protein enhances the ability of the aqueous to transmit light from the cornea to the lens. This is especially true for the absence of lipid whose increase in blood after a meal greatly contributes to the cloudiness of blood serum (blood's acellular component). It is probably true that the buffering capacity of aqueous is reduced by the loss of protein, but the pH is still maintained by the retention of sufficient phosphate and bicarbonate levels. The increased level of ascorbate is considered to act as an enhanced reservoir of antioxidant primarily for the lens.

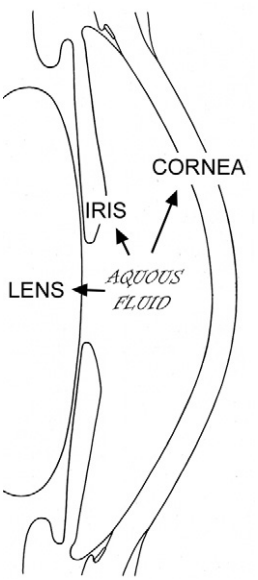


Figure 1–7
A cross section of the anterior chamber of the eye. ➤ The diagram shows some of the major tissues nourished by the aqueous fluid.

TABLE 1–3 ➤ COMPARISON OF SOME COMPONENTS OF BLOOD WITH AQUEOUS FLUID

Component	Blood Concentration[1]	Aqueous Concentration[2]
Albumin	4.4 gm/100 ml	.006 gm/100 ml
Ascorbate (vitamin C)	1.3 mg/100 ml	19 mg/100 ml
Bicarbonate	27 mmol/litter	20 mmol/liter
Calcium	4.8 mg/100 ml	.01 mg/100 ml
Cholesterol	195 mg/100 ml	_[3]
Globulin	2.9 gm/100 ml	.005 gm/100 ml
Glucose	98 mg/100 ml	47 mg/100 ml
Hemoglobin	15 g/100 ml	None
Hydrogen ions (as pH)	7.4	7.5
Phosphate	3.8 mg/100 ml	2.1 mg/100 ml
Potassium	105 mmol/liter in red blood cells	.005 mmol/liter
Sodium	150 mmol/liter	150 mmol/liter
Triacylglycerols	88 mg/100 ml	_[3]

[1]Tietz, 1976.
[2]Recalculated from Caprioli, 1992 and Berman, 1991.
[3]Kim and Cotlier, 1976. Reports vary, but the lipid content of aqueous is quite small due to the aqueous-ciliary body barrier to lipids.

THE VITREOUS (VITREOUS BODY)

The vitreous fluid is an interesting mixture of fluid and gel that varies between species as well as with the age of each species (Sebag, 1992). In humans, it begins as 80% gel/20% fluid and gradually changes to 40% gel/60% fluid. This rather peculiar composition occurs even though 98% of the vitreous is water (Mayne, Brewton, Ren, 1997)! The gel portion may be described as a stiff, but semi-rigid precipitate that retains the liquid in which it was initially formed (Daniels, Alberty, 1961). The peculiar characteristics of the vitreous are due to their inclusion of type II and other collagens (see Chapter 2) as well as proteoglycans (see Chapter 4). The change in proportion of gel and fluid with age is considered to be the result of the breakdown of type II collagen. Unfortunately, this breakdown can result in destabilization of the retinal surface and lead to retinal detachment (see Chapter 10). The gel-like nature of the vitreous gives it sufficient rheological (deformation) properties that it is able to cushion the eye from shock by absorbing exterior forces placed upon it. This absorption even includes the seemingly innocuous, continuous saccades of reading a book such as this one. This specific property of a gel, as such, is known as *viscoelasticity*. Viscoelasticity is a characteristic that is produced in the vitreous by both the proteoglycans (including hyaluronic acid) and collagens present there. As such the vitreal gel possesses both some *resilience* (the ability to reform its original shape after deformation) and some *flow* (movement without stored energy).

A comparison of some chemical components of vitreous with normal blood is shown in Table 1–4. Inspection of Table 1–4 reveals that ascorbate levels are elevated in vitreous. This is probably due to

TABLE 1–4 ➤ COMPARISON OF SOME COMPONENTS OF BLOOD WITH VITREOUS

Component	Blood Concentration[1]	Vitreous Concentration[2]
Ascorbate	1.3 mg/100 ml	7.6 mg/100 ml
Bicarbonate	27 mmol/liter	25 mmol/liter
Glycosaminoglycans (hyaluronin)	None	25 mg/100 ml
Protein	7.3 mg/100 ml	55 mg/100 ml (mainly as collagen)
Sodium	150 mmol/liter	137 mmol/liter
Potassium	105 mmol/liter (in red blood cells)	3.8 mmol/liter
Glucose	98 mg/100 ml	50 mg/100 ml

[1]Tietz, 1976.
[2]Recalculated from Berman, 1991 and Sebag, 1992.

the high levels present in the aqueous fluid, which dissipate into the vitreous. The amounts of protein and hyaluronin are comparatively elevated due to the presence of type II collagen (principally) and hyaluronic acid, which may contribute to the gel in the vitreous (see Chapter 10). Sodium and glucose are at levels that would be expected from the infusion of those substances from adjoining fluids (vitreous and retinal interstitial fluids). The small, but somewhat elevated level of potassium is probably due to the presence of a small concentration of cells (hyalocytes) in the outer layer (cortical region) of the vitreous. It is emphasized that the vitreous is a clear substance, which is important for the passage of light from the lens to the retina.

PRECORNEAL TEARS

The precorneal tears form a film between the inside of the lids and the cornea when the eyes are closed and remain as a film over the cornea for a limited time after the lids are opened (usually greater than 15 sec) (Vaughan, Ashbury, 1980). The tears act as a lubricating fluid for the lid-cornea interface and as an antibiotic medium to protect the eye. Therapeutically, the film serves as a temporary depository for the installation of topical drugs. The tears, as a film, are a complex mixture composed of three layers: lipid, aqueous, and mucin. Here, only the aqueous layer is dealt with. In Chapters 2, 5, and 9 additional information will be provided about the mucin and lipid layers as well as the immunochemical properties of the aqueous layer. A comparison of some components of blood with the aqueous layer of the tear film (Table 1–5) shows some useful information. Table 1–5 shows that, in general, components of the aqueous tears are more dilute than that of blood. A notable exception occurs with potassium where the level is concentrated nearly sevenfold by the ductal secretion of KCl from the lacrimal gland (Tiffany, 1997). The low level of ascorbate indicates that no contribution from the high levels of ascorbate in the aqueous is contributed to the tears. This may be compared with the relatively high level of ascorbate in the vitreous (see Table 1–4) where such an influence is seen. The very low level of glucose found in tears means that this fluid could not act as a nutrient

TABLE 1–5 ➤ COMPARISON OF SOME COMPONENTS OF BLOOD WITH AQUEOUS LAYER TEARS

Component	Blood Concentration[1]	Tear Concentration[2]
Ascorbate	1.3 mg/100 ml	0.4 mg/100 ml
Bicarbonate	27 mmol/liter	23 mmol/liter
Calcium	4.8 mg/100 ml	1 mg/100 ml
Glucose	90 mg/100 ml	6 mg/100 ml
Potassium	4.3 mmol/liter	30 mmol/liter
Protein	7 g/100 ml	0.7 g/100 ml
Sodium	150 mmol/liter	138 mmol/liter

[1]Data from Tietz, 1976.
[2]Data recalculated from Berman, 1991.

for corneal and conjunctival cells. In fact, corneal cells are dependent upon the aqueous for their nutrient glucose whereas conjunctival cells use interstitial fluid from their local blood supply.

SUMMARY

Water is a polar substance whose properties are also found in biological fluids such as blood, precorneal tear fluid, aqueous fluid, and the vitreous of the eye. The polar properties of water enable both simple and complex molecules (such as proteins) to be contained (solubilized) in each of the ocular aqueous media by weak interactions between the water and solute molecules. These weak interactions are: hydrogen bonds, ionic bonds, and van der Waals forces.

The hydrogen ion concentration (pH) of aqueous fluids is controlled by the partial ionization of weak electrolytes normally present in these fluids. The pH must be tightly controlled in order to preserve tissue structure and cell viability. This is achieved in the presence of three different buffer systems found both in the aqueous fluids and in the cells that are surrounded by these fluids: phosphate buffer, protein buffer, and bicarbonate buffer. The pH in such fluids can be measured instrumentally with a pH meter, but the pH may be determined mathematically by the use of the Henderson-Hasselbalch equation when buffered fluids are prepared for laboratory or drug use.

Blood is a complex fluid consisting of both cellular and biochemical components. Blood nourishes and removes wastes from tissues (ocular and nonocular). The aqueous fluid is a filtrate of blood that is formed and released into the anterior and posterior chambers of the eye. It generates the intraocular pressure and maintains the ocular shape. This fluid is largely devoid of protein and lipids to maintain its clarity, but has a

high concentration of ascorbate (vitamin C) possibly in an antioxidant role. The vitreous body is a fluid that is part gel in character. As such, it protects the retina from mechanical shock due to its viscoelastic characteristics. Those characteristics stem from the presence of both proteoglycans and collagen. The precorneal tear film is a three layered fluid whose largest component, the aqueous layer, contains largely diluted components of blood.

PROBLEMS

1. What buffer would you expect to be very important in the aqueous fluid? Explain your answer.

2. The phosphate buffer system: $H_2PO_4^-$ (dihydrogen phosphate) and HPO_4^{-2} (monohydrogen phosphate) has a $pK_a = 6.86$. At a pH of 7.4, what percentage of the dihydrogen form would be ionized? Would this be a good buffer system to use to formulate (make) a topical irrigating solution for the eyes? Explain.

3. What effect might rapid breathing have on cells exposed to the aqueous fluid of the eye such as the corneal endothelium or the lens epithelial cells?

4. Which component of the aqueous fluid is concentrated compared to that found in blood? What is the ratio of concentration in aqueous vs. blood? Speculate why this component may be necessary in aqueous fluid.

5. The gel/liquid ratio of the vitreous in cattle has been reported to be 100% gel in form. Based on this information, would you expect cattle to have more or fewer retinal detachments than humans? Explain your answer.

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CHAPTER 2

Proteins

ESSENTIAL COMPONENTS OF THE EYE

Proteins (meaning *primary* from the Greek) are polymers of amino acids linked together by peptide bonds (Figure 2–1). These polymers are arbitrarily called *peptides* when their molecular weight is below 10,000 (Figure 2–2A) and are referred to as *proteins* when their molecular weight exceeds 10,000 (see Figure 2–2B). The term *polypeptide is* also used for high molecular weight peptides that are often proteins by the above definition. Proteins were first described by Berzelius in 1838 (Stryer, 1988).

As end products of genetic expression, proteins serve many vital roles in cells and tissues of biological constituents from viruses to man. Such roles include mechanical support, control of growth and differentiation, catalysis, transport and storage, motion, nerve propagation, and immune protection. In the eye, proteins are known to support the structure and clarity of the cornea, to participate in the variable light refraction of the lens, to initiate the transduction of light into electrical signaling, to generate intraocular pressure, to lyse bacteria in the precorneal tear film, and other functions that indirectly sustain vision.

Proteins are composed of amino acids. Each amino acid consists of at least one carboxylic acid and one amino group attached to an adjacent carbon (α-carbon) as shown in Figure 2–3. Amino acids differ from each other in the groups that are attached to the α-carbon. It is reasonable to conclude that a protein is the sum of the functional properties of its constituent amino acids, but it is unreasonable to accurately predict what those conglomerate properties impart to an individual protein.

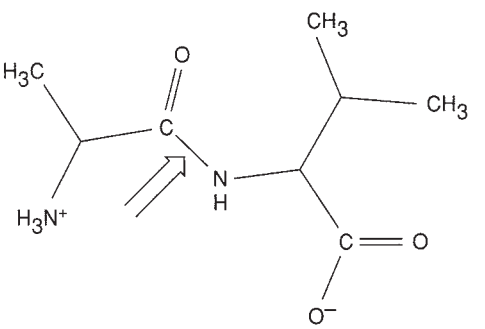


Figure 2–1
The dipeptide: alanylvaline. ➤ This peptide is made up of two amino acids linked by a peptide bond (*arrow*). Proteins consist of at least 80 amino acids joined by peptide bonds at their respective carboxylate and alpha amino groups.

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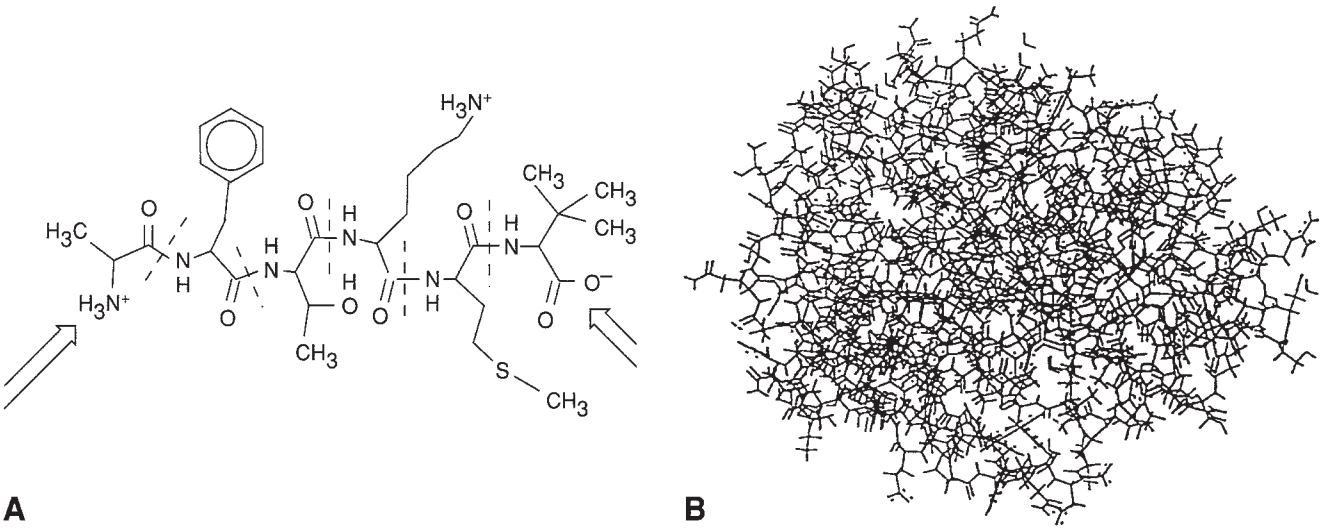


Figure 2–2
(A)The peptide: Ala-Phe-Thr-Lys-Met-Leu. ➤ This hexapeptide has five peptide bonds (*dotted lines*) and a molecular weight of 710 D. The N-terminal and C-terminal ends are indicated by *arrows*. The minimal complexity of a protein may be imagined by considering a molecule 14 times this large. (B) The protein: elastase. ➤ (Figure used with permission from the Protein Data Bank, Brookhaven National Laboratory, Upton, NY.)

Figure 2–3
Some representative amino acids. ➤ Refer to Table 1–1 for the class to which each belongs and some of their properties. The actual charge characteristic of each amino acid in a protein may be obtained by eliminating the charges on the carboxylate and alpha amino groups (except when they occur at the N- and C-terminal ends of a protein).

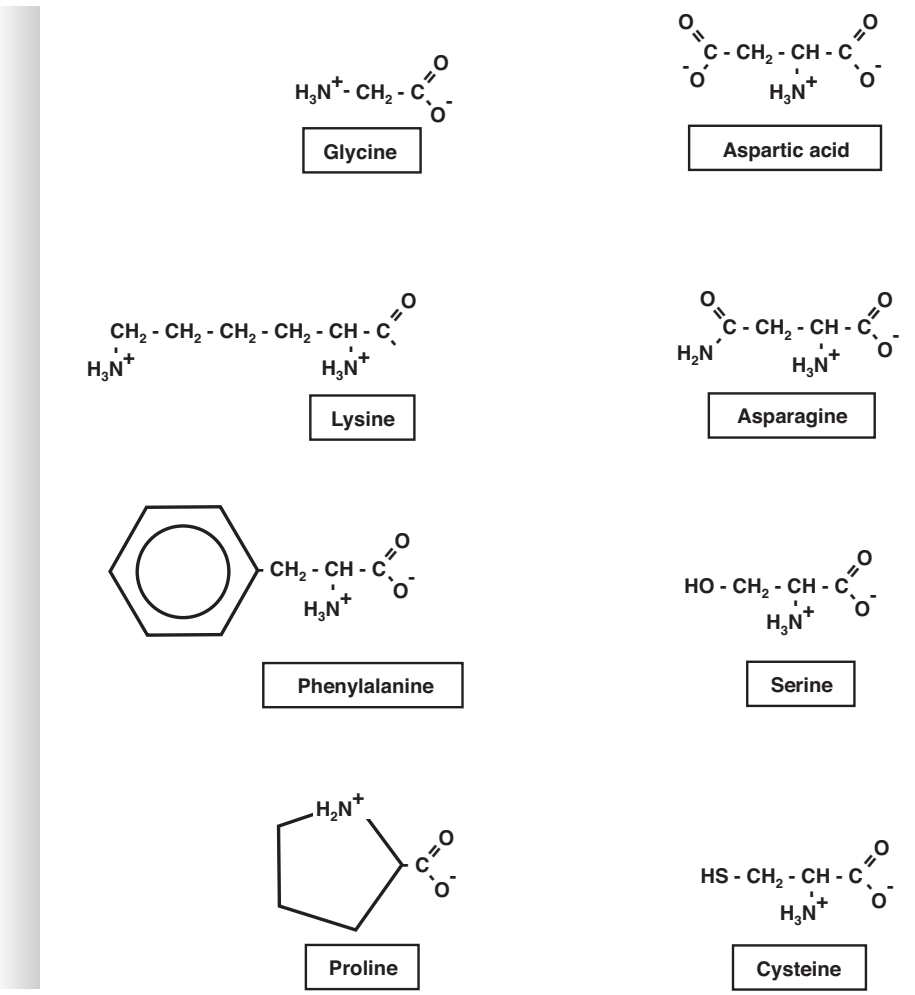


TABLE 2–1 ➤ AMINO ACIDS FOUND IN PROTEINS AND PEPTIDES

Type	Name	Abbreviation[1]	Remarks
Unsubstituted	Glycine	G/Gly	Impart hydrophobic characteristics to proteins. Glycine can give a "tighter" twist to proteins such as collagen.
	Alanine	A/Ala	
	Valine	V/Val	
	Leucine	L/Leu	
	Isoleucine	I/Ile	
Dicarboxylic	Aspartic (aspartate)	D/Asp	Impart negative charges to proteins due to ionization of one carboxylate.
	Glutamic (glutamate)	E/Glu	
Diamino	Lysine	K/Lys	Impart positive charges to proteins due to ionization of one amine.
	Arginine	R/Arg	
	Histidine	H/His	
Amido	Asparagine	N/Asn	Derived from D and E. They have hydrophilic character.
	Glutamine	Q/Gln	
Hydroxy	Serine	S/Ser	Hydroxy groups give them hydrophilic character.
	Threonine	T/Thr	
Aromatic	Phenylalanine	F/Phe	Impart hydrophobic characteristics to proteins. Y is less hydrophobic due to its OH group.
	Tyrosine	Y/Tyr	
	Tryptophan	W/Trp	
Sulfur	Cysteine	C/Cys	C forms disulfide bonds between polypeptide chains and is hydrophilic. M is hydrophobic.
	Methionine	M/Met	
Cyclic	Proline	P/Pro	P is nonaromatic and is found in collagen, elastin, and mucin.

[1]The first abbreviation is more conveniently used with lists of long sequences.

Certain generalizations can be applied, however. There are twenty amino acids (Table 2–1) commonly found in proteins, of which the dicarboxylic, diamino, amido, and hydroxy types contribute to the water solubility of a protein by their charged or polar nature. This is also true of amino acids occurring at the amino (N) and carboxyl (C) terminal ends of a protein. Cysteine also contributes to water solubility. In all peptides and proteins, amino acids have their α-amino and associated carboxylate groups bound up in peptide bonds except at the N- and C-terminal ends (see Figure 2–2).

Dicarboxylic, diamino, amido, and hydroxy amino acids (as well as cysteine) donate hydrophilic (i.e., water-soluble) properties to proteins. These amino acids are also important in controlling intra- and extracellular pH (see Chapter 1). Aromatic and unsubstituted amino acids as well as methionine and proline impart hydrophobic characteristics (i.e., lipid soluble properties) to proteins. These characteristics are important in the determination of conformational shapes, subunit binding (i.e., the association of more than one polypeptide chain), reactive site formation and function, as well as the existence of some proteins in cell membranes. Still other amino acids are involved in specialized functions that promote metal chelation (as histidine does in hemoglobin), chain binding (as cysteine does in the enzyme ribonuclease), and tight helical turns (as glycine does in collagen). All of these characteristics are significant, to greater or lesser degrees, in the function of each protein and are dependent upon where and how often each particular amino acid occurs in

sequence (*primary structure*). Proteins may be classified by several criteria. Function, solubility, size, and biological location are commonly used determinants. For example, using solubility as a factor, proteins are considered to be either water-soluble (e.g., blood plasma proteins such as albumin), lipid soluble (e.g., intrinsic membrane proteins such as rhodopsin), or insoluble (e.g., extracellular matrix proteins such as collagen). Proteins are capable of losing their function(s) by a process called *denaturation*. Denaturation can occur by changes of temperature, ionic composition, and other environmental parameters and means that, at least, some of the conformation of the protein has been altered from its native form. Denaturation can be temporary or permanent. Enzymes (catalytic proteins) are particularly prone to denaturation.

Contemporary understanding of proteins and their functions has been greatly aided by investigations of their genetic expression from deoxyribonucleic acid (DNA) and ribonucleic acid (RNA) as well as by studies of their structural representation *via* X-ray crystallography. More about the former will be included in Chapter 7. Crystallographic studies have yielded three dimensional representations of proteins, which may be exquisitely detailed with the aid of computer-driven molecular modeling (see Figure 2–2B and Figure 2–4). Protein structure may be considered to fall into four basic divisions: Primary, *secondary, tertiary,* and *quaternary*. As an example of these divisions, let us consider the general structure of immunoglobulins (proteins involved in binding to antigens or foreign bodies such as bacteria). Typically immunoglobulins are composed of four polypeptide chains each having its own particular sequence of amino acids. The sequence of amino acids (named left to right from the free amino group end or N-terminal end) is the primary structure of the protein (Figure 2–5). The immunoglobulins have at least four separate primary structures depending on type. Each sequence of amino acids is able to assume at least four different kinds of configurations or shapes due to the bulkiness, charge density, and hydrophobic regions of the amino acids in sequence. The shapes are further determined by hydrogen and disulfide bonding within and between amino acid chains.

In Figure 2–6 are seen three types of secondary structures that occur in immunoglobulins: *random coils, β-turns* and *β-pleated sheets*. They make up part of the shape of one heavy chain. Random coils are haphazard forms that connect other secondary structures whereas β-turns are 180° turns, which were first found to connect β-pleated sheets. The β-pleated sheets are characterized by parallel or antiparallel sequences of primary structures held together by numerous hydrogen bonds (see

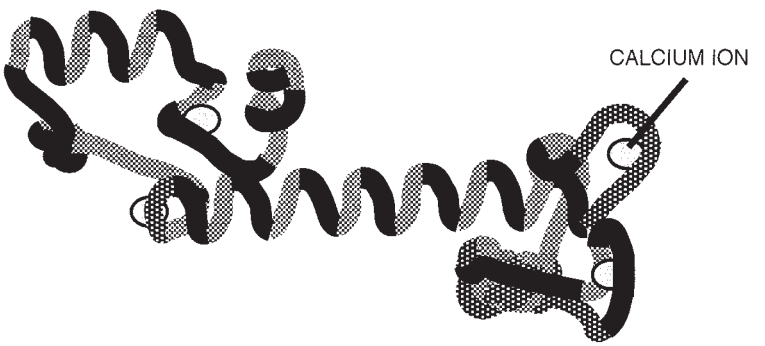


Figure 2–4
Redrawn model of calmodulin, a protein that stores calcium ions inside of cells. ➤ The location of four calcium ions is evident as well as the extensive helical structure of the protein. (Figure used with permission of Babu YS, Bugg CE, Cook WJ: *Calmodulin,* Cohen P, Klee CB, editors. Alsever, 1988, Amsterdam.)

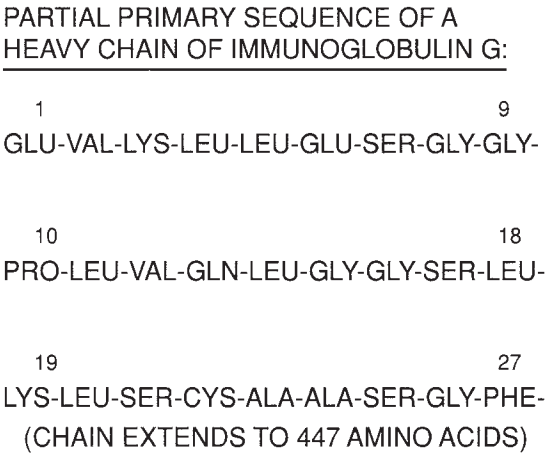


Figure 2–5
Primary protein structure. ➤ The structure consists of the sequence of amino acids named from the N-terminal end of each polypeptide chain in the protein.

Figure 2–6). Two antiparallel β-pleated sheets are shown in Figure 2–7. Other variations of β-pleated sheets exist. Another secondary structure present in proteins, but not in immunoglobulins is the *α-helix* (discussed in the following section).

A structural or functional part of one chain containing such secondary structures has been termed a *domain* as shown in Figure 2–6. A somewhat simplified version of a domain, called a *motif*, has also been described. The distinction between a motif and a domain, however, is not always absolute. The entire shape or conformation of one polypeptide chain constitutes its tertiary structure as seen in Figure 2–8. Here it can be seen that there are four globular masses or domains in the chain. These are the same domains as shown in Figure 2–6 except that they have been filled in. A domain may be considered, therefore, either as a subdivision of a tertiary structure or a discrete collection of secondary structures. Finally, there exists the quaternary structure, which is the total conformation assumed by all the polypeptides that make up a protein as seen in Figure 2–9. A quaternary structure assumes that the protein has two or more polypeptide chains. For the immunoglobulin, it is the three dimensional shape assumed by the two light and the two heavy chains. One other secondary structure found in other proteins is α helix as shown in Figure 2–10. Some helical

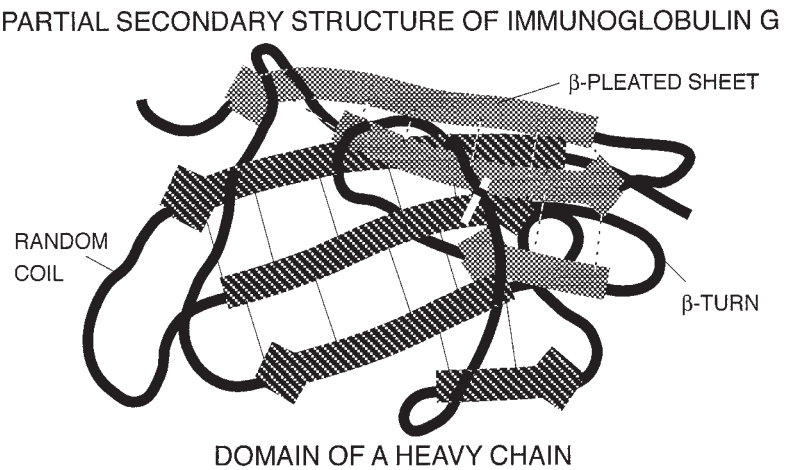


Figure 2–6
Examples of secondary protein structure. ➤ Such structures are configurations formed within a sequence of amino acids. Four types are generally recognized: helices, pleated sheets, random coils, and turns. Three are shown here. A domain is a collection of secondary structures with some functional importance.

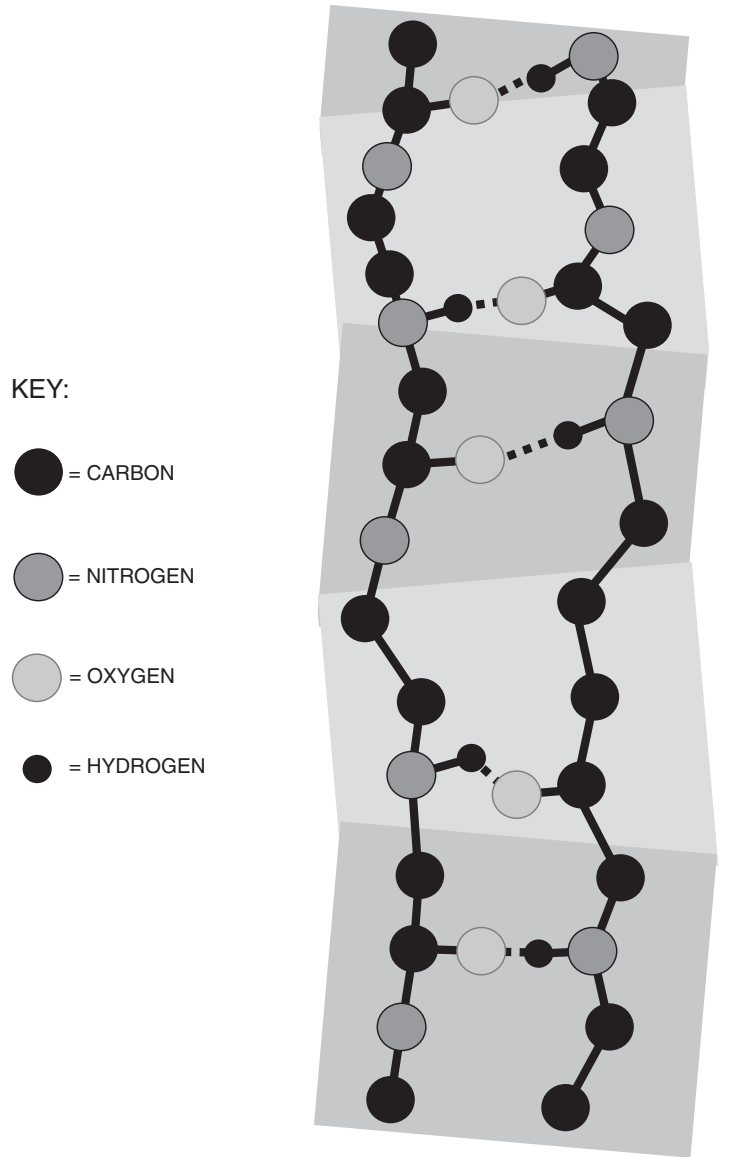


Figure 2–7
A β-pleated sheet. ➤ The name comes from the fact that planes of pleated sheets may be drawn through the figure and the sequence of amino acids must make a β-turn to form additional runs through the "sheet." The dotted lines represent hydrogen bonds, which strengthen the structure. (Redrawn from Mathews CK, van Holde KE: *Biochemistry,* Redwood City, CA, 1990, Benjamin/Cummings.)

structures may also be seen in the computer drawn protein of Figure 2–4. There are several kinds of helices that can be found in specific proteins. It is worthwhile to mention, however, that α-helices occur most commonly both in soluble proteins and in the portions of proteins that traverse membranes. In the eye, rhodopsin, for example, contains a considerable proportion of α-helical structure. On the other hand, β-pleated sheets dominate the lens soluble proteins known as crystallins. Both helices and pleated sheets are stabilized by hydrogen bonding. These are weak bonds that form between hydrogen and either oxygen or nitrogen. They occur as a result of the electron withdrawing properties of other oxygen and nitrogen atoms and give the hydrogen a partial positive charge (see Chapter 1).

Proteins vary enormously in their molecular weights from 10,000 to several million *daltons* (a dalton is a unit of mass close to that of hydrogen or 1.0000). Table 2–2 lists some representative proteins. Many proteins that contain more than one polypeptide chain are held together by

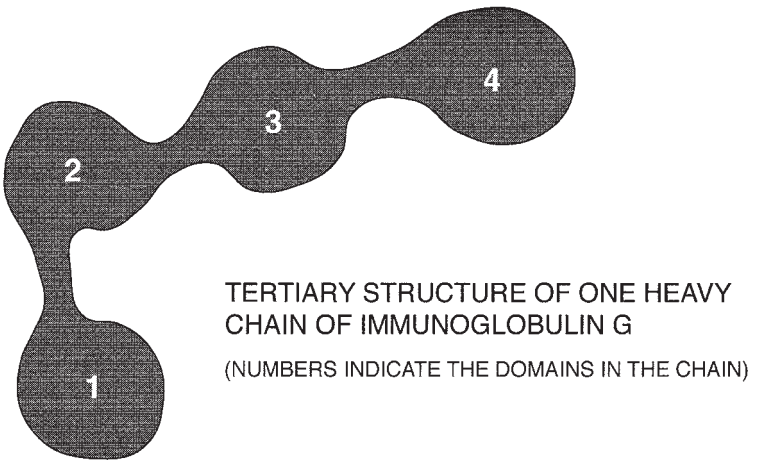


Figure 2–8
Tertiary protein structure. ➤ This structure represents the entire conformation assumed by one polypeptide chain. It may have several domains contained within its overall shape.

sulfur bridges known as *disulfide bonds*. The four chains of immunoglobulin G (see Figure 2–9) are joined in this manner. Some proteins have their peptide chains altered after synthesis. The process is called post-*translational modification*.

In addition to their specialized functions, proteins may act as reservoirs of positive and negative charges and, therefore, constitute both intra- and extracellular pH buffers. In general, the negative charges tend to predominate in proteins at the physiological pH of blood serum (i.e., 7.4), which may vary from some intracellular pH values. Proteins are an important source for the generation of osmotic pressure across cell boundaries. This is a significant function in the eye with respect to processes such as *corneal deturgescence* (the mechanism in which the corneal stroma is maintained in a clear state) and the generation of *intraocular pressure* (that contributes to ocular integrity and viscoelasticity). The discussion that follows considers six important proteins found in ocular tissues. However, it should be kept in mind that ocular cells and their surrounding tissues are made up of thousands of different kinds of proteins that are needed for their normal function. Many of these same proteins are also found in nonocular cells and tissues as well.

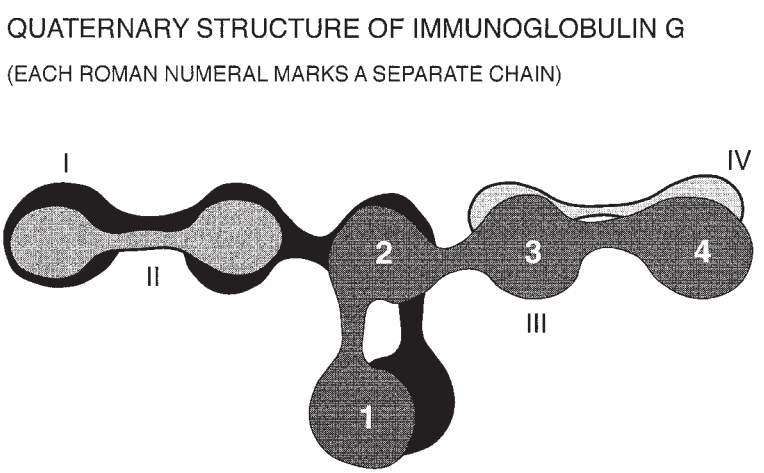


Figure 2–9
Quaternary protein structure. ➤ This is the entire conformation of a protein, which consists of two or more polypeptide chains. Single polypeptide chain proteins have no quaternary structure.

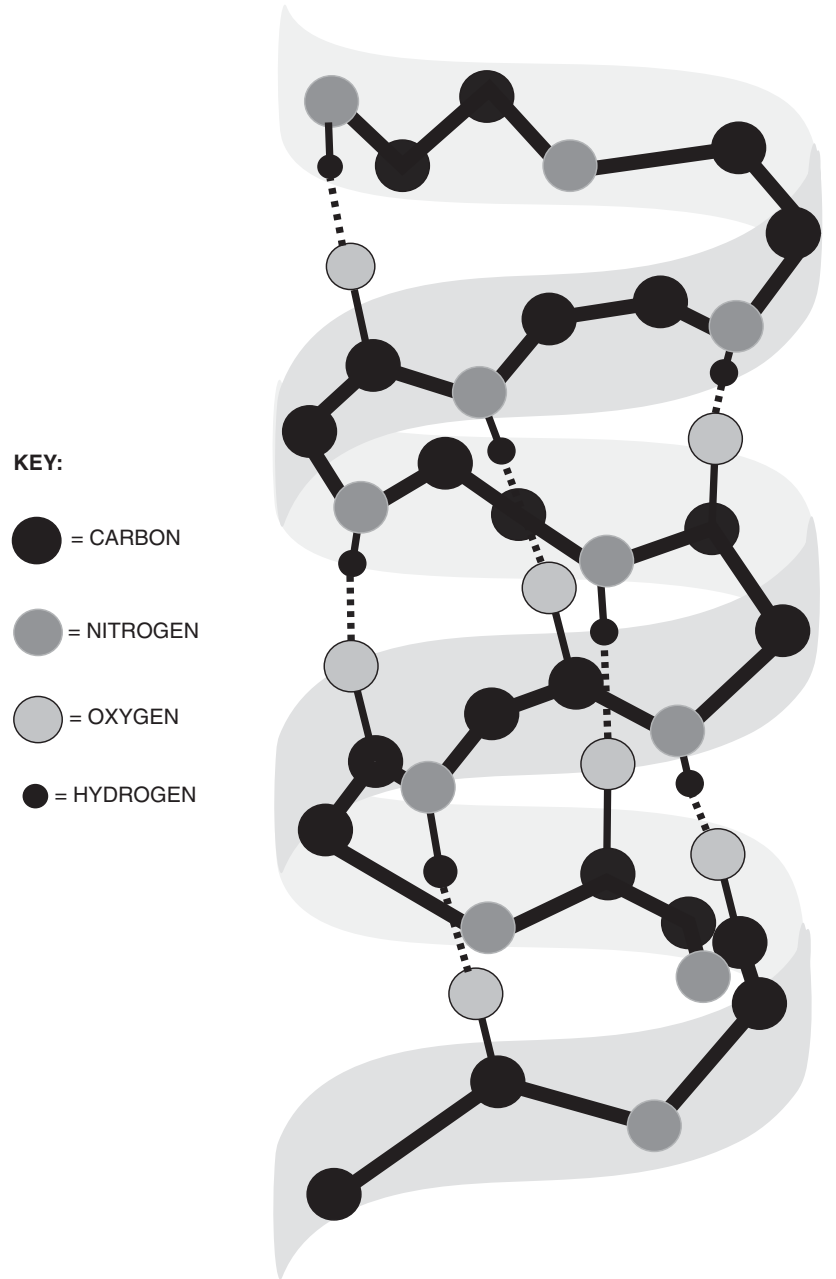


Figure 2–10
Alpha helical structure. ➤ This fourth type of secondary structure is usually an alpha helix in most cellular proteins. An alpha helix is defined as having a 1.5-angstrom rise for every 100^0 of rotation. The dotted lines represent hydrogen bonds. The figure is not to scale. Rhodopsin is an example of an ocular protein with considerable alpha helical structure. (Redrawn from Mathews CK, van Holde KE: *Biochemistry*. Redwood City, CA, 1990, Benjamin/Cummings.)

Crystallins

CHARACTERISTICS

The crystallins make up a group of proteins that are found in the epithelial and fiber cells of the ocular lens. They are water-soluble proteins whose major classification was originally made because of their relative ability to migrate in an electric field (i.e., *electrophoresis*). As such, they are broadly identified as *α, β, and γ-crystallins* in humans. A refinement in that classification (Harding, 1991) now divides the α-crystallins into αA and αB subunits while the β-crystallins are divided into β_H and β_L crystallins ($_H$ = heavy and $_L$ = light). The γ-crystallins consist of six human

TABLE 2–2 ➤ MOLECULAR WEIGHT RANGE OF SOME PROTEINS

Protein	Approximate Molecular Weight in Daltons	Function
Lysozyme	14,000	Enzyme (catalyst) in tears that lyses the peptidoglycan cell wall of Gram positive bacteria.
γ-Crystallin	20,000	Soluble protein found in lens fiber cells (that contributes to the lens structure).
Rhodopsin	40,000	Integral membrane protein of rod outer segments that mediates visual transduction.
Hemoglobin	64,000	Protein that carries oxygen to tissues.
two α-subunits each at	16,000	
two β-subunits each at	16,000	
Na^+K^+ATPase	304,000	Integral membrane enzyme that pumps sodium out of cells while pumping potassium inward.
two α-subunits each at	112,000	
two β-subunits each at	40,000	
Tropocollagen (type I)	400,000	Single unit of collagen structure found in corneal stroma and in many other ocular and nonocular issues.
Fibronectin	500,000	Connects cells with collagen.
two subunits each at	250,000	
Laminin	990,000	Connects cells with basement membrane.
three subunits each at	330,000	
H-M crystallin	>5,000,000	Aggregate in lens cell whose function is unknown. It may be a precataractous form.

subtypes known as γA to γF. The β- and γ-crystallins are actually considered to be members of the same family on a functional basis. Although crystallins had been long thought of as proteins unique to the eye, smaller amounts of crystallins have been found in nonocular tissues such as heart, brain, and lung (Horwitz, 1993; Harding, 1997). In fact, Wistow and Piatigorsky (1988) have hypothesized that the α-crystallins are related in origin to heat-shock proteins and a *Schistosome* (parasitic flatworm) egg antigen protein while β and γ-crystallins are related to a bacterial spore coat protein that binds calcium. In addition, other crystallin types are known, but are not found in the adult human. The role of these proteins, although water soluble, has been termed *structural* (Harding, Crabbe, 1984) meaning that they serve to maintain the elongated shape of lens fiber cells and, ultimately, the lens structure itself. This also means that these proteins affect the refraction of light that passes through the lens.

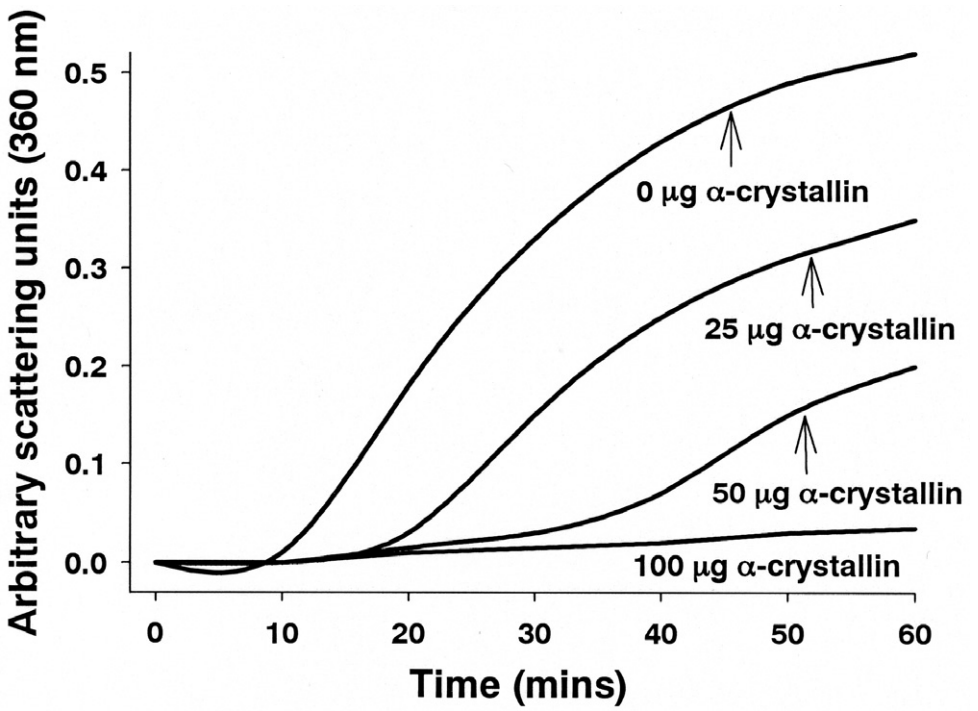


Figure 2–11
Decreased turbidity (light scattering) of β_L-crystallin in the presence of α-crystallin. ➤ The decrease in turbidity of the β_L-crystallin is a demonstration of the chaperone function of α-crystallin on the other crystallin. (Redrawn from the unpublished results of Derham and Harding as shown in Harding JJ, editor: *Biochemistry*. London, 1997, Chapman and Hall).

In addition, the α-crystallins have more recently been found to possess the function of *molecular chaperones* in which they help to maintain the normal molecular conformation of the other lens crystallins (Harding, 1997). This chaperone function is particularly important in the lens for the prevention of crystallin *aggregation,* which is considered a physical chemical event leading to the formation of senile cataracts. Figure 2–11 (Harding, 1997) demonstrates how *light scattering* (a phenomenon associated with the aggregation of β-crystallin) is inhibited in the presence of α-crystallin. The mechanism of chaperone function is not well understood, but involves repeated binding of the chaperone protein to its target protein when the latter is partially unfolded in order to induce proper refolding (Voet, Voet, 1995).

Crystallin concentrations in the lens cells are quite high with an average concentration that is about twice that of most other intracellular proteins (33% vs. 15%). The medical and scientific interest in crystallins has been dominated by their possible involvement in senile cataract formation. An examination of their chemical and physical characteristics is helpful in understanding their constitution (Table 2–3). In this table, acetylation (i.e., the addition of acetyl groups at the N-terminus) is a mechanism that prevents the cellular degradation of a protein. This means that the α- and β-crystallins, once synthesized, are considered to be stable. The primary structures of α- and β-crystallins are nonetheless altered, after synthesis, by the addition of phosphate groups (*phosphorylation*), the incorporation of sugars (*glycosylation* and *glycation*), *deamidation,* and possible *degradation* of the polypeptide

TABLE 2–3 ➤ CHARACTERISTICS OF HUMAN CRYSTALLINS

Properties and names	α	β	γ
Subtypes	αA, αB	β_L, β_H	γA, γB, γC, γD, γE, γF
Primary structure	N-terminus acetylated. Sequence varies and is modified.[1]	N-terminus acetylated. Sequence varies and is modified.[1]	N-terminus not acetylated. Sequence varies with subtype.
Secondary structure	α-helical and β-pleated sheets.[2]	β-pleated sheets.	β-pleated sheets.[3]
Tertiary structure	Possibly globular.	Probably globular.	Globular
Quaternary structure	Uncertain, consists of nascent and modified polypeptides each 20,000 daltons)	Large globe of polypeptides with a range of 23,000–35,000 daltons each.	None, it has a single polypeptide.
Molecular weight (daltons)	750,000[4]	50,000 (β_L) and 160,000 (β_H)[4]	20,000
Cysteine content	16 per 1000 residues[5]	25 per 1000 residues[5]	41 per 1000 residues[6]
pI[7]	~4.9	~6.4	~7.6
Relative lens occurrence[8]	~35%	~55%	~10%

[1]Proteins undergo post-translational modification (i.e., they are immediately modified after synthesis).

[2] Some studies suggest that α-crystallins also have α-helical structure (Farnsworth et al, 1993).

[3] See Figure 2–10.

[4]The actual molecular weight of the quaternary form of α-crystallin is in dispute (see Harding, 1997). Given a range of 600 to 900 kD reported by Bloemendal, 1977), an average of 750 kD was calculated. Harding (1997) lists the β-crystallins as having molecular weights of 40 kD (L) and 200,000 daltons (H).

[5]Data recalculated from Spector A, *Invest Ophthalmol Vis Sci* 25:130–146, 1984 from residues (i.e., amino acids or modified forms of amino acids after hydrolysis) per 1000 residues using an average amino acid molecular weight of 125.

[6]Data recalculated from Summers LJ, et al, *FEBS Letters* 208:11–16, 1986 as in [5] for one type of human γ-crystallin (γB, formerly known as γ1–2).

[7]*pI* is the pH at which the number of positive and negative charges on a protein are equal. The pI indicates the charge characteristics of a specific protein and can be used to separate proteins. The pIs given are approximate due to the existence of more than one subtype.

[8]Values are percentages of crystallins in whole lens. Exact percentages vary from species to species. α-Crystallin is the only crystallin type found in the lens epithelium. All three types occur in lens fiber cells (see Harding, 1981).

chains themselves. The special reactions involved with glycation will be considered in Chapter 4 in relation to diabetes.

The secondary structure of all human crystallins is characterized predominately by the existence of β-pleated sheets (see Figure 2–7 and Figure 2–12) although α-crystallins may have some helical structure (Farnsworth, et al, 1993). The β-pleated sheets of γ-crystallin consists of

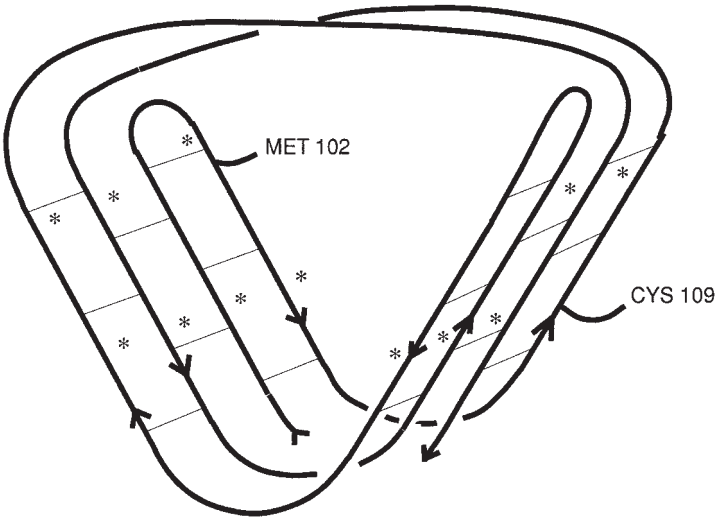


Figure 2–12
A domain of gamma crystallin (approximately one-half of the molecule). ➤ A domain is a portion of a protein that may contain one or more secondary structures. Here are seen two β-pleated sheets formed in a V-shape with hydrogen bonds (*thin lines*) connecting the polypeptide chains. There are two wide random turns at the top of the structure. Each asterisk indicates the presence of a hydrophobic amino acid. Methionine (#102) and cysteine (#109) are potential locations of oxidation of the protein. Oxidation can promote crystallin aggregation. (Redrawn from Summers et al, 1986)

rows of the same polypeptide chain, which run antiparallel to each other and are held together by hydrogen bonds (the thin lines in Figure 2–12). These β-pleated sheets also incorporate a number of hydrophobic amino acids in each sheet (shown by asterisks in the figure). Two sets of these sheets (referred to as motifs) are aligned in a V-shape to form a hydrophobic globular structure known as a domain (as previously mentioned for immunoglobulins). There are two domains for each γ-crystallin (tertiary structure), which, are remarkably water soluble although they also possess considerable hydrophobicity! This means that they are water soluble on their surface, but largely hydrophobic interiorly.

β-crystallins are thought to have a similar conformation. These secondary structures, therefore, determine the globular nature of all three crystallins (i.e., their tertiary structure). The α-crystallins, although incorporating β-pleated sheets, have not yet had their structures adequately described due to their complexity. However, they may also be globular. Several structures have been proposed such as the one in Figure 2–13. The structures are potentially important in determining lens fiber cell architecture and in understanding how cataract formation occurs.

The quaternary structure of α- and β-crystallins is often simply called an *aggregate*. This means that the final shape is determined by the binding of many polypeptide chains. In the case of α-crystallins, some 40 different subunits have been described, which consist of the primary gene products αA and αB as well as their post-translational modified forms (Harding, 1997). The modifications occur with aging (see following section). The αA form is an acidic polypeptide while the βA form is basic. These polypeptides are assembled in undefined combinations of the numerous kinds of chains that make up the native quaternary structure.

The number of polypeptides in β-crystallins is also complex. This is partly due to the revised consideration of β-crystallins as being included with γ-crystallins as a structural family (Harding, 1997). Strictly speaking, however, β-crystallins are divided into two groups by gel column chromatography: β_H (molecular weight approximately 160,000) and β_L (molecular weight approximately 50,000). Recent studies suggest that there are two β_L subfractions as well as a β_S fraction (with the latter existing as a monomer and now known incredibly as γ_S). Berbers, et al (1982) identified six different polypeptides as members of the β-crystallin

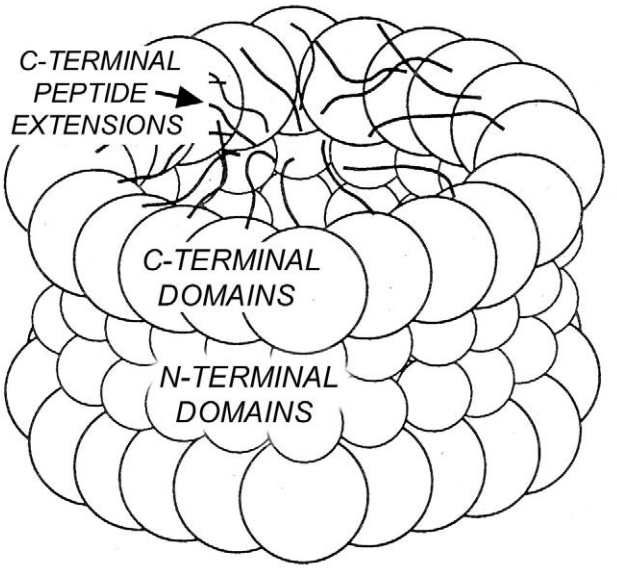


Figure 2–13
One of several proposed structures for α-crystallin. ➤ The model shows two bound rings of crystallin subunits laid on top of each other. The large subunit spheres are hydrophilic C-terminal domains while the small subunit spheres are hydrophobic N-terminal domains. Extending from the C-terminal domains are C-terminal peptide extensions that cover the central cavity where chaperone activity may take place. (Redrawn from Carver JA, Aquilina JA, and Truscott RJW: A possible chaperone-like quaternary structure for a-crystallin. *Exp Eye Res* 59: 231–234, 1994.)

family that are the primary gene products. Post-translational modifications augment this number of participants in β-crystallin aggregates so the exact compositions of these aggregates remain undefined. Life is indeed complicated for the lens protein chemist!

The crystallins account for about 90% of all soluble lens proteins. In the lens relative percentages of the crystallins, among each other, are: α-crystallins, 35%; β-crystallins, 55%; and γ-crystallins, 10% (Bours, Hockwin, 1976). It should be noted that α-crystallins are the only crystallin type found in the lens epithelium. All three lens crystallins occur in lens fiber cells.

THE AGING PROCESS AND EFFECTS ON CRYSTALLINS

As individuals age, crystallins are observed to undergo several biochemical transformations (Hoendess, Bloemendal, 1981; Lerman, Borkman, 1976). The transformations are significant inasmuch as lens proteins are not metabolically renewed (turned over) in lens fiber cells. These processes may be described as *phosphorylation*, *disulfide bond formation*, *deamidation*, and *peptide bond disruption*. *Glycosylation* (the enzymatic addition of sugar molecules) is not an age related change (Voet, Voet, 1995). However, *glycation* (the nonenzymatic addition of sugar molecules) is age related. Glycation will be considered in Chapter 4.

Phosphorylation is the process of adding phosphate groups most often to serine amino acids. Chiesa, et al (1989) found that phosphorylation of the αA chain of α-crystallin was maximal in mature lens fiber cells while phosphorylation of the αB chain was minimal in mature lens fiber cells. In general, though, the total amount of phosphorylated chains of α-crystallin is increased as lens fiber cells age. This addition amplifies the negative charges and potential energy of a protein. Although these observations have been made, no specific role for the phosphorylation of crystallins has been demonstrated (Harding, 1997). Phosphorylation has only been observed for α-crystallins and one of the β-crystallin polypeptides (Berman, 1991).

Disulfide bond formation (*crosslinking*) between different crystallin polypeptides has been a long studied phenomenon. It is an oxidative

process and it is well established that the formation of such crosslinking increases with aging (Harding, 1991; Spector, 1984). The cysteine content of the various crystallins is important inasmuch as each cysteine group is a potential source of molecular bridging or aggregation via disulfide bonding. In Table 2–3, under cysteine content, the γ-crystallins form the most likely crystallins for disulfide bond formation.

Deamidation is the loss of an amide group from Asn and Gln by oxidation to convert those amino acids to their corresponding dicarboxylic acids (Asp and Glu). It has been stated that this process may be an artifact of crystallin preparation. Some investigators, however, have identified it as part of the lens aging process. Takemoto (1999), for example, reported that there was increased deamidation of Asn-101 from αA crystallin in the normal human lens during aging.

Peptide bond disruption has been demonstrated in both β and γ-crystallins in human lenses with aging (Srivastava, 1998; Srivastava, Srivastava, Harrington, 1999; Lampi et al, 1997; Lampi et al, 1998). In the case of β-crystallins, the majority of peptide bond breakage occurred near the N-terminal region. However, the majority of peptide bond breakage occurred near the C-terminal region with γ-crystallin. Such peptide bond breakage suggests that an endopeptidase has catalytic access to these proteins over time.

CATARACT FORMATION

The formation of senile cataracts has been under investigation for a number of years, but significant progress has only taken place in the past 20 years. Observations about crystallins that have been made in the normal aging lens reveal changes that parallel, somewhat, the observations made on crystallins in the cataractous lens. The most general effect is the change in the amounts of soluble and insoluble lens proteins (Figure 2–14). In both normal and cataractous lenses both water soluble and insoluble proteins increase with age. The obvious difference between normal and cataractous lens proteins, however, is the larger increase in concentration of insoluble proteins with a concomitant decrease in concentration of soluble proteins in the cataractous lenses after an average age of 50 (Spector, 1984). In the normal aging process, crystallins of the three major types become incorporated somewhat into the insoluble fraction of lens proteins, presumably by disulfide bonding of their cysteine amino acids as well as by other crosslinking. Of the insoluble proteins, only about 1% are the normally insoluble proteins of the cell plasma membranes. However, with advancing age this insoluble fraction reaches a high amount of 50% or more of all the lens proteins in an individual who is 80 years old. It is known that several high molecular weight aggregates (HM) of crystallins can be isolated from the human lens with advancing age. These aggregates are termed: HM1, HM2, HM3, and HM4. HM1 and HM2, with molecular weights in excess of one million daltons, are soluble crystallin complexes. Although evidence has suggested that these fractions may be artifacts of preparation (Harding, Crabbe, 1984), nonetheless the aggregates occur more often in older lenses and then in the interior sections of those lenses.

The HM3 and HM4 aggregates, moreover, are only found in cataractous lenses. HM3 is insoluble and requires a strong denaturing agent such as guanidinium chloride for solubilization. This agent promotes unfolding and dissociation of the aggregate. HM3 is composed of

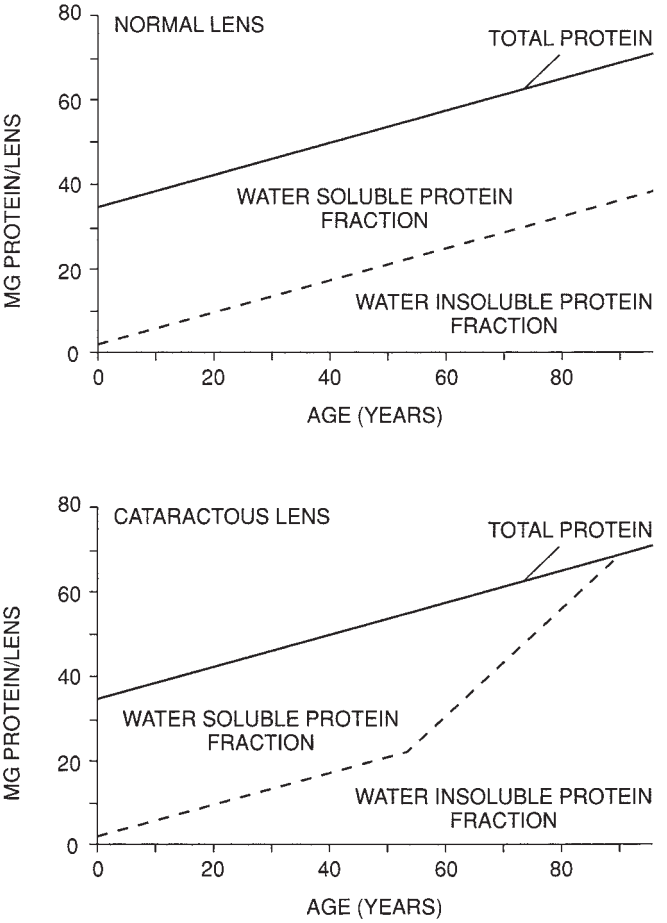


Figure 2–14
Graphs of protein concentrations in normal and cataractous lenses. ➤ The areas between the lines represent the amounts of soluble and insoluble protein (respectively) while the top line indicates total protein. (Adapted from Spector A, 1984)

a mixture of crystallins as well as a noncrystallin 43,000-dalton protein, a polypeptide located on the inner face of the plasma membranes of lens fiber cells. Spector (1984) has suggested that a 26,000-dalton intrinsic membrane protein may also be part of the HM fractions in cataracts. HM3 has a molecular weight range between 2 and 33 million daltons, a very large molecular species (Harding, Crabbe, 1984). HM3 is held together by disulfide bonds and is associated with cortical cataracts.

Fraction HM4 is composed of crosslinks that are not disulfide bonded and occurs exclusively in nuclear cataracts. HM4 is also insoluble and requires a denaturing agent for solubilization. This fraction is associated with the dark color of nuclear cataracts and, until recently, the source of the color was speculative, (Harding, 1981; Aquilina, et al, 1999). It is an important observation that the cataract formation associated with these fractions cause both light scatter as well as light absorption (Harding, 1991). This twofold component of cataract formation represents a considerable severity for the patient inasmuch as light scattering causes both the blurring of images as well as light glare (Bettelheim, Chylack, 1985). The properties of these HM fractions are summarized in Table 2–4.

It has been hypothesized that senile cataracts take place because of growing HM fractions that form at the inner layer of the lens cell plasma membranes and grow into the cell cytoplasm. The protein complexes continue to grow as the lens ages by forming either disulfide or other

TABLE 2–4 ➤ PROPERTIES OF HM AGGREGATES OF CRYSTALLINS

Fraction	Composition	Molecular Weight (10^6 d)	Solubility	Occurrence
HM1[1]	α-crystallin	>1x10^6	soluble	all
HM2[1]	α, β, γ-crystallins	>1x10^6	soluble	all
HM3[1]	α, β, γ-crystallins + 43 kD protein[3]	2–33x10^6	insoluble	cataractous (cortical)
HM4[2]	α, β, γ-crystallins + 43 kD protein[3]	>5x10^6	insoluble	cataractous (nuclear)

[1]Disulfide linked.
[2]Not disulfide linked, but containing one or more light absorbing (i.e., colored) components.
[3]May also bond to a 26 kD protein.

covalent bonds (Figure 2–15). This may be seen in A of the figure. This is the stage to which nuclear cataracts form, but with nondisulfide bonds. An important difference with cortical cataracts, however, is that the strain produced in the cell membrane by the growing HM aggregates, as seen in B of the figure, may lead to membrane rupture (arrow). The resultant cellular debris for the cortical cataracts would include membrane fragments such as the micellar form in C to which are attached HM crystallin aggregates. These would, at least partially, constitute the fragmented or globular formations, which are described clinically.

The reason why such an aggregation of disulfide bonded and other crosslinked crystallins occurs is not certain. Nor is it certain where the degree of formation must extend to begin membrane disruption. Some older patients are seen who have limited lens fiber cell disruption and yet have clinically clear lenses. In the case of disulfide bonded crystallins, Garner, Spector (1980) have shown that there is a difference in the oxidation state of both methionine and cysteine in the crystallins and the 43,000 dalton protein of normal 60 to 65-year-old human lenses vs. those that are cataractous. These investigators suggested that, in the noncataractous state, the sulfur containing amino acids are buried in normal conformation of crystallins and are prevented from oxidizing (that is, forming sulfoxide and disulfide bonds). Minimal oxidation of exposed groups, however, may cause conformational changes in the crystallins that expose other oxidizable groups, which, in turn, bring about aggregate formation. Glutathione (a tripeptide: γ-Glu-Cys-Gly), is thought to protect crystallins from crosslinking by binding to such exposed groups.

Evidence indicates, however, that its function may be overwhelmed in cataract formation (Spector, 1984). This crosslinking would apply only to cortical aggregates that form cortical cataracts and is indicated in Figure 2–16. The process of progressive oxidation might be introduced by extralenticular hydrogen peroxide in the aqueous (Spector, 1984).

The cause for nuclear cataracts is more cryptic and the nature of the color in brunescent nuclear cataracts, mentioned previously, has remained speculative until very recently. A review of some of the earlier studies is helpful in understanding the problem. The tryptophan amino acid components of crystallins had been suspected to be associated with nuclear cataracts (Harding, Crabbe, 1984). Normally, there is an increase in yellow coloration of the lens, which augments with age and which had been thought to be associated with changes in the tryptophan components of crystallins. Radiation in the range of 300 to 400 nm (including sunlight) has the potential of being cataractogenic since

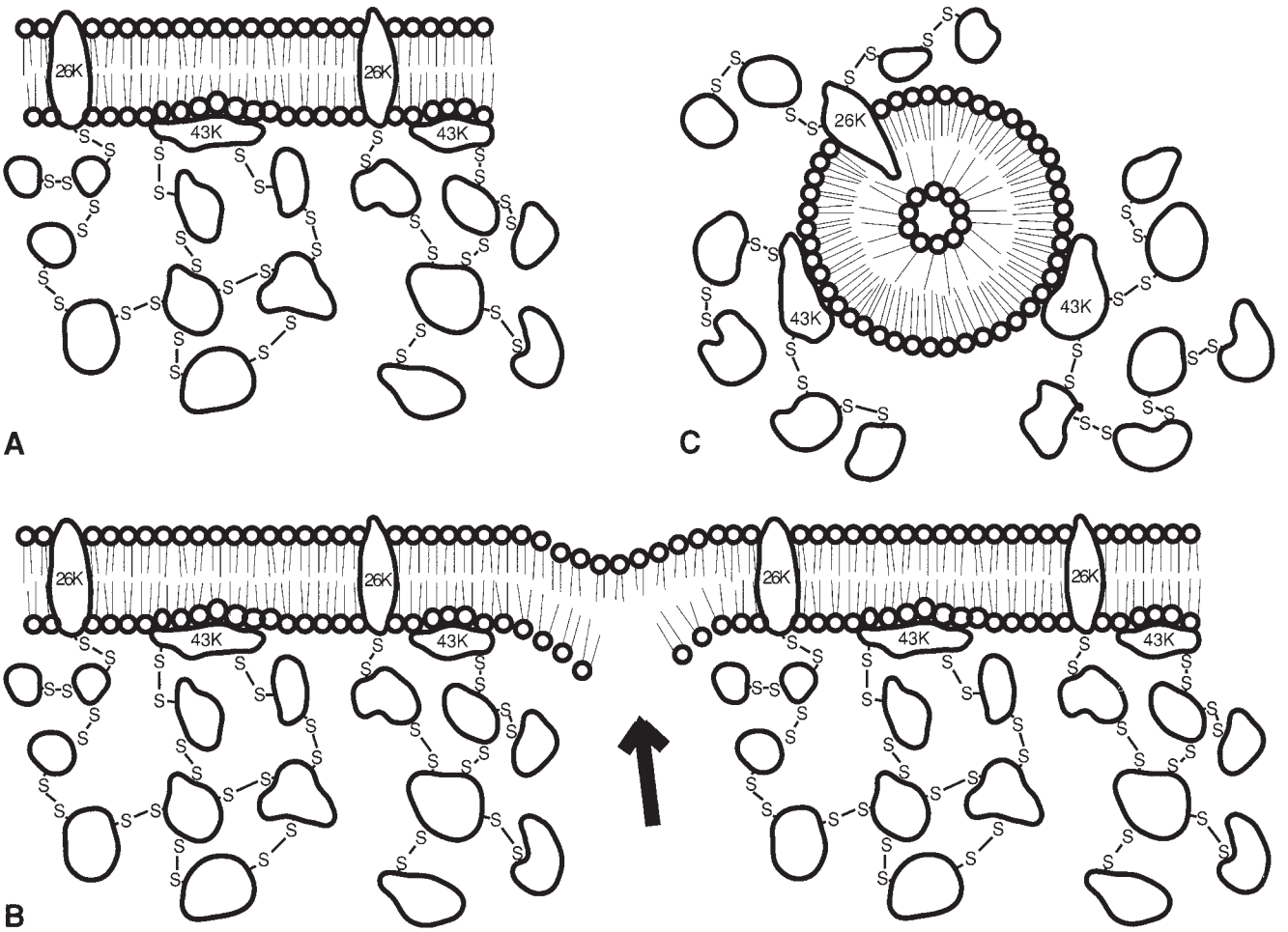


Figure 2–15
The aggregation of lens proteins proposed to occur in senile cataract formation. ➤ Both nuclear and cortical cataracts aggregate as at (A). The aggregated crystallins (*white blobs*) are seen bound to each other by disulfide (or other) bonds. They are also bound to 43K and 26K proteins at the plasma membrane of the lens fiber cells. In cortical cataracts, the aggregation proceeds to cause a rupture in the membrane (*arrow* at B), which destroys lens fiber cells and produces cellular debris as seen at (C). (Adapted from Spector A, 1984)

the lens absorbs these wavelengths. As a result of this, a possible connection between such ultraviolet light and tryptophan alteration was suggested by Pirie (1971, 1972) who reported that ultraviolet light was able to oxidize tryptophan to N'-formylkynurenine in proteins in vitro (Figure 2–17 A and B). She thought that such altered amino acids in crystallins may lead to the yellow to brown coloration in nuclear cataracts. It was also indicated that this process might eventually lead to the nondisulfide crosslinking that is characteristic of HM4 aggregates found in nuclear cataracts. Some candidate substances, found in lenses, which have been proposed to link tryptophan with nondisulfide crosslinking are anthranilate and β-carboline (Garcia-Castineiras, Dillon, Spector, 1978; Truscott, Faull, Augusteyn, 1977) (see Figure 2–17 C and D). Subsequently, van Heyningen (1973) demonstrated that hydroxykynurenine instead of kynurenine was the metabolite that could be linked to the interference of light passing through the lens acting as a UV filter.

Although there has been considerable confusion about the role of hydroxykynurenine in subsequent years, Wood and Truscott (1993),

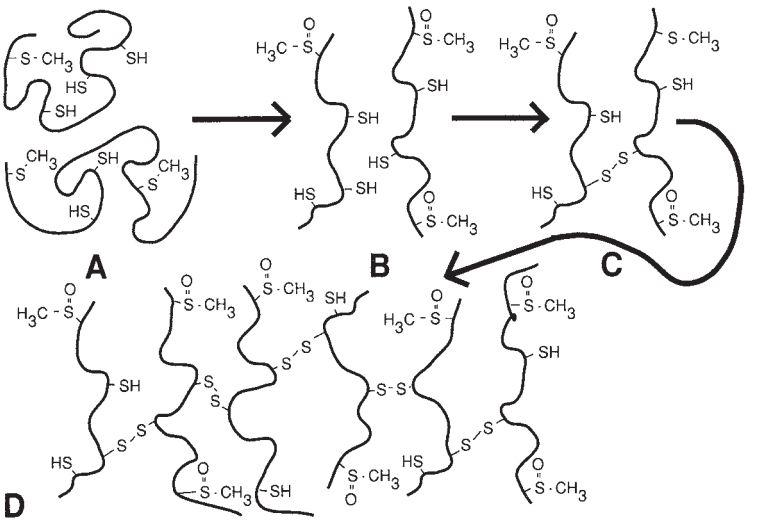


Figure 2–16
The progressive oxidation of lens crystallins. ➤ Some investigators believe that the normally folded (i.e., globular) form of crystallins protect the oxidizable amino acids: methionine (-S-CH_3) and cysteine (-SH) as seen at (A). In the course of aggregation, methionine is first oxidized to SO-CH_3, possibly by hydrogen peroxide in the adjacent aqueous. This action could partially unfold the protein as seen at (B). Subsequently, the exposed cysteines would oxidize, as -S-S-, forming disulfide bonds with other crystallins and membrane proteins in a series of chain reactions as seen at (C) and (D).

demonstrated that 3-hydroxykynurenine (3OHK) and its glucose bound analogue are produced by the catabolism of the amino acid tryptophan in the lens epithelial cells and, possibly, the most outer lens fiber cells. The metabolic pathway of tryptophan catabolism is shown in Figure 2–18. The normal function of these metabolites is to act as filters for UV light (300 to 400 nm) in order to protect the retina from UV damage (Dillon, 1994). Chen, et al (1997) found that at least the N-terminal domain of α-crystallins was involved in the formation of colored compounds that are associated with nuclear cataracts. Almost simultaneously, Aquilina,

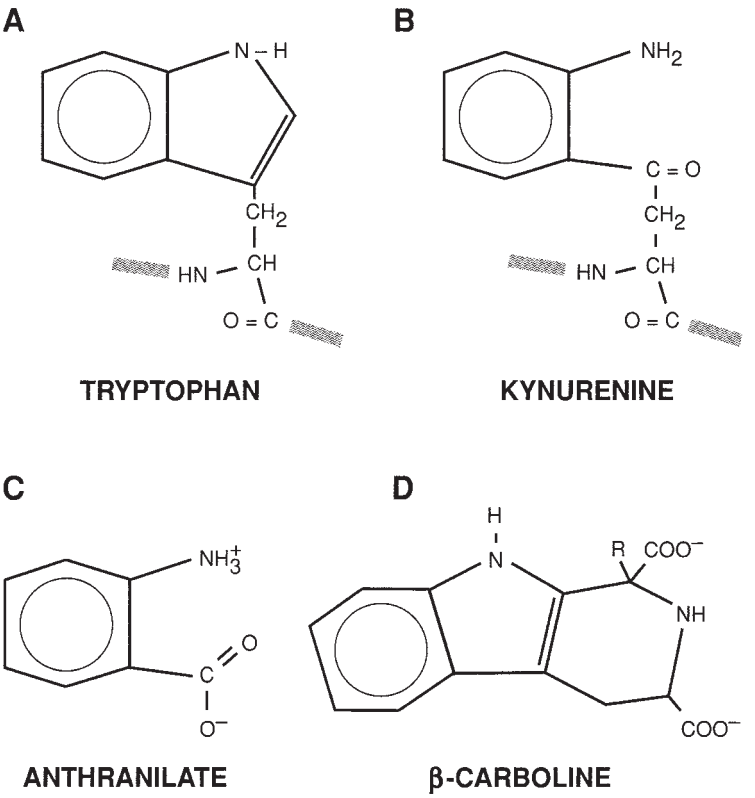


Figure 2–17
The oxidation of the amino acid tryptophan to kynurenine can be obtained in *in vitro* proteins by ultraviolet light (as in A and B). ➤ Anthranilate, a breakdown product of kynurenine (C) has been found in cataractous lenses. However, no relationship has been established between the possible formation of kynurenine itself in the lens and any lens coloration. β-Carboline, an example of one of many forms, is shown in (D). It has also been found in the lens and was suggested to be a fused ring form of tryptophan and another amino acid in crystallins, which produces yellow or brown coloration there. This is no longer thought to be true.

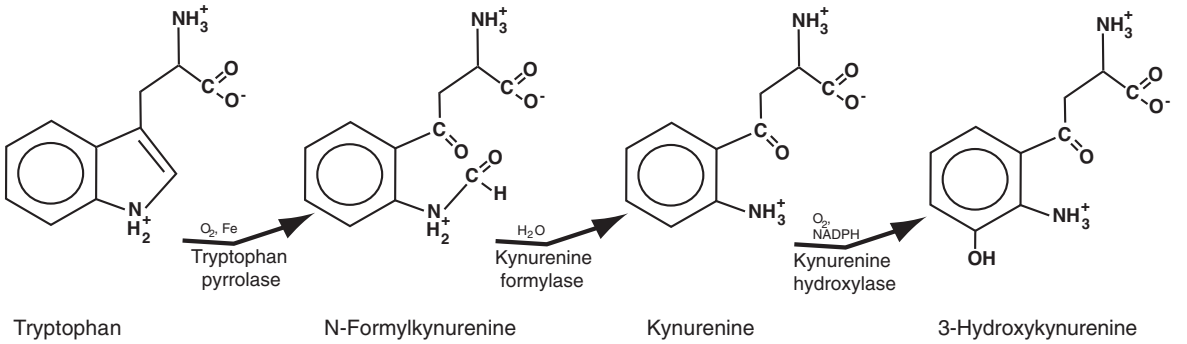


Figure 2–18
The metabolic pathway of tryptophan catabolism. ➤ Tryptophan is broken down to 3-hydroxykynurenine by means of three enzymes, which require oxygen, iron, and a coenzyme known as reduced nicotinamide dinucleotide phosphate (NADPH, see Chapter 3).

et al (1997) suggested that 3OHK, following oxidation, can form reactive intermediates that covalently bind with those lens proteins. More recently, the same group (Aquilina, Carver, Truscott, 1999) showed how 3OHK could be oxidized to an o-quinonimine that reacted with two Gly-Lys peptides to form the colored product quinilinobenzoxamine (QBA). QBA is, accordingly, a proposed covalent crosslink for nuclear cataracts, which may occur at the N-terminus of crystallins. An abbreviated pathway for this reaction is shown in Figure 2–19. Therefore, after many years of searching, it appears that the origin of the colored, nondisulfide crosslink of nuclear cataracts may now be known.

Rhodopsin and Cone Pigment Proteins

There was a time when virtually all ocular biochemistry was centered on the subject of rhodopsin. This was the case because rhodopsin is at the heart of the process of visual transduction, that is, the conversion of light energy into electrical signaling, which the brain interprets as sight. It is now known that there is a much more complex process involved in visual transduction and that rhodopsin only initiates that process. Here, the focus will be on rhodopsin and cone pigment proteins per se (the visual pigments). In Chapter 7, the connection of rhodopsin with visual transduction will be described in detail. Rhodopsin is a protein that was first described by the German scientist Franz Boll in 1876 as a red-purple visual pigment. Afterwards, Willy Kühne (a contemporary of Boll) described the pigment as "regenerable" in a light-dark cycle. He extracted the pigment from the retina and was the first to describe its spectral properties. In more recent times, the American scientist George Wald showed the relationship of vitamin A to rhodopsin (Bridges, 1970).

Rhodopsin is an *intrinsic membrane protein* found in the discs and, to a lesser extent, the plasma membranes of the rod outer segments of photoreceptor cells in the retina. As an intrinsic membrane protein, its structure extends completely through the disc membrane (Figure 2–20). Rhodopsin, like many membrane proteins, is analogous to a boat floating in a sea of phospholipids and those lipids that constitute the disc and plasma membranes of the rod photoreceptor. There are some constraints to this "floating," however. The protein is always oriented such that its N-terminal end is facing either the intradiscal space on the disc membrane or the interphotoreceptor matrix (extracellular space between

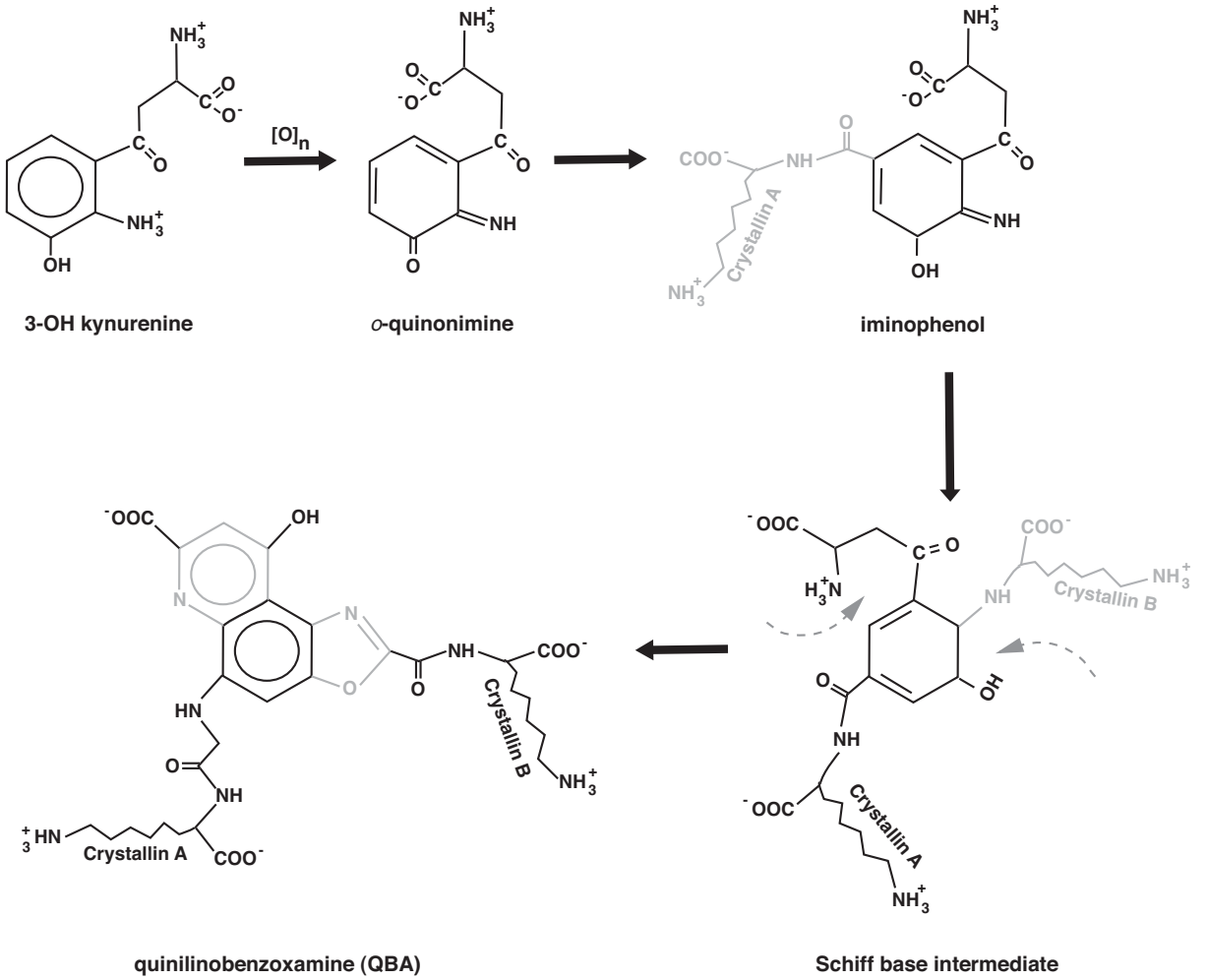


Figure 2–19
The formation of nondisulfide crystallin crosslinks by 3-OH kynurenine (3-OH K). ➤ 3-OH K is oxidized to the corresponding ortho-quinonimine. The first crystallin binds to the closed ring to form an iminophenol. Subsequently, the second crystallin binds to the ring and forms an intermediate Schiff base. Finally, the bridged structure is stabilized by forming two additional ring structures to give quinilinobenzoxamine or QBA.

photoreceptors) on the plasma membrane. This is indicated by the tail-like appendage on each rhodopsin molecule in Figure 2–20. This orientation is necessary so that the protein can maintain its functional role. Rhodopsin molecules are further constrained from migrating around the ends of the discs by the proteins: peripherin/rds-Rom 1, originally called "peripherin," and ABC/RIM (or simply "rim protein") present at the apex or rim of each disc (Molday, 1998). Peripherin is thought to contribute to integrity of the disc structure while the role of rim protein, as such, is not understood. However, the constraint of these proteins controls the population of rhodopsin molecules on each side of the disc. Rhodopsin has a molecular weight of approximately 41,000 and has conformational features as shown in Figure 2–21.

The N-terminal region of rhodopsin is located inside the disc and has two short-chain sugars (oligosaccharides) that are bound to Asn amino acids. These sugars anchor or orient the molecule and may stabilize the disc structure itself (Gordon, Bazan, 1997). The C-terminal

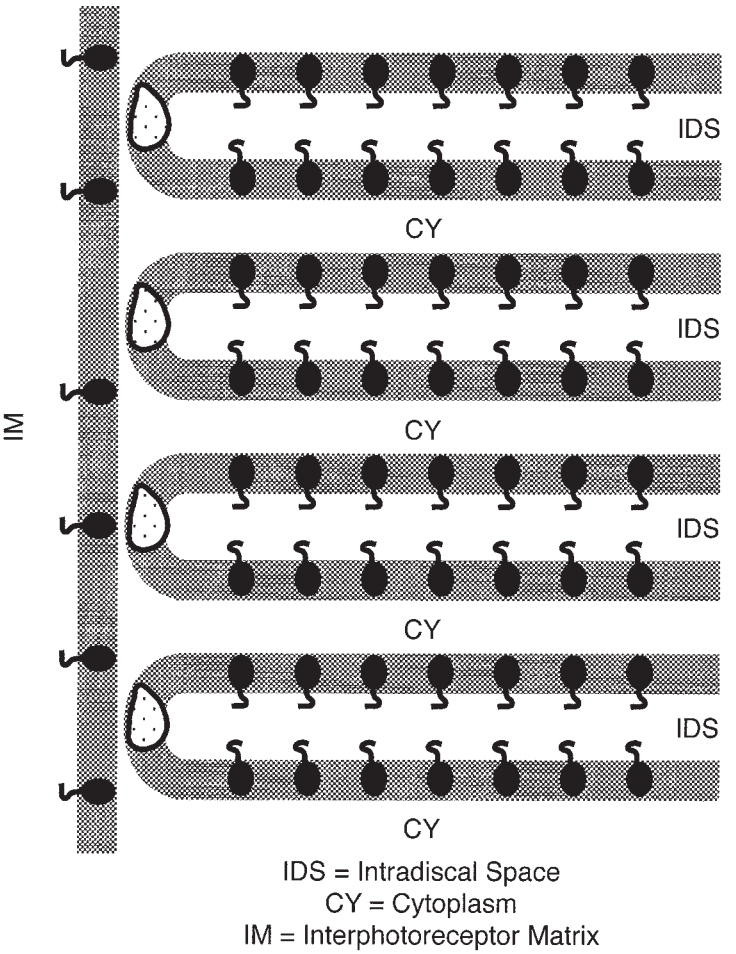


Figure 2–20
Partial diagrammatic cross section of a rod outer segment showing the placement of rhodopsin molecules (*black eggs with hooks*) in the discs and plasma membrane of the rod. ➤ The hooks represent sugar-containing N-termini of the molecules, which prevent molecular flip-flopping and maintain the orientation of the molecules. It is well known that the vast majority of rhodopsin molecules are in the discs. Proteins, such as peripherin/rds (located at each end of the disc [*speckled blob*]), prevent rhodopsin from migrating around the ends of the discs. (Adapted from Anderson RE: Biochemistry *of the eye*. San Francisco, 1983, American Academy of Ophthalmology.)

region contains several hydroxy amino acids, which can be phosphorylated. Phosphorylation acts as a mechanism for turning off the sensitivity of the activated protein to light as described in Chapter 7. The portion of the molecule that traverses the membrane consists of 7 α-helices (secondary structure), whose sequences (primary structure) are composed of substantial amounts of hydrophobic amino acids. Having α-helices that traverse membranes is a characteristic common to many intrinsic membrane proteins and these helices impart a strong association of the protein with the lipids that make up the membrane environment.

Rhodopsin has an additional, nonprotein component: vitamin A. This lipid soluble vitamin is contained within the membrane lipid-associated portion of the molecule (see arrow, Figure 2–21). The vitamin A component is bound to the protein at the amino acid lysine, #296 from the N-terminal end of the molecule. As part of the rhodopsin molecule, vitamin A is present as an aldehyde (known also as retinal) and consists of the 11-*cis* form (Figure 2–22). This form represents the most energetically favorable configuration for its confinement among the helices of the protein. The bond linkage of the aldehyde form with the protein is that of a *protonated (H^+ added) Schiff base*:

$$[\text{retinal} - \text{CH} = \text{NH}^+ - \text{protein}]$$

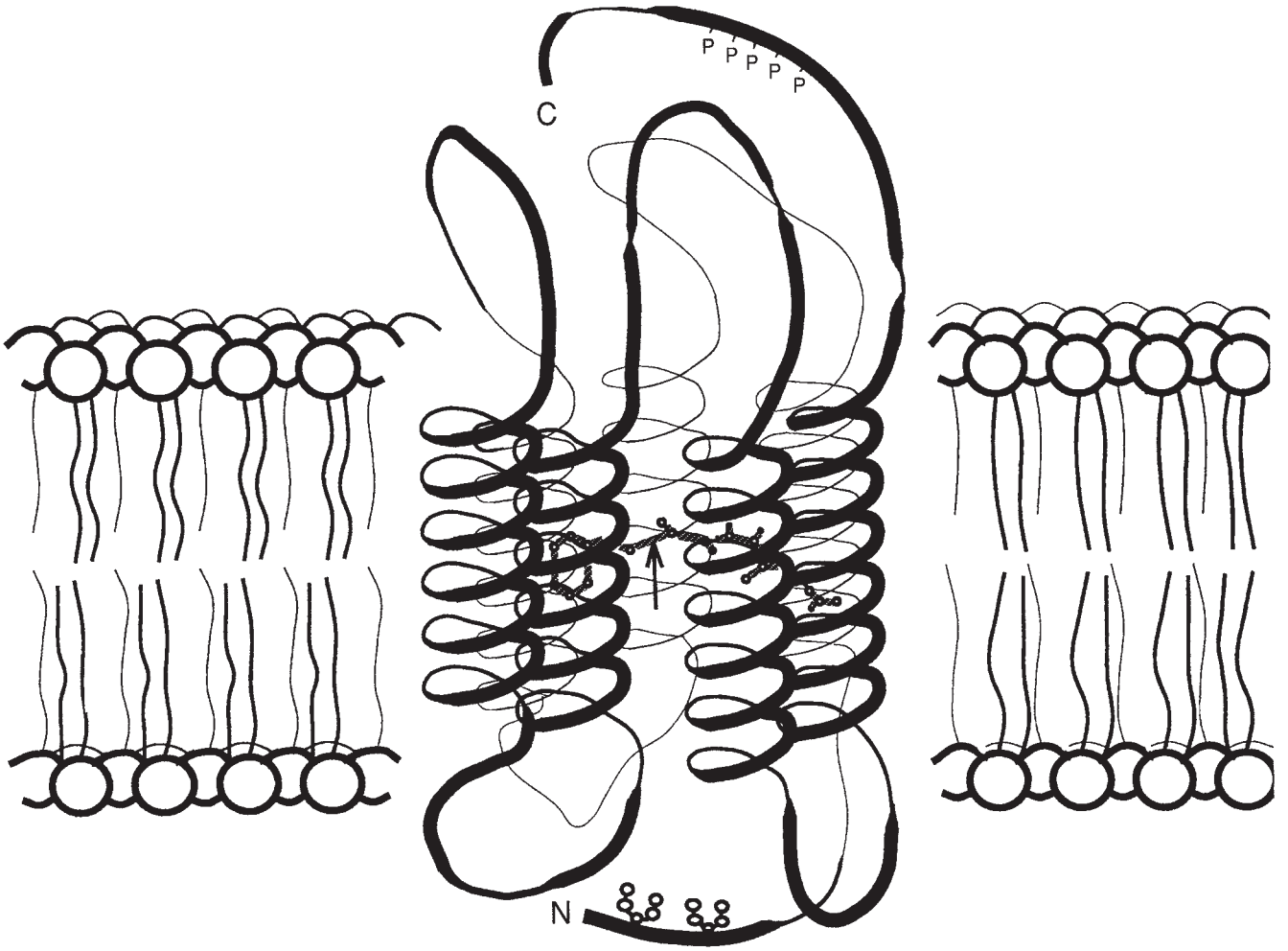


Figure 2–21
Representation of a rhodopsin molecule situated in a rod disc membrane. ➤ Note the presence of seven alpha helices in the area of the membrane, the 11-*cis* retinal buried within the helices (*arrow*), the small sugar chains at the N-terminus (*small spheres*), and the phosphorylated serine amino acids near the C-terminus (where the phosphate groups are located). (Adapted from Dratz EA, Hargrave PA: *Trends Biochem Sci* 8:128, 1983.)

A Schiff base has the characteristic of being less "permanent" or stable than many covalent bonds so that vitamin A can be more easily detached upon light stimulation.

Rhodopsin is referred to as a *holoprotein* when retinal is attached to lysine #296. In 1958, George Wald (1968) demonstrated that light isomerizes the 11-*cis* retinal group of rhodopsin to all-trans retinal (see Figure 2–22). This creates an energetically unstable molecule that rapidly proceeds through several intermediate forms: bathorhodopsin, lumirhodopsin, metarhodopsin I and II in which the Schiff base linkage is deprotonated and finally broken (see Figure 2–22). The completion of the reaction results in the release of vitamin A from the protein as all-trans retinal. The vitamin-detached protein then is called *opsin*. When any nonprotein portion of a holoprotein (such as rhodopsin) is released from its protein, the remaining protein portion is labelled an *apoprotein*. Opsin is, therefore, an apoprotein. The nonprotein portion, such as vitamin A, is termed a *prosthetic group*. Table 2–5 summarizes the properties of rhodopsin.

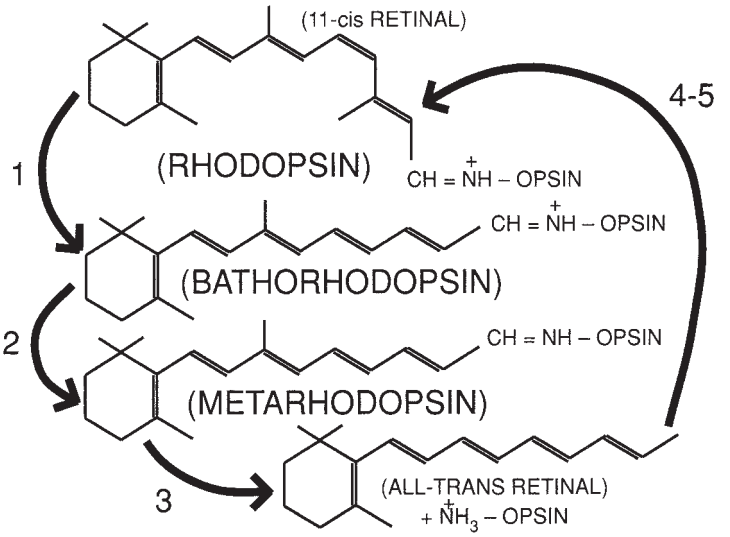


Figure 2–22
The conversion of rhodopsin to all-*trans* retinal and opsin. ➤ Rhodopsin is shown at the top with 11-*cis* retinal bound to opsin via a protonated Schiff base. The initial reaction (1), caused by a photon of light striking the molecule, is the conversion of 11-*cis* retinal to all-*trans* retinal while bound to the protein producing bathorhodopsin (a form in which the structure of retinal is strained). Three intermediate forms are then rapidly made in sequence (2): lumirhodopsin, metarhodopsin I, and metarhodopsin II (the last form shown). Each of these forms, with its own spectral properties, produce slightly different protein conformations (not shown). In the conversion of metarhodopsin I to metarhodopsin II, the Schiff base is deprotonated. Metarhodopsin II is also known as photoexcited rhodopsin and is further discussed in Chapter 6. Finally, the Schiff base is broken (3) with the release of all-*trans* retinal and opsin. Rhodopsin is regenerated in two stages (4–5). All-*trans* retinal is isomerized to 11-*cis* retinal by the protein enzyme: retinal isomerase (4) and the 11-*cis* retinal recombines with opsin (5).

It has been known for many years that rhodopsin also has certain spectral or light absorbing properties that are not part of the visual transduction process *per se*. Figure 2–23 shows the absorption spectrum of frog rhodopsin before and after exposure to light, that is, before and after the conversion of rhodopsin to all-trans retinal and opsin. This process is also called the *bleaching* of rhodopsin. Three peaks are seen. The α-peak (approximately 515 nm) is the absorption of 11-*cis* retinal

TABLE 2–5 ➤ PROPERTIES OF RHODOPSIN

Molecular weight	
holoprotein	41,399
apoprotein[1]	39,049
oligosaccharides	2,114
retinal	284
Number of amino acids	348
Helical content	~66%
Oligosaccharides (carbohydrates)	mannose and *N*-acetyl glucosamine[2]
Amino acid bound to retinal	Lys # 296
Amino acids bound to phosphate	Ser (near C-terminus)
Amino acids bound to oligosaccharides	Asp (near N-terminus)

[1]Less retinal and the oligosaccharides.
[2]See Chapter 3 for structures.
Adapted from Shichi H, 1983.

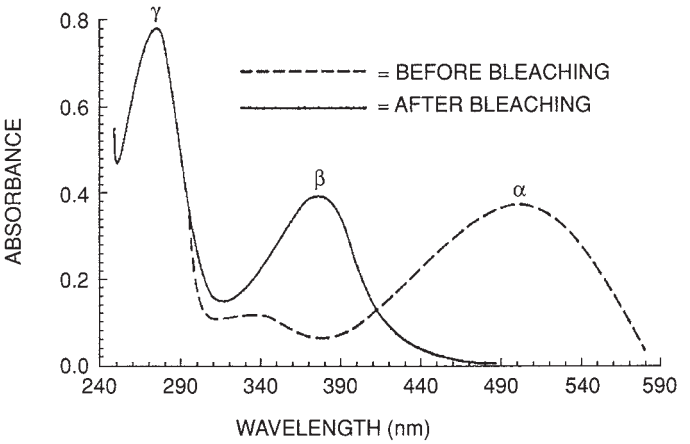


Figure 2–23
The absorption spectrum of rhodopsin and opsin. ➤ The spectra of intermediate forms are not shown. The γ-peak is characteristic of all proteins, while the β-peak is the absorption peak of unbound all-*trans* retinal and the α-peak is bound 11-*cis* retinal.

bound to opsin (as rhodopsin). The β-peak (approximately 375 nm) is the absorption of all-trans retinal, which is not bound to opsin. The γ-peak (280 nm) is the protein absorption peak common to both opsin and rhodopsin. The shifting of these absorption signals led investigators to discover the intermediate forms of rhodopsin and its final products in the bleaching process. Rhodopsin is able to absorb light by virtue of having that light raise the electronic and other energy levels inherent in the rhodopsin molecule. This is only possible at specified wavelengths of light and the relationship of absorption to the protein concentration can be expressed by an equation developed from Beer's law (Meites, Thomas, 1958):

$$A = abc$$

where *A* is the absorbance, *a* is the absorptivity, *b* is the light pathlength, and *c* is the *concentration*. The *absorbance* may be considered as the ratio of the light shone on a sample compared to the light that is transmitted through the sample. The *absorptivity* is a quantity that mathematically contains the physical-chemical characteristics of the sample and its surroundings. Absorptivity must be determined empirically (i.e., experimentally). For example, the molar absorptivity of rhodopsin = 40,000 cm^{-1} M^{-1}. The light pathlength is the distance that the light travels in passing through a sample and is usually 1 cm. This light passes through the sample in a special container known as a *cuvette*. The concentration of the sample is the most useful part of the equation inasmuch as it gives the amount of sample present in a given volume. Accordingly, one can determine the amount of rhodopsin present when the absorptivity is known together with the light path and the absorption. Absorption values are obtained in an instrument known as a spectrophotometer (Figure 2–24).

The conversion of rhodopsin to opsin is only the beginning of the visual transduction process. In Chapter 7 the remainder of the mechanism will be described. Rhodopsin is the visual transduction protein of rod photoreceptors whose role is principally that of visual sensation, particularly at lower light levels. Color vision, however, is realized by light

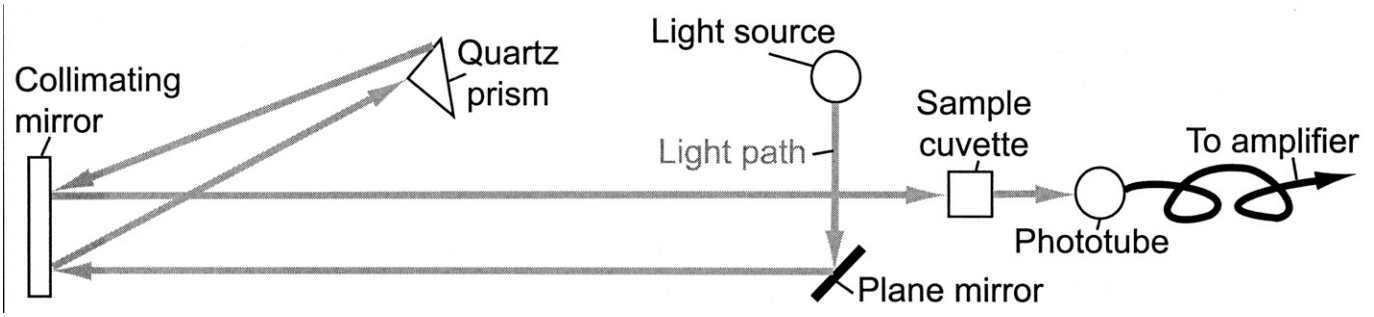


Figure 2–24
Simplified diagram of a spectrophotometer. ➤ In operation, light from a tungsten or hydrogen lamp passes through a quartz prism where it is broken up into its component wavelengths. The collimating mirror is used both to fix the light on the prism and to gather and focus light of a particular wavelength from the prism onto the sample cuvette (chamber). The collimating mirror and the prism are part of a device known as a *monochromator.* The light that passes through the sample is detected and amplified with a photomultiplier tube coupled to an amplifier.

transduction involving proteins in cone photoreceptors of which there are three types: blue, green, and red light absorbing.

These photopigments are maximally sensitive at wavelengths of 440 to 450 nm (blue), 535 to 555 nm (green), and 570 to 590 nm (red) as shown in Figure 2–25. Until recently, cone pigment proteins had not been as well characterized as rhodopsin due to difficulties of sampling the very small amounts of these proteins. The chromophore, which acts as the prosthetic group in these proteins, is also 11-*cis* retinal. However, the sensitivity of these cone visual pigments to light of specified wavelengths (essentially what we interpret as color) is controlled by varied amino acid environments around the 11-*cis* retinal and its protonated Schiff base linkage (Nathans, 1999). Two factors are important for variations in color discrimination: (1) modulation of the interaction between the protonated Schiff base with a counter ion present on one of the α-helices nearby; and (2) modification of the environment of the conjugated chromophore of vitamin A with neutral amino acids also present in the nearby α-helices (Figure 2–26).

For example, substituting Ser for Ala at position 180 in the sequence of the red pigment protein conveys greater sensitivity to red light. That is, there is a much greater likelihood that visual transduction will be initiated by red light when Ser is present rather than Ala at this

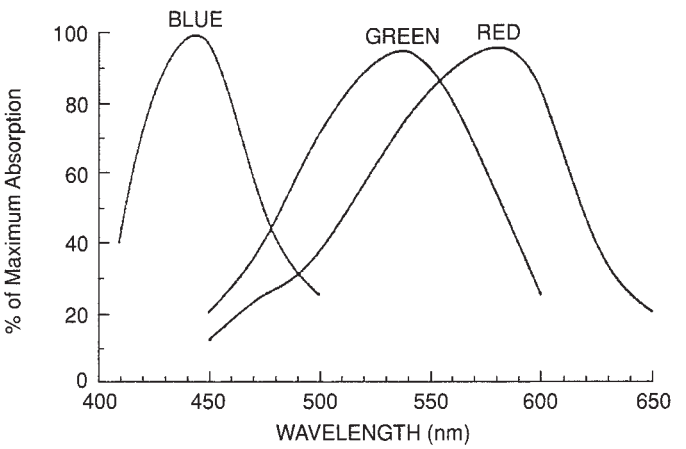


Figure 2–25
Absorption maxima of the blue, green, and red pigment proteins of the cones of the retina. ➤ These proteins bind to 11-*cis* retinal in the same manner as opsin does. However, the amino acid sequences vary from opsin to absorb light at different wavelengths. Genetic variations also occur as seen in Figure 2–26.

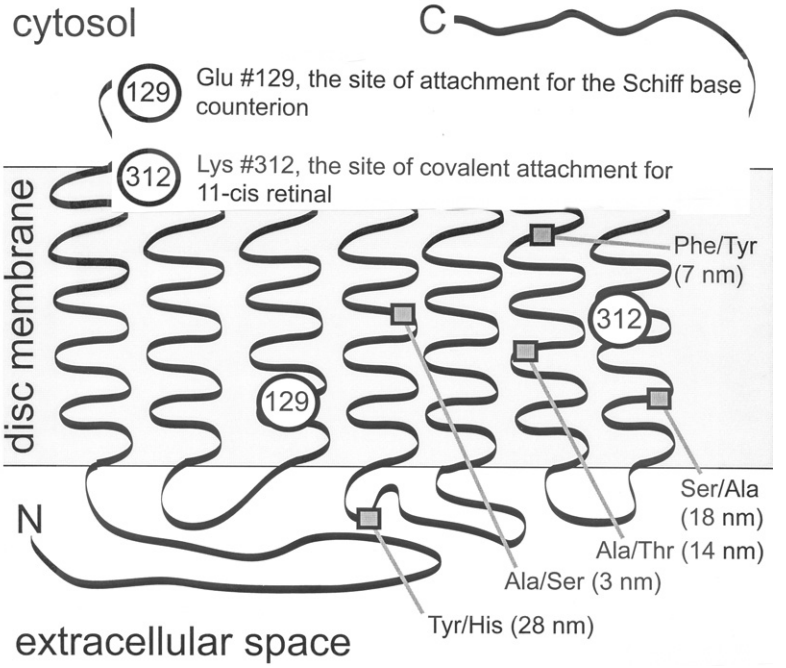


Figure 2–26
Diagram of a red or green cone pigment protein inserted into a cone membrane showing the site of attachment of the Schiff base counter ion and covalent attachment for the 11-*cis* retinal. ➤ Also illustrated are shifts in color vision produced by a substitution of certain amino acids (e.g., Thr for Ala to produce a shift of 18 nm). The figure was redrawn from Nathans J: The evolution and physiology of human color vision: insights from molecular genetic studies of visual pigments, *Neuron* 24:299–312, 1999.

position. This is accomplished by enhancing the process of removing the protonation on the Schiff base, which forms the bond between 11-*cis* retinal and its Lys on the protein. The process, which involves shifting the sensitivity by some 5 nm, is called *spectral shifting*. Shifts, which occur in the blue region (less than 500 nm), however, are not as well understood.

In color blindness or color sensation "abnormalities," male individuals inherit variations of red and/or green pigment genes arrayed on the X chromosome. This in turn produces cone pigments that are abnormal in 3% to 8% of the male population. The pigments are abnormal in the sense that their amino acid sequence varies along with their sensitivity to red and/or green light. Variations in only 15 different amino acids are responsible for such profound effects (Nathans, 1999).

Mucous Glycoproteins

Glycoproteins are proteins to which short chains of sugars (or oligosaccharides) are bound. Rhodopsin, previously considered, is an example of a glycoprotein. Such proteins are to be distinguished from *proteoglycans* in which the carbohydrates consist of at least one long polymer of alternating sugar-aminosugar units known as a glycosaminoglycan (see Chapter 4). Glycoproteins have a variety of roles that include structure orientation, immunological recognition, and biological lubrication. In the precorneal tear film, the mucoid layer (and to a lesser extent, the aqueous layer) contains mucus glycoproteins (also known as *mucins*). These proteins are similar to the mucins that are found in other mucus secreting tissues such as the gastrointestinal tract, nasal passages, and trachea. Mucins have larger amounts of carbohydrates than serum and plasma membrane glycoproteins. However, they are still considered glycoproteins as they are composed of short-chain sugars. The carbohydrates are attached in numerous short chains along the length of the

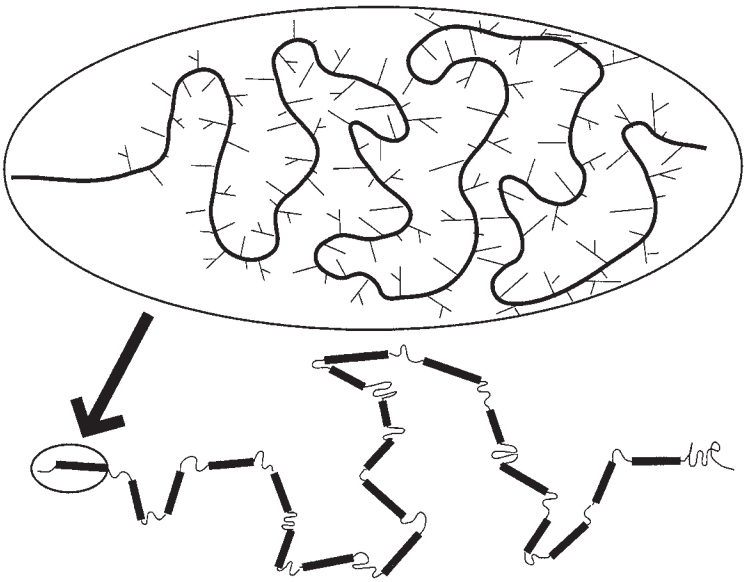


Figure 2–27
Molecular diagram of a mucin protein of the precorneal tear film. ➤ The top portion shows an enlargement of a section of the molecule. Segments of the twisted polypeptide chain have short lengths of carbohydrate chains known as oligosaccharides bound to them (as indicated by the *thin lines*). There are also intervening segments with no oligosaccharides. (Adapted from Berta and Török, 1986)

polypeptide chain unit as shown in Figure 2–27 (top). Each peptide chain unit, that contains these carbohydrates, is separated by a small length of peptide chain having no carbohydrates. This results in a heterogeneous molecule as shown at the bottom of Figure 2–27. Berta and Török (1986) stated that, typically, there is a significant proportion of the amino acid Pro in these proteins and this seems to support the random nature of their structure. Representative molecular weights of nonocular mucins are 0.5 to 16×10^5 daltons. Ocular mucins are estimated to be greater than 2.5×10^5 daltons (Chao, Butala, Herp, 1988; Tiffany, 1997). About 55% of the composition of ocular mucins are carbohydrates. The composition and linkages of these carbohydrates are discussed in Chapter 4. Ocular mucins are primarily secreted by the conjunctival goblet cells. They maintain the stability of the tear film; act as a biological lubricant at the epithelial surface; and are a viscoelastic buffer against mechanical shock. Mucins support tear film stability by increasing tear film viscosity and by trapping lipids within their structure so that they can be reused after blinking. Several pathological conditions will cause mucins to become decreased or absent from the tear film, for example: vitamin A deficiency, ocular pemphigoid (conjunctival ulcerations), Stevens-Johnson syndrome (an acute attack of the mucous membranes and skin), and alkali burns. All of these conditions bring about destruction of the goblet cells with the resultant loss of mucin production. This results in a rapid break up of the tear film and occurs even when there is an adequate volume of the aqueous layer of tears.

Collagen

Collagen is a protein of great structural importance to the eye just as it is for other parts of the body. Approximately 80% to 90% of the bulk of the eye contains collagen. This protein is an extracellular, insoluble molecular complex that has a variety of morphological roles. Collagens act as: (1) supporting members or *fibers* to form and maintain tissue

structure (including wound repair collagens); (2) as *scaffolding* upon which *basement membrane* is constructed; and (3) as *anchoring devices* to hold cells onto noncellular areas. In the eye, collagen also makes up the *semiliquid gel* of the vitreous humor and, therefore, has a fourth role there.

There are about 19 different types of collagen of which a few are not well defined (Eghbali-Webb, 1995). At least 12 types have been found in the eye (Berman, 1991). Collagen may be classified according to whether it is fibrous or nonfibrous with the nonfibrous types subdivided into three areas: *f*iber *a*ssociated *c*ollagens with *i*nterrupted *t*riple helices (FACIT collagen); sheet forming collagens; and miscellaneous collagens (which include types involved in tissue anchoring).

All collagens are protein complexes whose basic units consist of at least some triple helices (*tropocollagens*) in which three polypeptide chains are wound around each other like a piece of rope. These units make up part of a larger assembly to be described in the following discussion. The helical form of the unit chains is different from that of an α-helix. In fact, there exists both a helical structure for each chain (minor helix) and a helical structure formulated by the three chains together (major helix). The molecular weight of a single tropocollagen unit of type I collagen is 400,000 daltons.

The assembled structures of collagen molecules are best understood in terms of how cells make them. As with all proteins, collagen peptide synthesis occurs by "reading" their specific code (sequence) from messenger RNA (ribonucleic acid) at ribosomes located along the cell's rough endoplasmic reticulum. These polypeptides also undergo post-translational modifications during and after synthesis as diagrammed in Figure 2–28. In step 1 of the figure, each of the three chains being assembled contains substantial amounts of the amino acids Pro and Lys as indicated by small lines protruding from each chain. These amino acids are partly hydroxylated during peptide synthesis by the catalytic action of hydroxylase enzymes. The added OH groups are indicated by asterisks in the figure. In addition, the major portion of each chain contains Gly as every third amino acid. This frequent occurrence of a small Gly molecule is necessary to fit the three chains within a triple helical structure. The next events occur at steps 2 and 3 in which the three chains first become associated hydrophobically and then form disulfide bonds at their C-terminal regions. Upon disulfide bond formation, the three chains immediately and rapidly twist into a triple helix with a zipper-like motion in which the hydroxyl groups protrude outward. This occurs to stabilize and lower the high potential energy induced by the disulfide bonds. The triple helix is further stabilized by interchain hydrogen bonding in which the hydroxyl groups participate. Some of the hydroxyl groups subsequently become bound to small sugar chains (oligosaccharides), but the reason for this is unknown.

The type of collagen to be formed will be partially determined by the kinds of post-translational modifications that occur to the triple helix as well as the degree to which the chains are hydroxylated. In the fiber forming collagens (types I, II, III, and V), and similarly in some other types, large portions of the nonhelical N- and C-terminal peptides (*extension peptides)* are removed by the catalytic action of peptidase enzymes after transport out of the cell. The remaining structure, as shown in step 4 of Figure 2–28, is known as *tropocollagen*. Some evidence indicates that the released extension peptides regulate the synthesis of new procollagen chains (Miller, Gay, 1992). Not all collagens lose

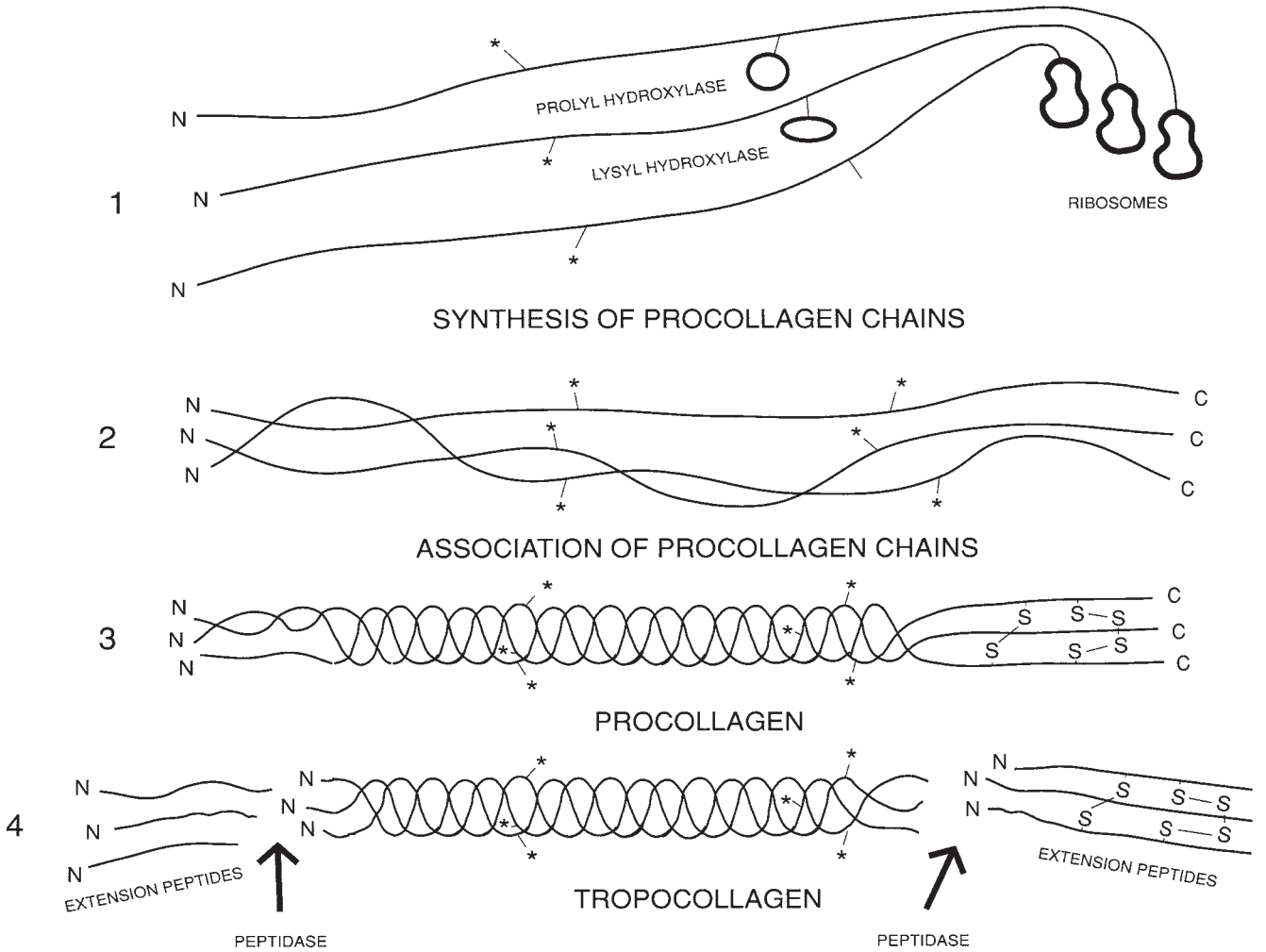


Figure 2–28

Cellular synthesis of collagen. ➤ Single collagen chains are synthesized, as are all proteins, in the usual manner on ribosomes (1). During synthesis, some of the Lys and Pro amino acids have hydroxy groups added to them. After synthesis three chains associate together along common hydrophobic areas (2). The formation of disulfide bonds (at the C-terminal areas) cause the three chains to wrap around each other in a triple helix (3). Note that the ends of the molecules do not form a helix. This procollagen form is ready for transport outside the cell. Synthesis of the unit (known as tropocollagen) is complete with the hydrolysis of the N- and C- extension peptides (4). Nonfiber forming collagens, however, do not lose their extension peptides. (Figure used with permission from McGilvery RW: Biochemistry, *a functional approach*. Philadelphia, 1983, WB Saunders.)

their extension peptides, however. The scaffold-associated type IV collagen, for example, is not subject to the molecular truncation of step 4.

The kinds of polypeptides (i.e., the sequences) that compose collagen may be similar (homopolymeric) or dissimilar (heteropolymeric). Type I collagen is heteropolymeric and consists of two α1(I) chains and one α2(I) chain designated as $[\alpha 1(I)]_2$ [α2(I)] whereas type II collagen is homopolymeric and consists of three α1(II) chains designated: $[\alpha I(II)]_3$ (Table 2–6). Once outside the cell the tropocollagen units of the fiber forming collagens go through a process of assembly in close vicinity to the cell's surface. The process consists of the lateral association of tropocollagen units, which are staggered lengthwise in three dimensional space as shown in Figure 2–29. This association is made up of hydrophobic interactions and the formation of crosslinks of lysine and hydroxylysine as shown by the

TABLE 2–6 ➤ TYPES OF COLLAGEN IN OCCULAR TISSUES

Type	Major molecular species (Chain types)	Molecular characteristics	Ocular sites	Function
I	$[\alpha1(I)]_2$, $\alpha2(I)$	Low content of hydroxylysine, low content of carbohydrate	Corneal stroma, sclera	Structural fibers
II	$[\alpha1(II)]_3$	High content of hydroxylysine, high content of carbohydrate	Cornea, sclera, vitreous	Structural fibers
III	$[\alpha1(III)]_3$	High content of hydroxyproline, low content of hydroxylysine, low content of carbohydrate	Blood vessels, cornea, iris, lids	Limits diameter of fibers and repairs fibers
IV	$[\alpha1(IV)]_2$, $\alpha2(IV)$	High content of hydroxylysine, high content of carbohydrate, terminal ends retained	Descemet's membrane, lens capsule, capillaries	Amorphous or basement membrane scaffold
V	$[\alpha1(V)]_2$, $\alpha2(V)$	High content of hydroxylysine, high content of carbohydrate	Corneal stroma, endothelium	Limits diameter of fibers and cell shape
VI	$[\alpha1(VI), \alpha2(VI), \alpha3(VI)]$	Two substantial noncollagen sequences at each end	Corneal stroma	Adhesion
VII	$[\alpha1(VII)]_3$	Forms antiparallel fibers	Anchoring fibrils of Bowman's membrane	Adhesion
VIII	$[\alpha1(VIII), \alpha2(VIII), \alpha3(VIII)]$	Low content of hydroxylysine, low content of hydroxyproline, noncollagenous sequences at each end	Descemet's membrane	Scaffold of basement membrane

slanted vertical lines of the microfibril. Crosslinking adds considerable strength to the growing fibers. The *microfibril* is an association of five staggered lengths of collagen units. The next larger unit, the collagen *fibril*, is made up of many microfibrils with a diameter range of 10 to 300 nm depending on collagen type and tissue location. The further association of several fibrils together is termed a *fiber*.

Apart from the retention of the extension peptides on the procollagen triple helix, the assembly of the nonfibrous collagens has not been quite as well described (van der Rest, Garrone, 1991). The FACIT collagens (e.g., type IX) participate in the formation of fibrils of other

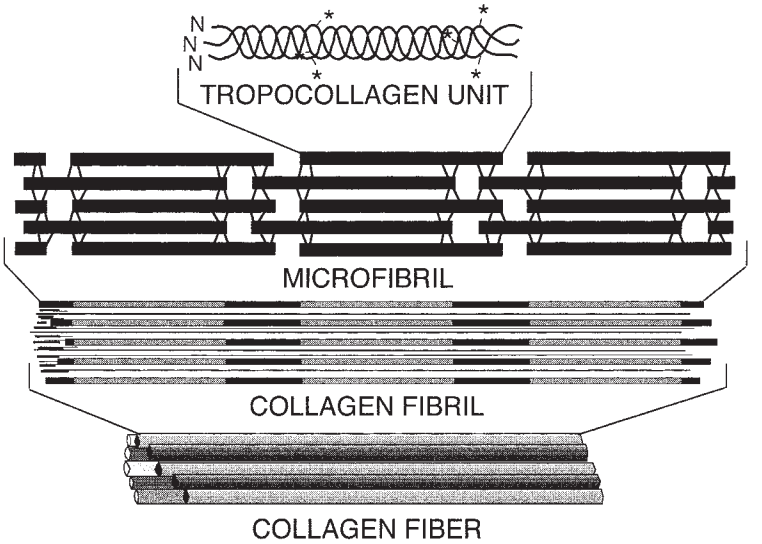


Figure 2–29
Collagen fiber formation. ➤ Tropocollagen units associate hydrophobically and are strengthened by covalent crosslinks (*small lines* in the microfibril). Note that the units are staggered with open spaces between the ends of the units. Electron micrographs of these fibrils and fibers show a banded pattern (as indicated for the *collagen fibril*) in which the electron dense material enters the empty spaces making them appear dark. A microfibril consists of five rows of crosslinked tropocollagens. A fibril is arbitrarily defined as being 10 to 300 nm in diameter. A fiber is composed of several fibrils.

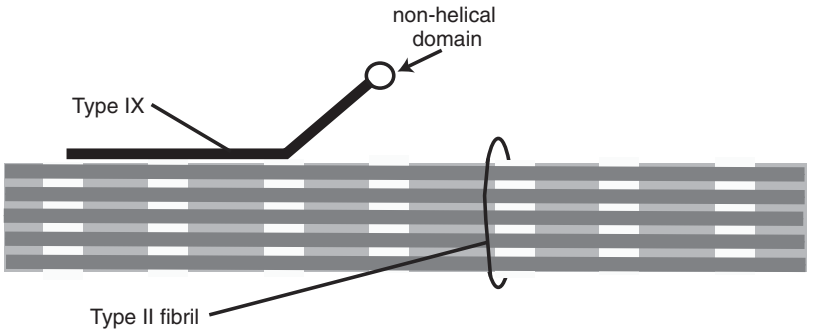


Figure 2–30
FACIT (*f*iber *a*ssociated *c*ollagens with *i*nterrupted *t*riple helices) collagens. ➤ The collagen typically attaches to a fiber forming collagen (as shown for type IX), but has nontriple helical regions (domains) that cause bends away from the fiber (arms) and which associate with other tissue matrix components (such as proteoglycans).

collagens (e.g., with type II collagen) by interacting with their fibrils, but do not by themselves form their own fibril structures (Figure 2–30). The sheet forming collagens, such as IV and VIII, define nets or sheetlike structures either by making spider web structures or polygonal surfaces (Figure 2–31). Anchoring fibrils are dimers that connect cells and tissues at their globular end domains (Figure 2–32).

OCULAR, STRUCTURAL ROLES

Collagen structures take on many forms in the eye. In the corneal stroma the fibers form sheets (termed *lamellae*) whose long axes are parallel within the sheet, but taken together are at different angles from

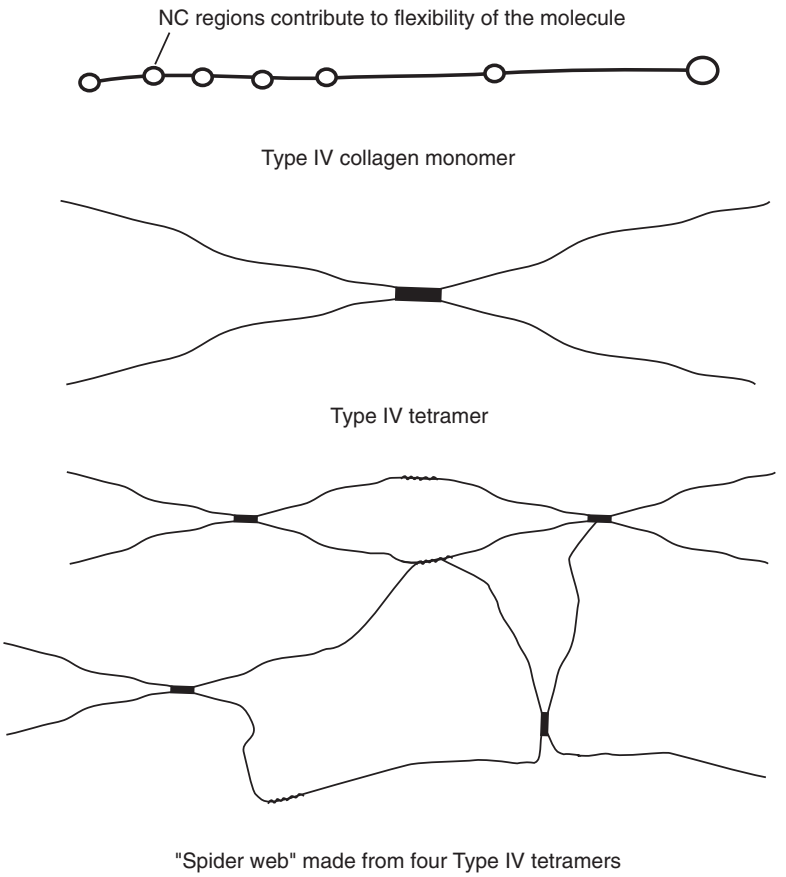


Figure 2–31
Sheet forming collagens. ➤ These collagens, such as type IV, have numerous nonhelical (or noncollagenous domains labelled *NC*) at which the molecule may bend. Such highly flexible collagens form spider structures or meshes upon binding together. The top figure shows a single molecule. The middle figure is a tetramer while the bottom figure illustrates a mesh of four tetramers. (Adapted from van der Rest M, Garrone R: Collagen family of proteins. *FASEB J* 5:2814–2823, 1991.)

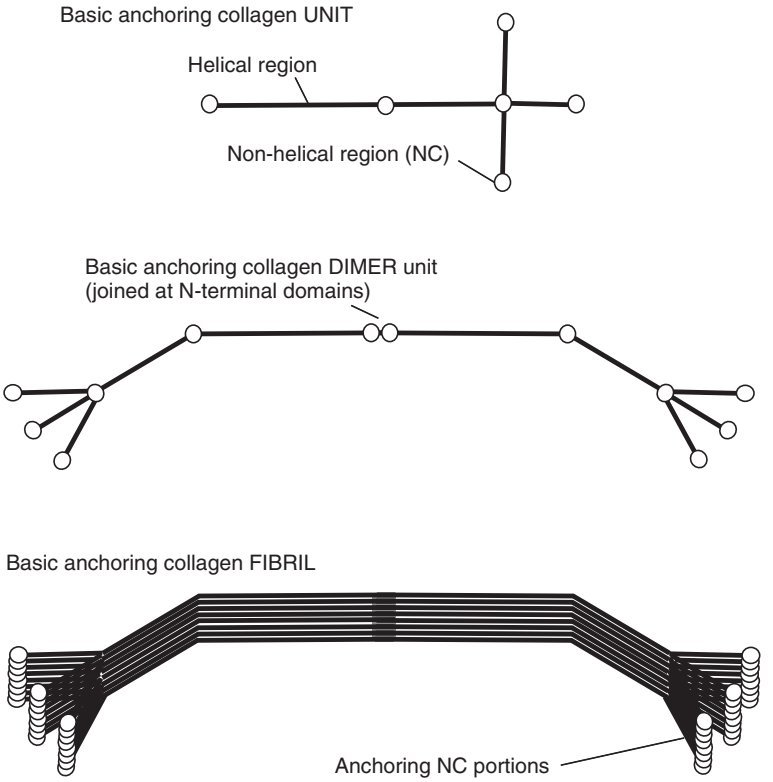


Figure 2–32
Anchoring fibril type collagen. ➤ Collagens such as type VII form anchors to connect cells to tissues. The basic molecule has a flexible three-pronged foot at one end (top figure). The dimer is joined at the end terminal NC domain leaving a foot at either end. Multiple dimers then form (bottom figure) to produce large feet that are able to become enmeshed in the anchoring plaques of extracellular tissues and the hemi-desmosomes of cells. (Adapted from van der Rest M, Garrone R: Collagen family of proteins, *FASEB J* 5:2814–2823, 1991.)

their adjacent lamellae (Figure 2–33). Such a structure imposes considerable cross-sectional strength upon the cornea. This stromal collagen is described by Borcherding, et al (1975) as having fibers with a uniform diameter of about 30 nm. This is compared with adjacent scleral collagen whose fibers vary between 40 and 140 nm (Figure 2–34). The majority of this collagen, about 70%, belongs to type I (see Table 2–6). Other collagens, which are present there in significant quantities, include types V (approximately 15%) and VI (approximately 15%).

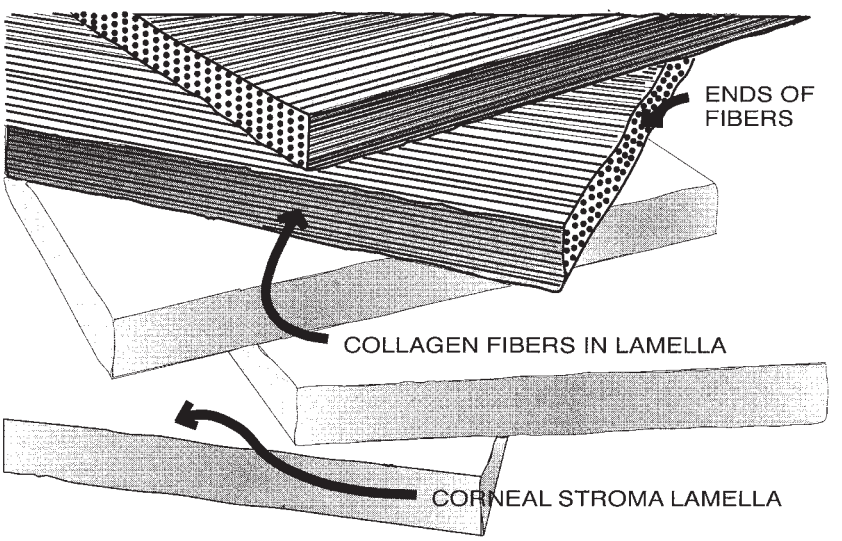


Figure 2–33
Collagen fibers contained in the lamellae of the corneal stroma (predominately type I collagen). ➤ The fibers have their long axes run at angles to each other in adjacent lamellae. (Adapted from Hogan MJ, Alvarado JA, Weddell JE: *Histology of the human eye*. Philadelphia, 1971, WB Saunders.)

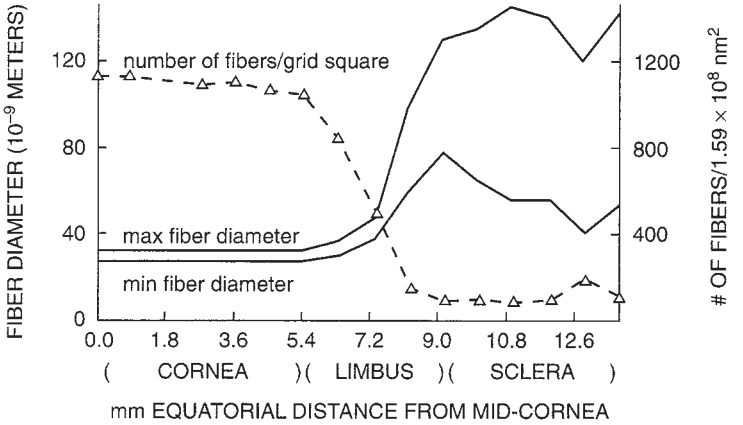


Figure 2–34
Graph of fiber diameter vs. distance from the central cornea. ➤ It can be seen that the stromal fibers are small, regular, and numerous in comparison to the sclera. This contributes to the clarity to the cornea. (Adapted from Borcherding MS, et al: *Exp Eye Res* 21:59–71, 1975.)

While type I collagen forms oblique running lamellae in the stroma, type V may serve to limit the diameter of the type I fibers (Birk, Linsenmayer, 1994). It might accomplish this by assuming an exterior conformation that prevents the attachment of additional fibrils (Miller, Gay, 1992) such as seen in Figure 2–30. This role is extremely important for the role of collagen in preventing light scattering in the corneal stroma by having its diameter tightly controlled. Type VI collagen, an extremely unusual and beaded form of the collagen family, is considered to act as a stabilizing molecule for the proteoglycans and keratocytes located between the collagen type I lamellae in the stroma (Figure 2–35).

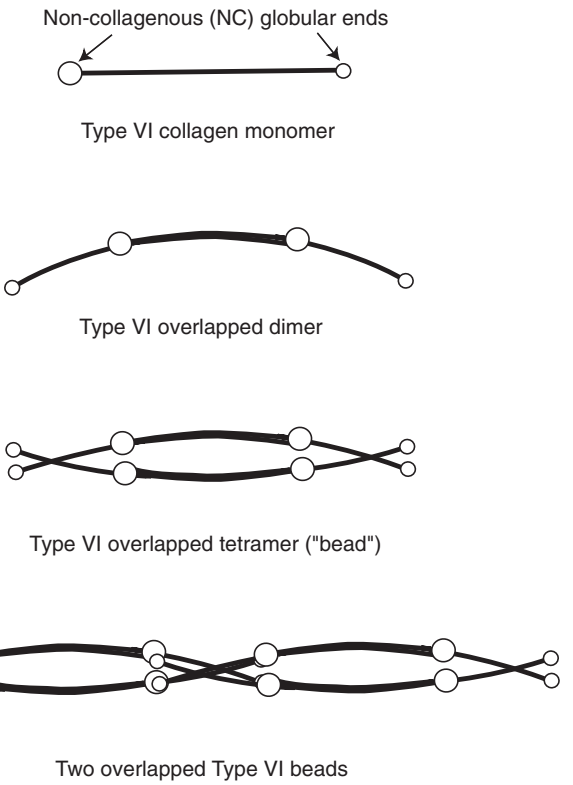


Figure 2–35
Type VI collagen. ➤ This collagen forms beaded filaments and has noncollagenous domains like types IV, V, IX, and XI. Unlike the other collagens, it combines to make overlapped tetramer beads (as seen from the top to the bottom figures). The role of these beads is somewhat uncertain, but it may stabilize proteoglycans in the corneal stroma (Adapted from van der Rest M, Garrone R, 1991.)

An entirely different collagen structure occurs in the ocular vitreous (Figure 2–36). In the gel-like milieu of the vitreous, collagen fibers are arranged in a roughly parallel orientation extending across the entire volume of secondary vitreous anteriorly to posteriorly (Sebag, Balazs, 1989). At a few locations, somewhat random fibrils are seen joining the fibers. Although less dense and without any regular lamellae as found in corneal collagen, the fibers establish firm attachments to surrounding tissues (most notably near the ora serrata and the macula). The principal vitreal collagen has been classified as type II (Swann, 1980) and is known as *vitrosin*. Some differences in vitreal type II collagen suggest that it may retain portions of its extension peptides. More recently, type IX and a type V-XI hybrid have been found associated with type II collagen in the vitreous (Brewton, Wright, Mayne, 1991; Mayne, Brewton, Ren, 1997). Type IX collagen represents about 10% to 15% of the total collagen. This collagen is thought to associate with both type II collagen and surrounding *proteoglycans* in the vitreous. These proteoglycans (PGs) are protein-sugar polymer complexes that are negatively charged and help to form the aqueous gel of the vitreous. The PGs will be described in Chapter 4. The type V-XI hybrid collagen has been suggested to limit the diameter of the type II fibers. The hybrid is analogous to what type V does to type I in the cornea. The relationship of all of these fibers is suggested by Figure 2–30. See also Chapter 10.

Basement membranes are thin structures that occur extracellularly next to certain cell types. They function to separate cells from adjoining tissues, but also to support those cells in their location. In addition, they act as sieves and barriers for molecules that may approach their associated cells. The collagen found in ocular basement membrane structures is usually type IV (see Figure 2–31). This includes Bowman's membrane, the lens capsule and blood vessels (Newsome, Gross, Hassell, 1982; Brinker et al, 1985). Type IV collagen in these structures may be thought of as a somewhat unstructured spider web in which one

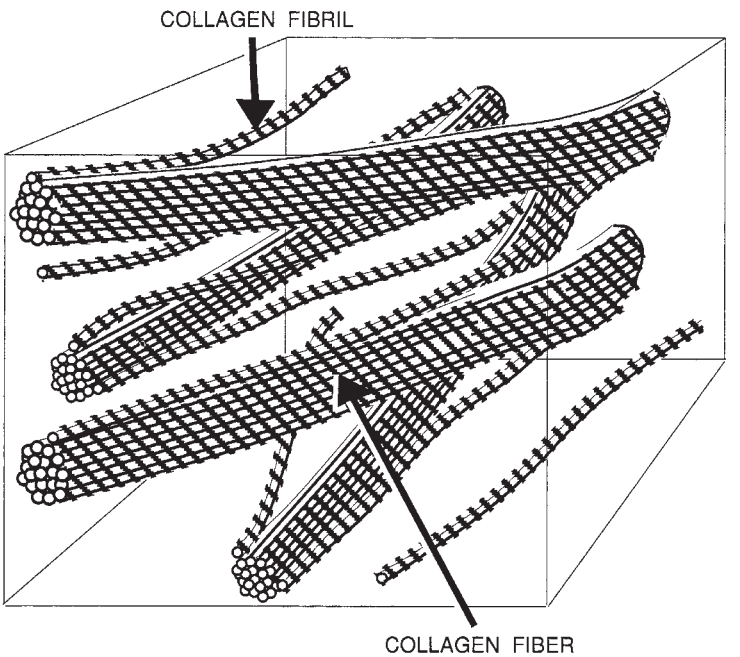
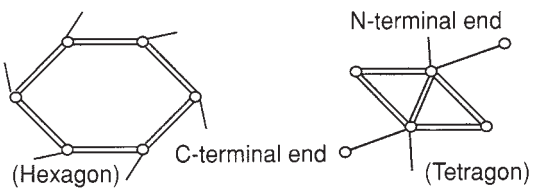


Figure 2–36
Three-dimensional representation of a cross section of the vitreous gel. ➤ The gel is predominately composed of type II collagen *fibers* of low density and type IX collagen/proteoglycan (not shown). A limited number of collagen *fibrils* also occurs. Ninety-nine percent of the gel is water. (Used with permission from Sebag J, Balazs EA: *Invest Ophthalmol Vis Sci* 30:1867–71,1989.)

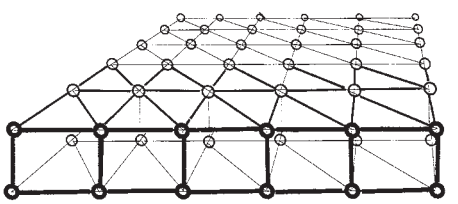
type IV collagen joins to other type IV collagens by the association of its noncollagenous (NC) peptide extensions. The structure of the web and its collagens is flexible in comparison with those collagens found in fibers. When joined, the molecules form an open mesh rather than a fiber (Hulmes, 1992).

The collagen found in Descemet's membrane is an exception to the rule for basement membrane collagens and is represented by type VIII (Kapoor, Bornstein, Sage, 1986). This collagen forms very geometric patterns and its structure resembles a box-spring mattress (Figure 2–37). The nonhelical regions of the collagen are capable of forming bonds with type IV collagen that is also associated with the cornea. Miller and Gay (1992) have described the important end-to-end bonds of type VIII that result in an open meshlike network. Although the structures are difficult to visualize, Figure 2–37 indicates two possible geometric forms. The hexagon was described by Miller and Gay. The tetragon approximates a basic form for the suggested structure of Descemet's membrane and resembles the electron micrographs obtained by Jakus (1964).

A fourth structural type of collagen found in ocular tissue is the anchoring fibril. An example of anchoring fibrils is found extending between the basal epithelial cells of the cornea and the outermost lamellae of the corneal stroma. The fibers extend through Bowman's membrane and serve to attach the epithelium to the stroma as shown in Figure 2–38. Type VII collagen has been identified (Gipson, Spurr-Mauchaud, Tisdale, 1987) with these fibrils. The type I collagen fibers in the anterior most lamella (as shown in the figure) are somewhat randomly oriented. The anchoring fibers are attached from the *hemidesmosomes* of the epithelial basal cells to anchoring plaques on the stromal type I collagen fibers. Other anchoring fibrils extend from one anchoring plaque to the next. It is thought that the diabetic state may affect the synthesis of adequate anchoring fibrils resulting in loose adhesion of the epithelium to its underlying stroma (Kenyon, 1979).



A. POLYGONAL FORMS OF TYPE IV AND IV COLLAGENS



B. PARTIAL LATTICE STRUCTURE OF DESCEMET'S MEMBRANE (based on the above tetragon structure)

Figure 2–37
Type VIII collagen and Descemet's membrane. ➤ Descemet's membrane is a basement membrane with a lattice structure with units similar to the tetragon shape in the upper right hand corner of the figure.

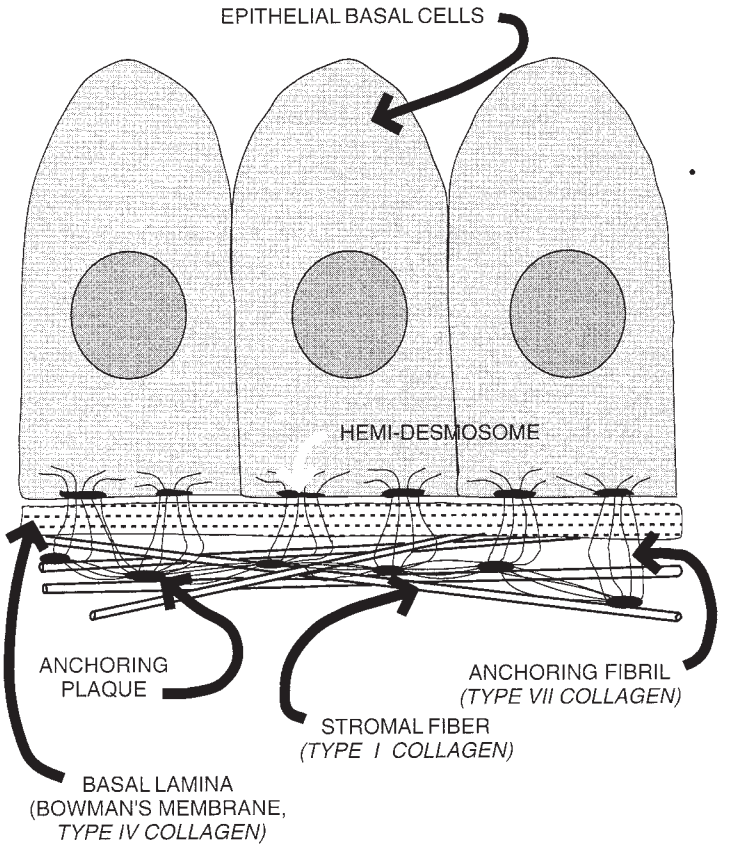


Figure 2–38
Anchoring fibrils (type VII collagen) attach epithelial basal cells (at hemidesmosomes) to the outermost stromal lamella (at anchoring plaques). Note participation and location of other collagen types.

SUMMARY

Proteins possess a variety of functional roles in ocular tissue. Although there are a huge number of proteins in the eye, some deserve special attention. Crystallins are soluble lens proteins whose normal function is supportive in the maintenance of elongated lens fiber cells. These proteins are considered to be involved in the manifestation of senile cortical cataracts by the oxidation of the disulfide bonds although the process is incompletely understood. They have also been implicated in the formation of nuclear cataracts. Rhodopsin and cone pigment proteins act as the initial participants in phototransduction. They are membrane proteins found on the discs of rods and cones. Vitamin A aldehyde (retinal) is a prosthetic group on these proteins whose release triggers the cascade of phototransduction. Mucus glycoproteins known as mucins are found in the precorneal tear film (mucous layer) and act to stabilize the tear film. Collagen composes the major type of ocular protein and is found in 80% to 90% of the bulk of the eye. It forms complex structures from basic tropocollagen units, which may form fibers, ground substances, or anchoring rods. It is also a constituent in the gel of the vitreous humor.

PROBLEMS

1. In Beer's Law, *a* is termed the absorptivity and is a property of the molecular characteristics of a given molecule. This term, when used to refer to molar concentrations of specific molecules, is called the molar absorptivity or molar extinction coefficient and is found experimentally. If the molar extinction coefficient for rhodopsin = 40×10^3 cm^{-1} M^{-1}, what would be the molar concentration of a laboratory purified rhodopsin solution when A = .660 as measured in a spectrophotometer? Assume the standard light path length of 1 cm.

2. What is QBA and what do the initials of the compound stand for? Hint: Look in the Aquilina et al (1999) reference. What is the origin of QBA and what is important about it in clinical disease?

3. Ehlers-Danlos syndrome is a collagen disease that affects many parts of the body including the eyes. In one form of the disease, the sclera becomes very thin. In another form, the corneal tissue may perforate. The disease has been at least partially linked to genetic abnormalities for the enzymes: lysyl hydroxylase (Heikkinen et al: *Am J Hum Genet* 65:308, 1999) and procollagen peptidase [called procollagen I N-proteinase] (Colige et al: *Am J Hum Genet* 65:308, 1999). Give a biochemical explanation of how deficiencies of these enzymes might affect collagen tissues.

4. A new ocular protein has been discovered in your laboratory. Upon placing the protein in a gel and subjecting it to electrophoresis, you find that the protein migrates as a single band to a point corresponding to a molecular weight of approximately 84,000 D. You previously found that there were four Cys amino acids in the protein. After you treat the protein with mercaptoethanol, a compound that breaks disulfide bonds, you again run the protein on the gel and find two bands corresponding to molecular weights of 25,000 D and 34,000 D. The density of the 25,000 D band is about 2× that of the 34,000 D band. What can you conclude about the tertiary and quarternary structures of this protein?

5. Alpha crystallins have been found to have chaperone activity. What is chaperone activity and why would you think that such a function would be necessary in a lens protein? [Hint: Look up the normal physiology and metabolism of lens fiber cells.]

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CHAPTER 3

Enzymes

OCULAR CATALYSTS

Enzymes are proteins that have the ability to optimize the rates of biochemical reactions in cellular tissues of both plants and animals. Enzymes, as catalysts, have been known since ancient times. Their activity was actually described in the Codex of Hammurabi of Babylon about 2100 BC in association with wine fermentation (Copeland, 2000). Since these proteins remain unaltered, they are true chemical catalysts. Some ribonucleic acids (RNA) have also been found to possess catalytic activity, but that will be taken up in Chapter 7. The intervention of enzymes in cellular metabolic reactions is essential to the survival and operation of every class of cell in the body. Some enzymes also function outside of cells. Accordingly, enzymes are involved in thousands of biochemical reactions, from the conversion of glucose into cellular energy, to the very synthesis of enzymes themselves as proteins. In the eye, enzymes are also instrumental in the visual transduction process, the generation of intraocular pressure, the maintenance of a clear cornea (***deturgescence***), the destruction of bacteria in the precorneal tear film, the development of lens fiber cells and many other functions that support vision. At a normal cellular temperature (37°C), and especially at the somewhat cooler regions of the cornea (30° to 35°C), biochemical reactions without enzymes would be virtually at a standstill. ***Kinetically***, an enzyme (or any catalyst) increases the rate of a reaction by lowering the energy required (that is, the ***activation energy***) to convert a reactant (***substrate***) into a new compound (***product***). This is indicated in Figure 3–1. In the case of an enzyme, at least one intermediate is formed prior to product formation. The advantage of an enzyme-catalyzed reaction is that there are comparatively small amounts of energy required ($Y_1 + Y_2$) to form the product vs. the energy needed (X) when no catalyst is present.

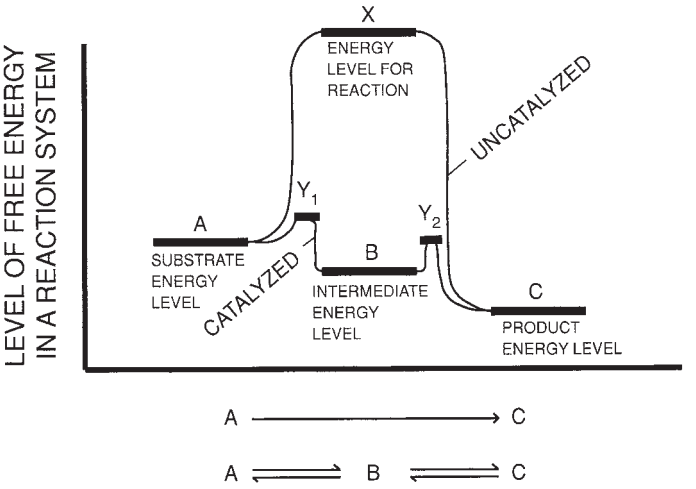


Figure 3–1
In enzyme catalyzed reactions, there are two small energy-requiring steps between the formation of an intermediate (B) from the substrate (A) vs. the formation of a product (C). ➤ Compare this with the energy required (X) for the uncatalyzed reaction.

Therefore, in the figure, the energy levels of $Y_1 + Y_2 << X$. These reactions are usually reversible, meaning that a large amount of substrate (A) will drive the reaction to form product (C) and vice versa. In a series of enzyme driven reactions, however, usually one reaction tends to proceed in a given direction regardless of substrate and product concentrations. This reaction will drive all the reactions in the series or pathway in that same direction. The enzyme involved in the principal driving reaction is called the *rate limiting enzyme*.

On the molecular level, what actually occurs in an enzyme catalyzed reaction is a binding of a substrate (Figure 3–2) to a region of the enzyme known as the *active site*. This is usually a cleft, on the outside, or a small "cavern" on the inside of the structure of the catalytic protein. The molecular architecture of this site is suitable for holding the substrate in an orientation favorable for a rapid conversion first to (an) intermediate(s), then a product. Small changes to the enzyme (e.g., transfer of protons, conformational strain, etc.) occur as part of the catalytic event, but the enzyme is always returned to its original form at the end of each reaction (i.e., it is unchanged). Enzymes, just as proteins, may be classified by several criteria. One criterion is based on the kinds of reactions supported: (1) *oxidation-reduction*; (2) *transfer of molecular groups*; (3) *hydrolytic cleavage*; (4) *double-bond alteration*; (5) *isomerization*, and (6) *condensation* (also known as *synthesis* or *ligation*). One can also classify enzymes on a kinetic basis and the classifications of *Michaelis-Menten* and *allosteric* types give a better idea of how enzymes actually function.

Michaelis-Menten Enzymes

Michaelis-Menten enzymes have kinetic, functional properties that were described mathematically by Henri in 1903, by Leonor Michaelis and

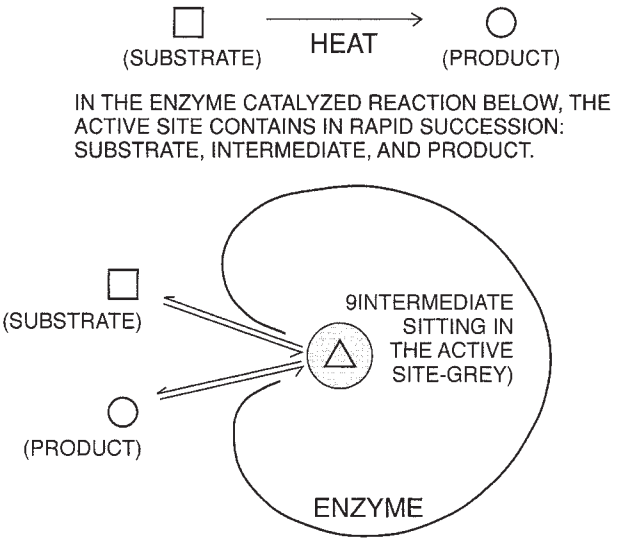


Figure 3–2
Noncatalyzed reactions are often driven by heat and molecular collision whereas enzyme catalyzed reactions align and hold reactants (substrates) at the active site for reactions to occur. ➤ Although some heat is involved (usually at 37°C), the molecular precision of alignment and containment is far more efficient.

Maude Menten in 1913 and, finally in 1925, by G.E. Briggs and J.B.S. Haldane (Palmer, 1981). The reaction kinetics are derived from the equation.

$$\left[\quad [E] + [S] \underset{k_2}{\overset{k_1}{\leftrightarrow}} [ES] \overset{k_3}{\rightarrow} [P] \quad\right] \tag{3–1}$$

in which [*E*], [*S*], [*ES*] and [*P*] are the molar concentrations of enzyme, substrate, enzyme-substrate complex, and product respectively while k_1, k_2, and k_3 are the rates for each conversion to and from [ES]. In particular, the term k_3 is also known as k_{cat} (turnover number) when [*S*] is high and $v \rightarrow V_{max}$ (defined on the next page) (Copeland, 2000). The molecular forms, when in brackets, refer specifically to the molar concentrations of each form. The concentration relationships for these conversions over time may be visualized in Figure 3–3. Notice at the arrow in the figure that the enzyme becomes saturated very early in time with substrate (enzyme-substrate complex [ES]) and remains at a fairly steady level. Accordingly, the change in [ES] with time may be described as:

$$\left[\quad \frac{d\,[ES]}{dt} \cong 0 \quad\right] \tag{3–2}$$

Since the change in [ES] is very small or nearly zero, d[ES]/dt may also be described in terms that cause both its formation: k_1 [E][S] and its dissolution: $-(k_2\,[ES] + k_3[ES])$. Moreover, these terms may also be made nearly equal to zero, rearranged, and substituted in the following first order reaction equation:

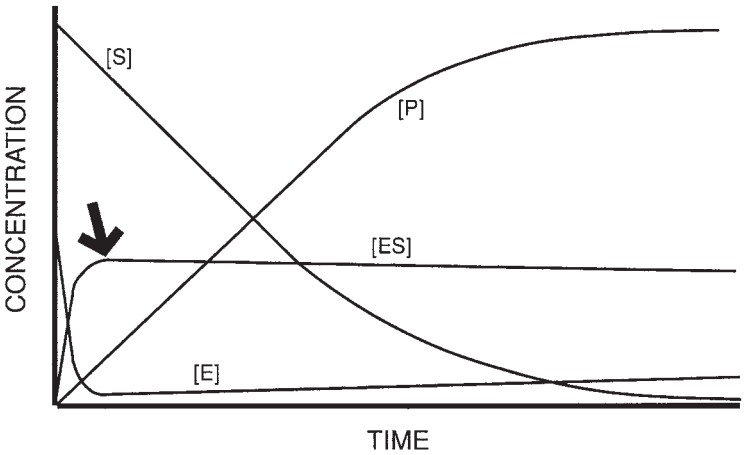


Figure 3–3
Concentrations of participants in an enzyme catalyzed reaction over time. ➤ The concentration of the enzyme-substrate complex [ES], indicated by an arrow, is held nearly constant as the substrate [S] is consumed and the product [P] is formed. This is another way of showing that the enzyme's active site is saturated.

$$\text{velocity of the reaction} = v = k_3[ES] \qquad (3\text{–}3)$$

This may be used to derive the equation:

$$v = \frac{V_{max}\,[S]}{[S] + K_m} \qquad (3\text{–}4)$$

where V_{max} is the maximum velocity of the enzyme and:

$$K_m = \frac{k_2 + k_3}{k_1} = \textit{Michaelis-Menten constant}$$

The derivation of equation 3–3 is given in the Glossary under *velocity*. Equations (3–3) and (3–4) are both rate equations. Equation 3–4 is known as the *Michaelis-Menten equation* and mathematically defines the maximum velocity (V_{max}) and the dissociation constant (K_m) of an enzyme. These terms are useful in measuring the rate properties of an enzyme (how fast they catalyze reactions) and in comparing the relative affinity ("stickiness") of different substrates for the same or different enzymes. The Michaelis constant is, however, actually a *dissociation constant* for the [ES] complex in which [ES] → [E] + [S]. Since this term is *opposite* in meaning to affinity, one must be aware that the lower the K_m value, the greater will be the affinity of substrate and enzyme provided that k_3 is sufficiently small. Sometimes this is not the case and enzymologists have used the term *apparent* K_m or $K_{apparent}$ to indicate this. This term is also used to indicate the presence of other substances or conditions, which may modulate the enzyme's activity. (For more details on the meaning of $K_{apparent}$ one may refer to the discussion by Mathews and van Holde, 1990 as well as by Palmer, 1981.) In practical terms, the K_m is also equal to the substrate concentration [S] when v is 1/2 V_{max} (Figure 3–4). For most enzymes, K_m lies between the substrate concen-

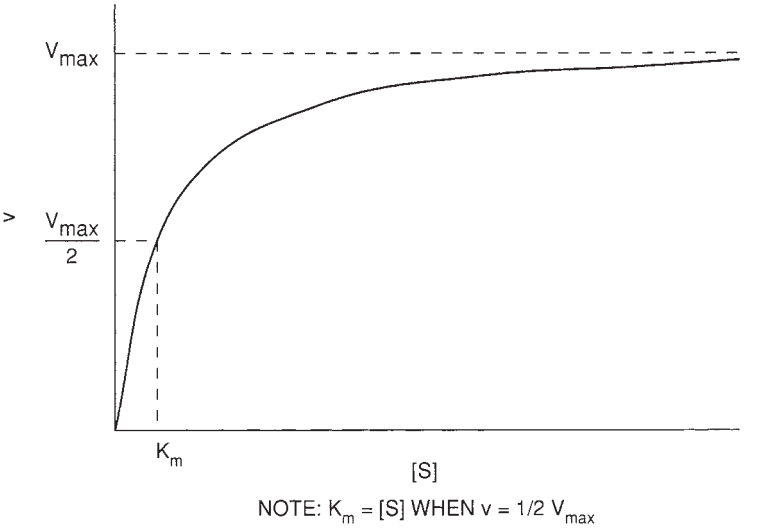


Figure 3–4
Graph of enzyme activity (v) vs. substrate concentration [S] showing the difficulty of obtaining a true value of V_{max}, the maximum velocity of the enzyme. ➤ The velocity of the enzyme increases with [S], but never quite reaches its V_{max}. At 1/2 of V_{max}, one can measure the affinity of the enzyme for the substrate (K_m).

trations of 1×10^{-1} and 10^{-7} M (Stryer, 1988). However, the V_{max} and the K_m of an enzyme are not usually measured by constructing graphs as shown in Figure 3–4. This is due to the difficulty in obtaining the value of V_{max} as it approaches an asymptotic limit. Another approach to finding V_{max} and K_m is the use of a double-reciprocal plot such as a Lineweaver-Burk plot (Figure 3–5). The plot is constructed by using the reciprocal of the Michaelis-Menten equation:

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

This equation allows one to obtain a straight-line graph having two intercepts (x and y), which give the reciprocal values for K_m and V_{max}, respectively. In the eye, aldose reductase, an enzyme involved in the formation of sugar cataracts, serves as an example of a Michaelis-Menten enzyme.

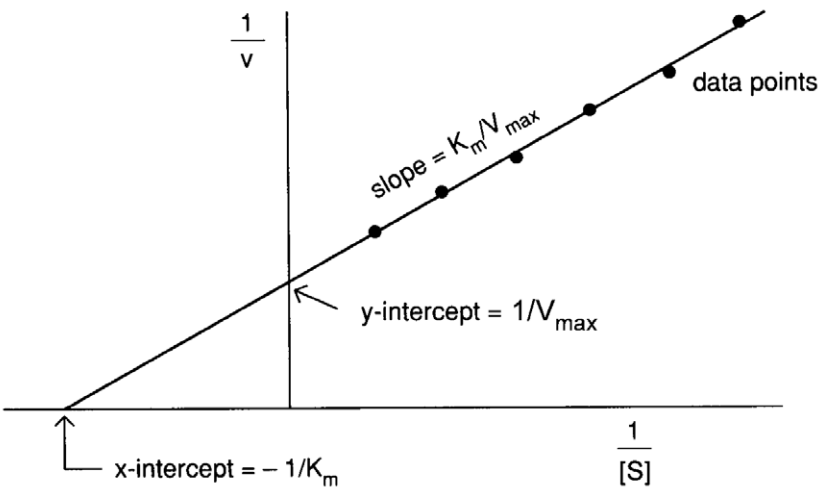


Figure 3–5
A Lineweaver-Burk plot of 1/v (reciprocal of enzyme velocity) vs. 1/[S] (reciprocal of the molar concentration of the substrate). ➤ Here it is possible to accurately determine both the V_{max} and the K_m. Enzyme velocities are measured as the concentration of substrate consumed or product formed per unit time at stated conditions of temperature and bathing solutions. The term "units of velocity" (U) may also be used. Note that the x-intercept is a negative value.

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Allosteric Enzymes

The name *allosteric* means "other site" and refers to the fact that the kinetics of these enzymes is influenced by substances bound to the enzyme at locations other than the active site. This can occur in two different ways. Since allosteric enzymes are proteins with quaternary structures (meaning that they consist of more than one polypeptide chain), each chain has its own active site. When only one, or a few, active site(s) are occupied, the affinity of enzyme and substrate is low. In this situation, the velocity of the reaction is also low. As the number of active sites occupied increases, an overall conformational change in the protein enzyme becomes more favorable to increased binding of the substrate to the remaining active sites. Therefore, the velocity of the enzyme increases. The change in the enzyme conformation is described as pro-

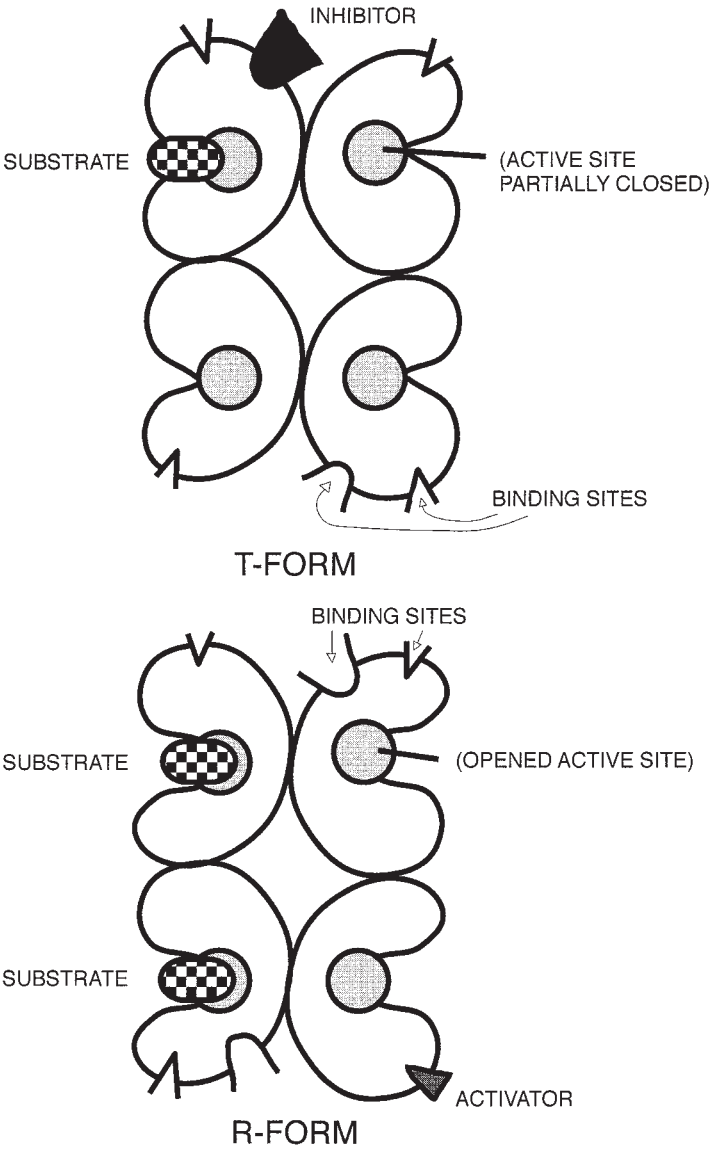


Figure 3–6
Schematic diagram of an allosteric enzyme in its T- and R-forms. ➤ The enzyme is represented as four polypeptides with an active site (grey circle) in each polypeptide. In the T-form, the substrate has difficulty entering the active site while in the R-form access to the active site is easily gained. This is shown by the narrow and broad openings to the active sites. Inhibitors maintain these enzymes in their T-forms, but activators convert them to their R-forms. Substrates force a conformational change in the enzyme from T-form to R-form after enough substrate has entered the active sites available.

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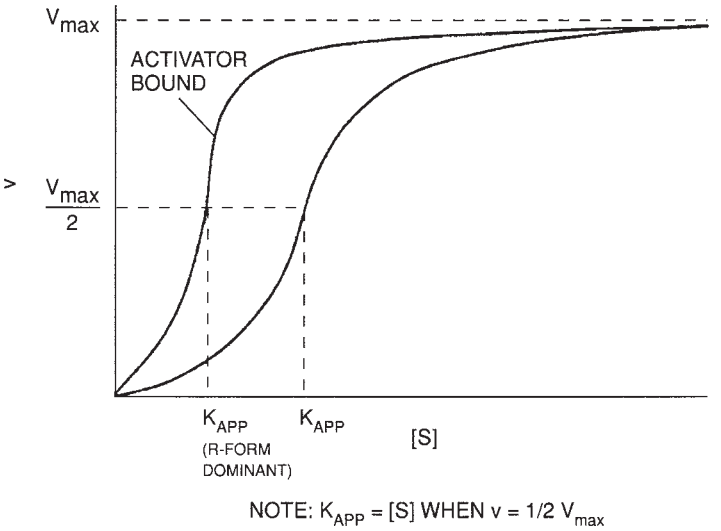


Figure 3–7
The effect of an activator upon the velocity and substrate affinity of an allosteric enzyme. ➤ When the activator is bound (left hand curve). The apparent K is decreased (i.e., the affinity is increased) while the velocity is increased.

ceeding from a *T-form (T = tense)* to an *R-form (R = relaxed)*. *A* second way that the kinetics is influenced is with the binding of activator substances that occupy sites on the enzyme away from the active sites themselves. In this way, such an enzyme is also induced to change from its T-form to its R-form at much lower substrate concentrations. This is a biological way of jump-starting an enzyme to high velocities at lower substrate concentrations. Figure 3–6 shows a hypothetical enzyme in the T- and R-forms when bound to activators or multiple substrates (*inhibitors*, also shown, are described later).

Figure 3–7 indicates a graph of velocity versus [S] concentration for an allosteric enzyme without (on the right) and with (on the left) bound activator substances. Note that with such enzymes K_m becomes known as $K_{apparent}$ (also known as K_{app} or $K_{0.5}$) since the K value with allosteric enzymes is dependent upon activators as well. In the eye, sodium-potassium activated ATPase, whose role is of special significance in the cornea and the ciliary body, serves as an example of an allosteric enzyme. When one attempts to make a Lineweaver-Burk plot with allosteric enzymes, however, the line becomes nonlinear and it is impossible to determine K_{app} (Figure 3–8). There are other graphing techniques that can solve this problem such as an Eadie-Hofstee plot (Figure 3–9), (Whikehart, Montgomery, Hafer, 1987).

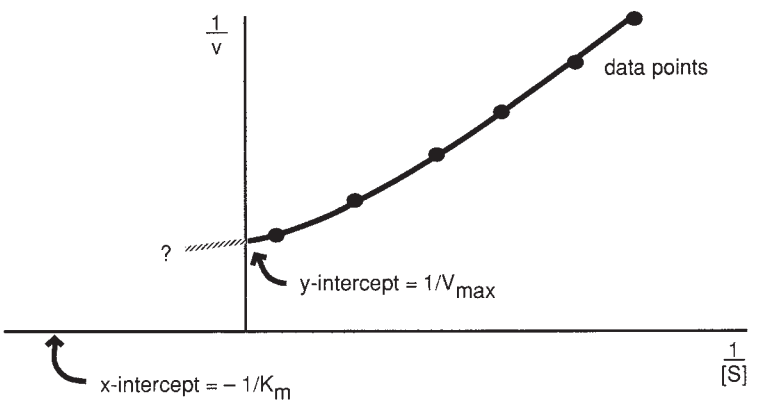


Figure 3–8
The inability of a Lineweaver-Burk plot to give accurate information about the apparent K of an allosteric enzyme. ➤ The degree of curvature and any intersection with the x-axis cannot be determined.

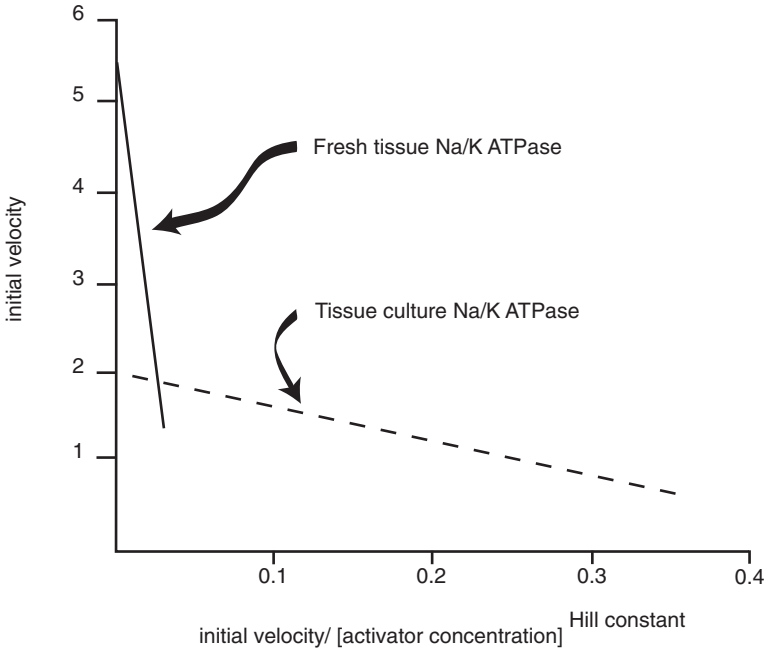


Figure 3–9
Two typical Eadie-Hofstee plots used to determine the K_{app} of Na activation for Na,K-ATPase present in fresh tissue and tissue cultures of bovine corneal endothelial cells (Whikehart et al, 1987). ➤ The initial velocity is plotted against the initial velocity divided by the substrate or activator concentration of the enzyme. In this version of the plot, the value of v/[S] or v/[A] is refined by using a Hill coefficient, which is described in the Glossary. The values of the K_{app}, in mM, for Na activation, are at the y-intercepts of the plot.

Enzyme Inhibition

In addition to controlling the rates of enzymes positively (i.e., by stimulation), negative control may also be realized by the inhibition of activity both naturally, by substances within tissues, and artificially, by substances introduced into tissues. The latter represents a pharmacological technique that is useful, for example, in the treatment of glaucoma by the inhibition of acetylcholinesterase, an enzyme that normally lyses acetylcholine in the autonomic nervous system. Michaelis-Menten enzymes are usually inhibited by one of three mechanisms: *competitive*, *noncompetitive,* and *uncompetitive*. The pattern of Lineweaver-Burk plots, as shown in Figure 3–10, indicate how the apparent K_m and V_{max} may be influenced by the mechanism of inhibition. The expression $(1+ [I]/K_i)$ becomes a factor in all three forms of inhibition and may be used to determine the *concentration of the inhibitor* and *its affinity for the enzyme*. [I] stands for the molar concentration of inhibitor and K_i is a measure of the affinity of the inhibitor for the enzyme in the same reciprocal sense as K_m is for [S]. In competitive inhibition, the inhibitor replaces or competes with substrate for the active site. In noncompetitive inhibition, the inhibitor binds to a site close to the active site and prevents catalytic action on the substrate even though it may bind to the enzyme. In uncompetitive inhibition, two substrates are usually required for catalytic action and the inhibitor binds to an area close to the active site after the first substrate binds there. The inhibitor then prevents the second substrate from binding. The latter mechanism occurs in the enzyme aldose reductase, an enzyme involved in cataract formation of diabetics and people who have *galactosemia*. Figure 3–11 demonstrates these mechanisms. Allosteric enzymes are inhibited by substances binding to allosteric sites resulting in a more pronounced T-form (which makes it more difficult for the substrate to bind to the enzyme). This is shown in Figure 3–12 and may also be seen in Figure 3–6. Inhibition also serves to slow down the rates of metabolic reactions (a series of enzyme

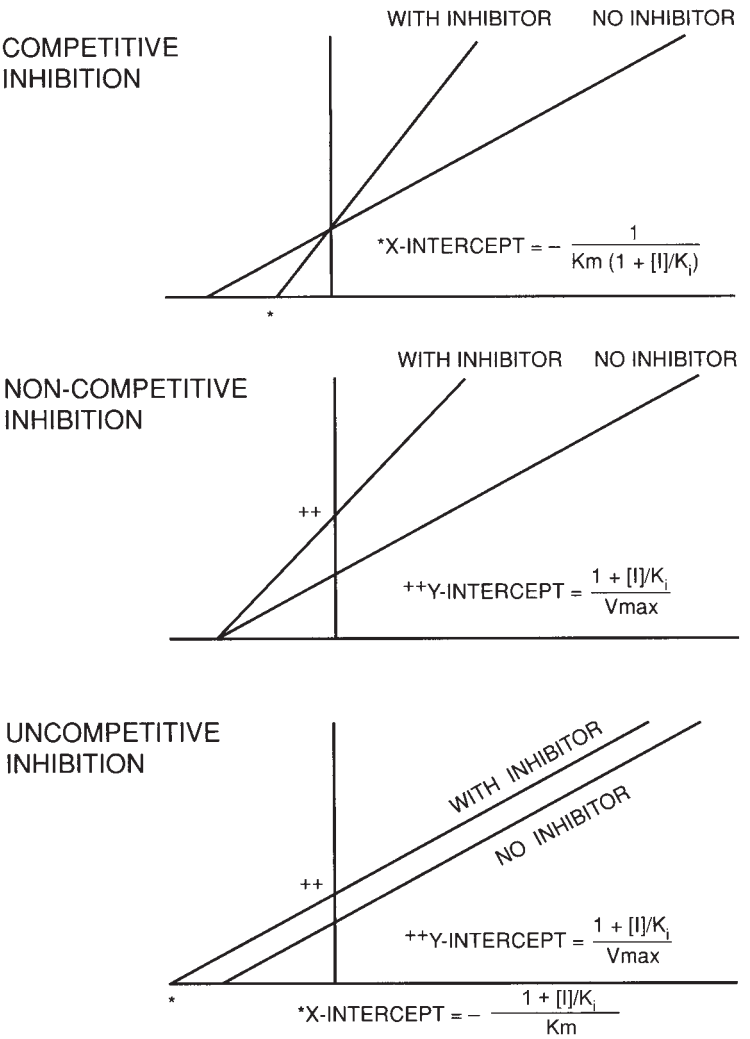


Figure 3–10
Lineweaver-Burk plots for three inhibition mechanisms found in Michaelis-Menten enzymes. ➤ In competitive inhibition, the apparent K is affected by competition of the inhibitor for the active site. In noncompetitive inhibition, the velocity of the enzyme is affected by the proximity of the bound inhibitor to the active site. In uncompetitive inhibition, both the apparent K (for one of the substrates) and the velocity of the enzyme are affected by the binding position of the inhibitor.

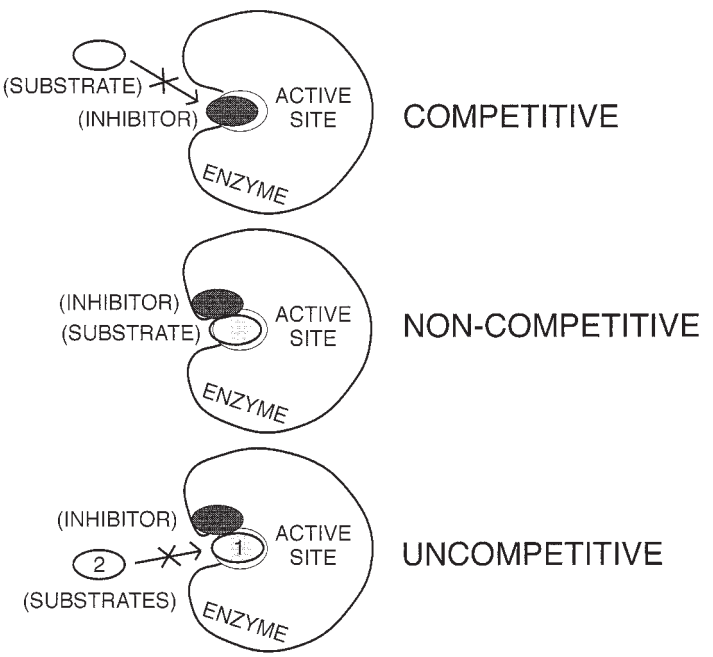


Figure 3–11
The binding positions and effects on enzyme catalysis produced by the three kinds of inhibition of Michaelis-Menten enzymes. ➤ In competitive inhibition, the substrate is blocked from entering the active site. In noncompetitive inhibition, the substrate enters the active site, but cannot easily be converted to product. In uncompetitive inhibition, a second necessary substrate is blocked from entering the active site.

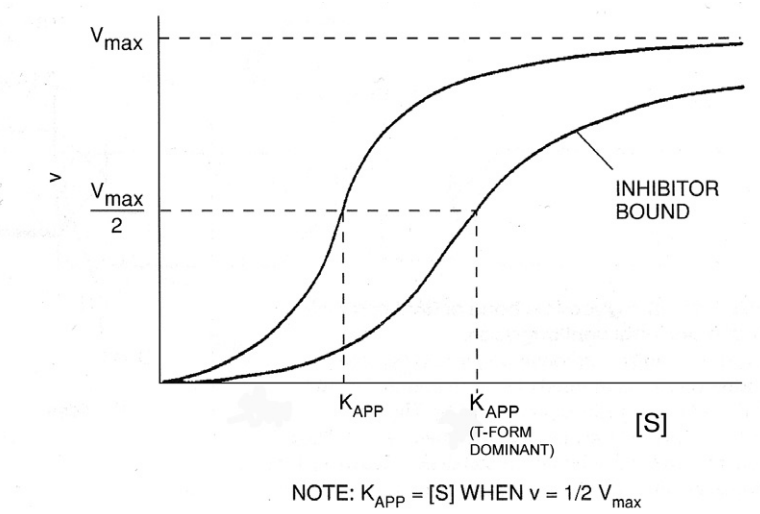


Figure 3–12
Plot of v vs. [S] when an inhibitor binds to an allosteric enzyme. ➤ The curve is shifted to the right causing both a decrease in velocity and a higher apparent K. The enzyme is in its T-form in the presence of an inhibitor.

catalyzed reactions having a specific purpose such as the breakdown of sugar molecules). In a series of such reactions, the end product of the series may actually act as an inhibitor for the first reaction in that series (*feedback inhibition*). The hydrogen ion content (pH) also influences enzyme reaction rates both positively and negatively. Intracellular pH varies from one cell type to another and is not necessarily equivalent to the extracellular or *physiological* pH of 7.4. This is important since most enzymes function intracellularly to control normal cell operation. Some enzymes act at cell membranes to facilitate transport and a number also catalyze reactions extracellularly. Tear film lysozyme is an example of an extracellular enzyme associated with the eye.

Lysozyme

Lysozyme is an enzyme of the precorneal tear film that is instrumental in destroying certain kinds of bacteria (namely, those which are positively stained with a crystal violet-iodine complex for *peptidoglycans* known as *Gram stain*). These Gram-positive bacteria possess an outer coat of a peptideglycan (sugar) polymer (or peptidoglycan), which, in Gram-negative bacteria, is only transiently stained since those bacteria are covered up by a second, outer lipid membrane. Lysozyme is able to hydrolyze or break up the glycan (sugar polymer) components of the peptidoglycan of Gram-positive bacteria as shown in Figure 3–13. The enzyme was initially described in 1922 by Alexander Fleming, a British bacteriologist (Stryer, 1988). He first found it in nasal mucous, but later discovered that tears are a rich source of the enzyme. The concentration has been estimated at 1.3 mg per ml of tears, which are unstimulated by external or internal sources such as onion odor or emotional stress (Sen and Sarin, 1980). Specifically, the enzyme breaks the β1→4 glycosidic bond of the oxygen bridge between the repeating glycan units of N-acetylmuramic acid (NAM) and N-acetylglucosamine (NAG) as indicated in Figure 3–14. Lysozyme itself is a globular protein with a

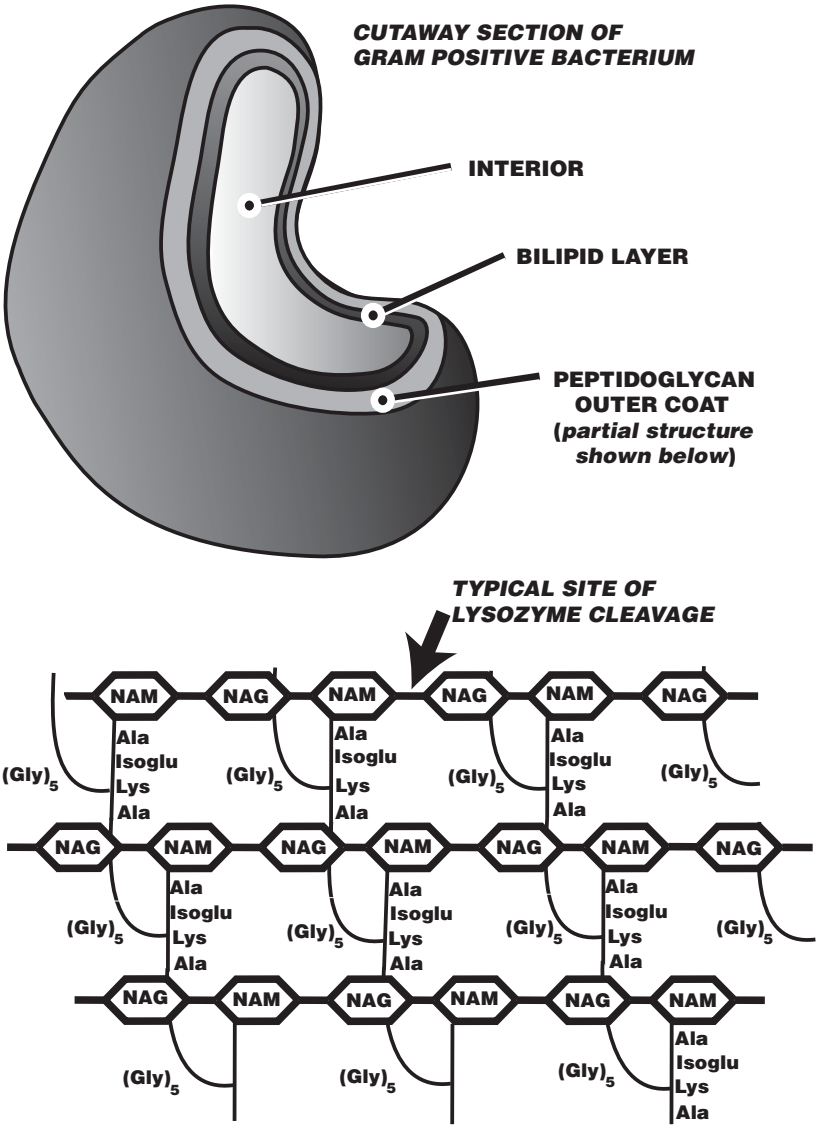


Figure 3–13
Cut-away diagram of a Gram-positive bacterium, which has two boundary layers: a bilipid layer (membrane) and a peptidoglycan layer (outer coat). ➤ The latter, composed of a molecular cross weaving of sugars and peptides, is represented below. A representative site of lysozyme cleavage is shown by an arrow and a unit of two of the sugars is represented in Figure 3–14.

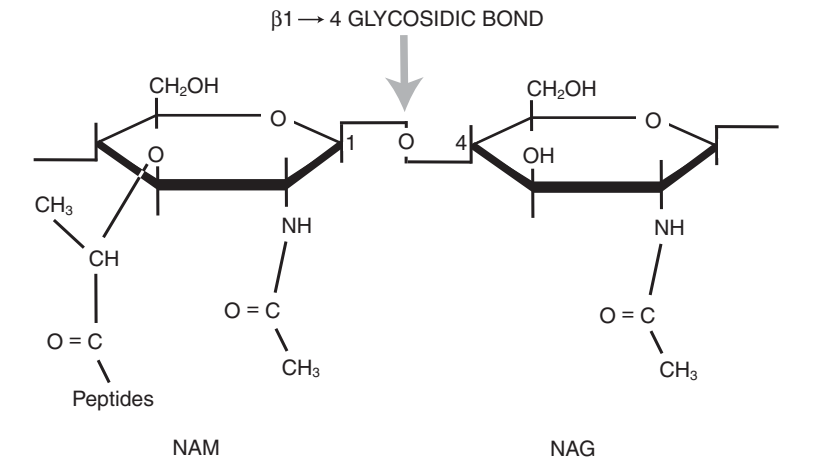


Figure 3–14
A basic two-sugar (carbohydrate) unit of bacterial peptidoglycans. ➤ It is composed of N-acetylmuramic acid (NAM) and N-acetylglucosamine (NAG). These two carbohydrates (and the ones that join them) are held together by an oxygen bridge (*arrow*) designated as a β(1→ 4) glycosidic bond.

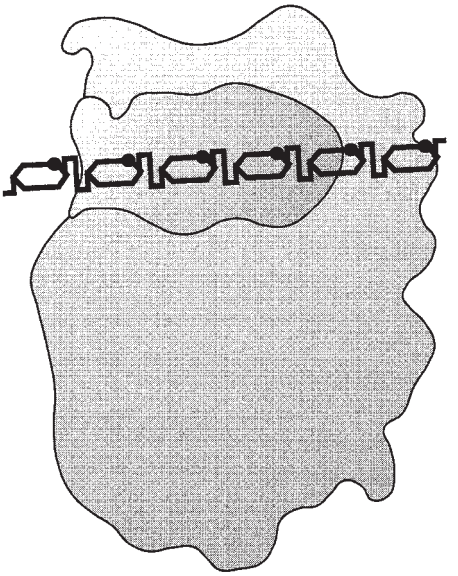


Figure 3–15
Outline diagram of a lysozyme molecule. ➤ The enzyme is a globular polypeptide that has a groove (darker area near the top of the enzyme) into which the carbohydrate units of the peptidoglycans of bacteria can fit. Hydrolysis (or rupture) of the peptidoglycan units occurs there.

molecular weight of about 14,000. A portion of the bacterial peptidoglycan is able to fit in a groove on the outer face of the enzyme that contains the active site (Figure 3–15). This is an enzyme in which the detailed mechanism for hydrolysis is known and can be described in three stages. Figure 3–16 shows the mechanism in which the disaccharide unit is hydrolyzed. The active site contains two amino acid components (Glu and Asp) whose carboxylate groups participate in the hydrolysis. Initially, a proton (H^+ from the Glu) breaks the bond by binding to the oxygen between the two sugar rings leaving an unbound, positively charged carbonium ion (carbon #4) in the right hand sugar ring. This carbonium ion is temporarily stabilized by the negative charge on Asp located above it (see Figure 3–16 A and B). Then, a nearby water molecule ionizes and donates its proton to the negatively charged Glu while the hydroxy group (–OH) binds to the carbonium ion and the reaction is complete (see Figure 3–16 B and C). At completion, the original forms of the enzyme are regenerated and the hydrolyzed (split) chains of the peptidoglycan leave the active site of the enzyme.

Once the peptidoglycan cover is split open (hydrolyzed) by lysozyme, the bacterium is no longer able to contain its high, internal osmotic pressure with its plasma membrane alone and it bursts open. Other protein components of tear film have also been implicated in bacteriocidal action (Selsted, Martinez, 1982), but none of them are as efficient as lysozyme.

In addition to its bacteriocidal activity, lysozyme also serves as an important analytical indicator of tear dysfunction. Measurement of lysosomal activity reflects the productivity of the main and accessory lacrimal glands (Gillette, Greiner, Allansmith, 1981) as well as the status of aqueous deficiency of the tear film (Van Bijsterveld, 1974; Van Bijsterveld, Westers, 1980). In the application of a *Micrococcus* agar diffusion assay (also known as a Schirmer Lysoplate Assay), for example, tear film is collected on filter paper discs and placed on a dish with agar containing 5×10^7 organisms of *Micrococcus lysodeiticus*. The lysozyme in the tear sample is allowed to hydrolyze the peptidoglycans and destroy

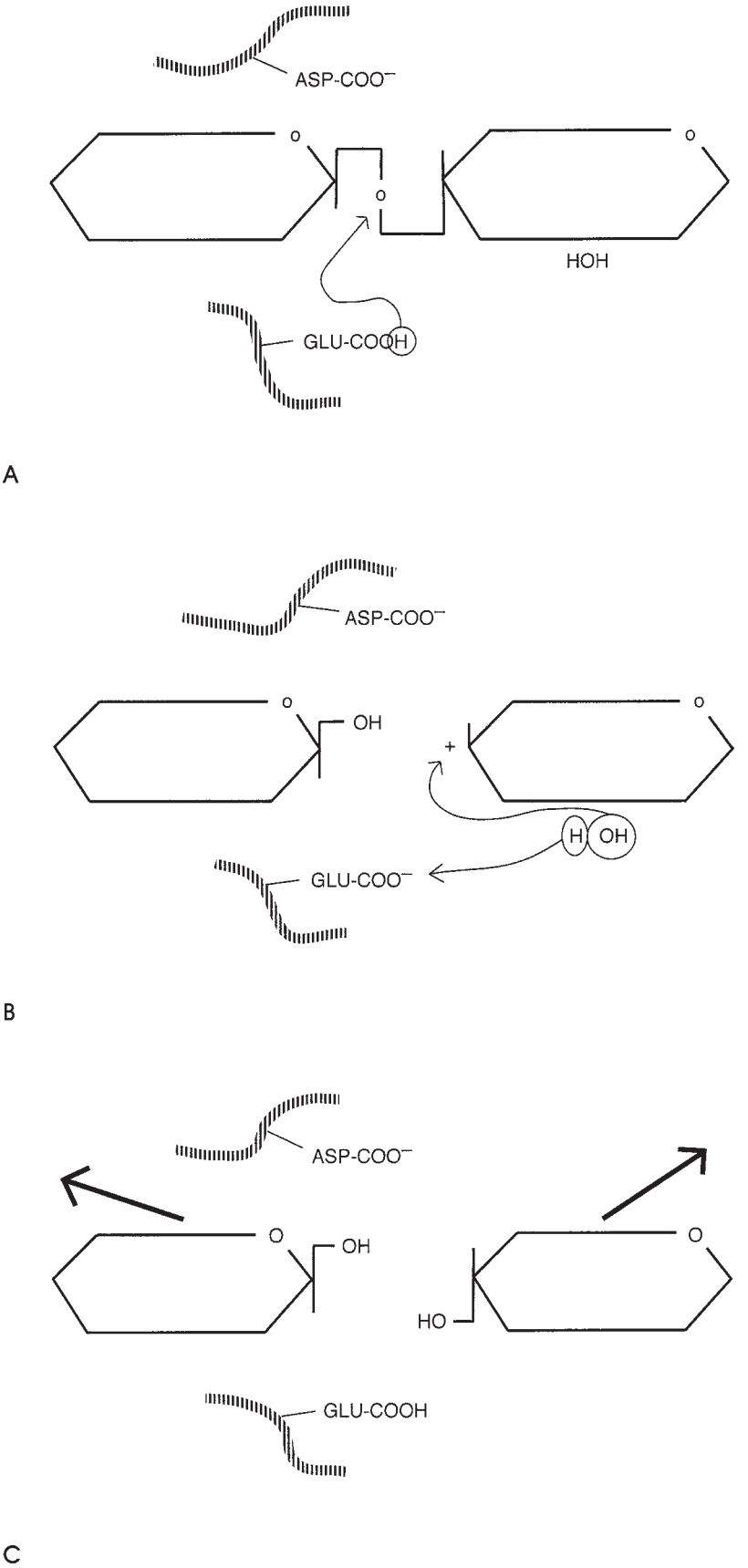


Figure 3–16
Molecular mechanism of lysozyme catalysis at the active site. ➤ (A) A proton is donated by an uncharged Glu residue breaking the glycosidic bond while forming a hydroxyl group in the left hand sugar and a charged carbonium ion (also known as a carbocation) at carbon 4 of the right hand sugar. A negatively charged Asp on the enzyme stabilizes the carbonium ion. (B) A nearby water molecule ionizes to add a hydroxyl group to the carbonium ion and a proton back to the Glu on the enzyme. (C) The two fragments of the peptidoglycan are released from the active site.

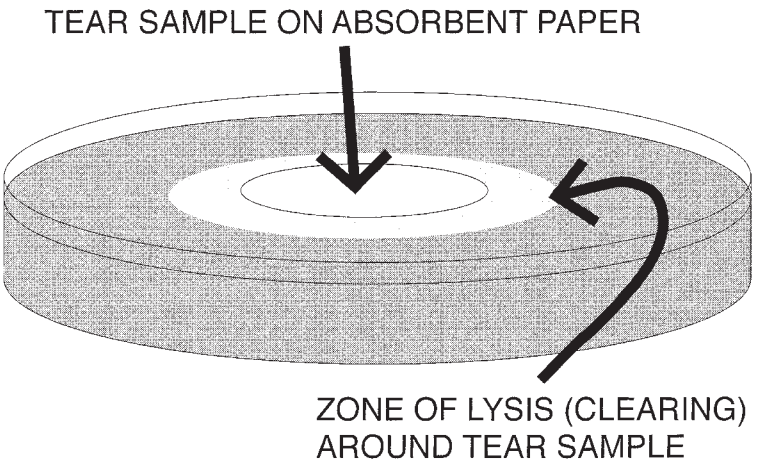


Figure 3–17
The Micrococcus agar diffusion assay. ➤ The tear sample containing lysozyme clears an area (zone of lysis) in an agar base (a gelatin-like product of seaweed) containing a standard amount of the bacterium: *Micrococcus lysodeiticus*. The amount of lysozyme is proportional to the area cleared.

the bacteria for 24 hours at 37°C. Then the diameter of the clear area of agar (i.e., destroyed bacteria) surrounding the tear sample is measured (Figure 3–17). This cleared diameter may be converted to units of enzyme activity or simply interpreted either as normal or indicative of tear dysfunction. More recently, Klaeger and coworkers have developed a more reliable and efficient assay in which enzyme activity is measured from collected tears with the substrate: p-nitrophenyl penta-N-acetyl β-chitopentaoside. This substrate releases the colored product: p-nitrophenol upon enzyme hydrolysis and tear sample lysozyme activity can be analyzed within one hour.

Na, K-ATPase

Sodium, potassium-stimulated adenosine triphosphatase is an enzyme located in the plasma membranes of a wide variety of cells, but in ocular tissues it has two special functions: (1) control of corneal hydration and; (2) the production of aqueous fluid. The enzyme is membrane-bound, which is to say that it is an integral protein that spans the width of cell plasma membranes. Its minimal quaternary structure is strongly postulated to consist of four polypeptide chains: two α and two β chains. This is shown in Figure 3–18. The α chains are the actual catalytic molecules for which the substrate is the high energy compound: ATP (adenosine triphosphate, see Chapter 4). The catalytic reaction is:

$$ATP \xrightarrow{Na,K-ATPase} ADP + P_i$$

Beyond this, the catalyzed reaction is energetically coupled to an ion transport process. Inorganic phosphate (P_i) becomes bound to one of the α-subunits and in the process supplies the energy necessary to transport three sodium ions out of a cell and two potassium ions inward. The exact detailed mechanism remains elusive. However, it is postulated that this may take place either by a conformational shuttle within the α subunits or by the existence of pores in the subunits through which the ions are pumped.

The corneal stroma is a tissue that readily takes up water due to the osmotic pressure generated by the large amount of negatively charged sugar polymers there (see Chapter 4 under "glycosaminoglycans"). In the control of corneal hydration (known as *deturgescence* or literally: "declouding") excess water that leaks into the stroma *via* aquaporin proteins (Li et al, 1999) is pumped back out of the stroma by the counter osmotic pressure generated by the net flow of sodium ions that are transported by Na,K-ATPase into the narrow channel (200 Å wide) (Hogan, Alvarado, Weddell, 1971) between adjacent endothelial cells. That is to say, that Na,K-ATPase pumps sodium ions into the channel where they flow into the anterior chamber following the path of least resistance (diffusion). Water follows the sodium ions as an osmotic function (Figure 3–19). It is reasonable to postulate ionic flow in this direction since a higher density of trapped ions already exists in Descemet's membrane and beyond in the stroma. If the pumping mechanism did not exist, Na^+ ions and water would continuously enter the corneal stroma, causing it to swell and become opaque. A similar mechanism is thought to operate in the nonpigmented epithelial cells of the ciliary body (Figure 3–20). In that case, a surplus of Na^+ ions are pumped into the posterior aqueous chamber by Na,K-ATPase causing water to flow into the chamber osmotically. The enzyme, therefore, indirectly generates an intraocular pressure (Davson, 1990). Bicarbonate ions have also been shown to contribute to this mechanism, but the manner in which this occurs is speculative.

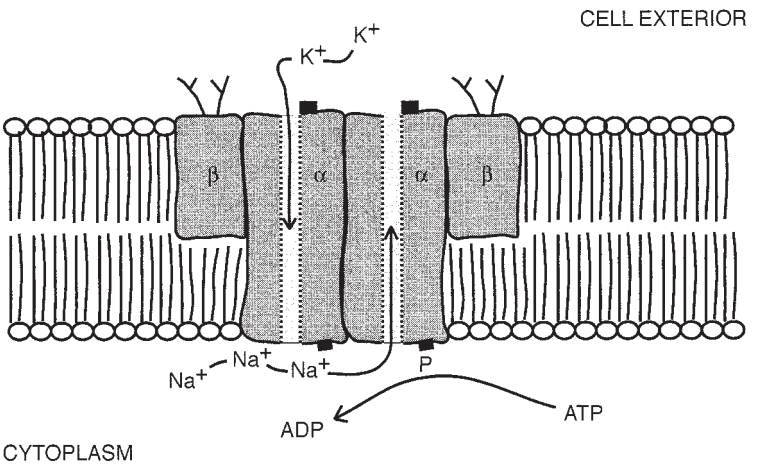


Figure 3-18

Diagram of Na,K-ATPase incorporated in the plasma membrane of a cell. ➤ The two α-subunits catalyze the transport of two K^+ ions inward and three Na^+ ions outward. The two dark rectangles on the cytoplasmic side are binding sites for phosphate and supply energy to drive the transport of these ions. The phosphate is obtained from the hydrolysis of ATP (adenosine triphosphate) to ADP (adenosine diphosphate), which is the true catalytic event of this enzyme reaction. The two dark rectangles on the cell exterior side are binding sites for a cardiac glycoside known as: ***ouabain*** (an inhibitor of this enzyme). The two β-subunits, containing small chains of carbohydrates (the branchlike objects projecting into the cell exterior), are thought to be necessary for the membrane insertion, stabilization, and orientation of the α– subunits.

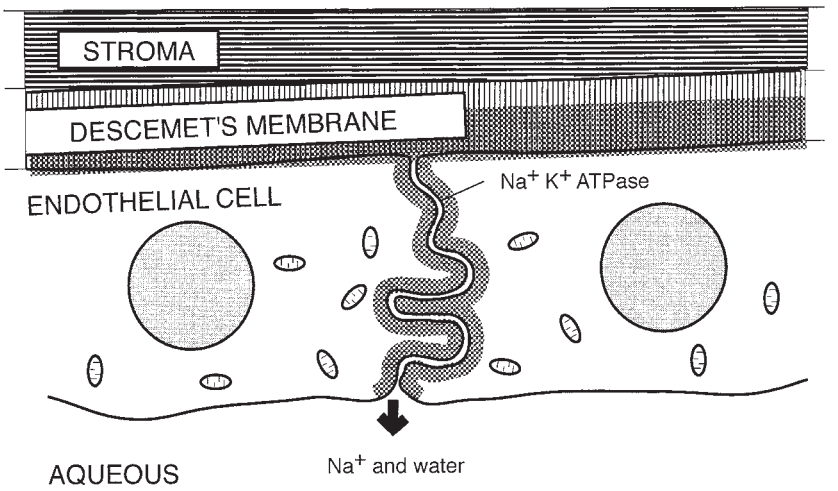


Figure 3–19
Diagram of the endothelial cell side (posterior side) of the cornea. ➤ The location of Na,K-ATPase along the baso-lateral portions of the endothelial cells is indicated by wide grey lines. Sodium ions are believed to be pumped from the space between these cells in order to remove excess water from the cornea osmotically. The osmotic flow of water across the plasma membranes of the corneal endothelium occurs by means of water transport proteins known as *aquaporins.*

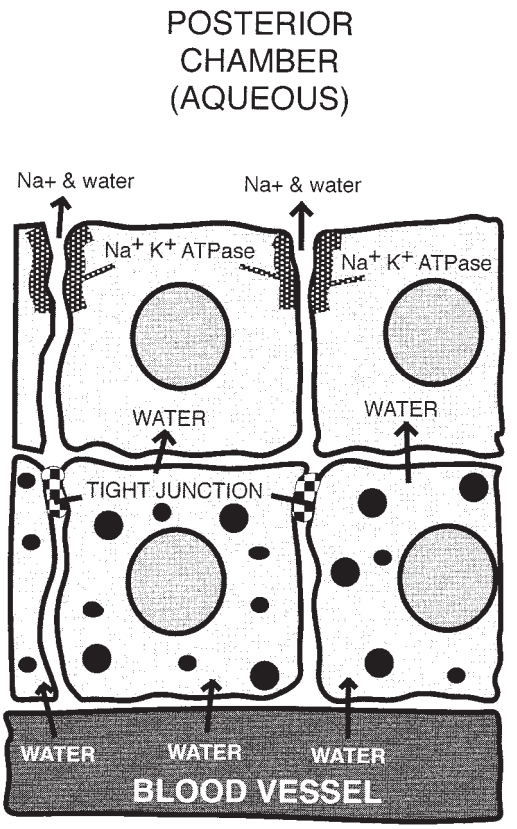


Figure 3–20
Diagram of the ciliary body showing nonpigmented (top) and pigmented (bottom) epithelial cells. ➤ Na,K-stimulated ATPase is located on the baso-lateral membranes of the non-pigmented cells (dark grey portions) where the enzyme pumps excess Na^+ ions into the posterior chamber. Water flows from the adjacent blood vessels (shown on the bottom) through the pigmented and nonpigmented cells and into the posterior chamber because of the osmotic gradient made by this flow of Na^+ ions. The tight junctions between pigmented cells represent a barrier to molecules larger than 20 angstroms. Water flows freely through the cell membranes (*via* aquaporin proteins) and barriers when osmotically directed.

Lactate Dehydrogenase

During the metabolism of carbohydrates (sugars) for the production of cellular energy, a point is reached at which the carbohydrate metabolite will be further processed either aerobically or anaerobically. The aerobic process (or pathway) has the advantage of efficiently producing energy in a high yield while the anaerobic pathway has the advantage of producing energy very quickly without oxygen, but inefficiently in which many more sugar molecules are required. Cells, both ocular and non-

ocular, make use of both pathways. In particular, the corneal epithelium (especially during contact lens wear) and lens fiber cells (which normally are distant from nourishing blood vessels) make use of substantial fractions of anaerobic sugar metabolism. Surprisingly, photoreceptor cells also process a considerable fraction of sugar metabolites along the anaerobic pathway in a process that is actually dependent upon oxygen! However, that process takes place because of the photoreceptor's very high rate of metabolism, which overloads the normally efficient aerobic pathway. Photoreceptors are strongly bent on obtaining energy in any way possible to maintain their primary function of visual transduction. The enzyme that operates at the metabolic junction of aerobic and anaerobic metabolism is lactate dehydrogenase and is found in the cytoplasm of all eukaryotic cells.

The reaction is diagrammed in Figure 3–21. Pyruvate is the metabolic substrate for the reaction and is ultimately formed from dietary carbohydrates such as glucose, fructose, and galactose. A second substrate, the coenzyme NADH (or reduced nicotinamide adenine dinucleotide), provides electrons for the reduction of pyruvate to lactate. The coenzyme is shown in Figure 3–22. NADH is one of a number of coenzymes that are metabolically derived from water-soluble vitamins (Table 3–1). The enzyme itself, which we will abbreviate as LDH, is a protein tetramer (i.e., four polypeptide chains) with a total molecular weight of 140,000. A diagram of the enzyme is shown in Figure 3–23. It might be presumed, since LDH has four subunits, that it is an allosteric enzyme. However, binding studies of the four subunits have shown that there is no interaction between the binding sites of those subunits (Palmer, 1981).

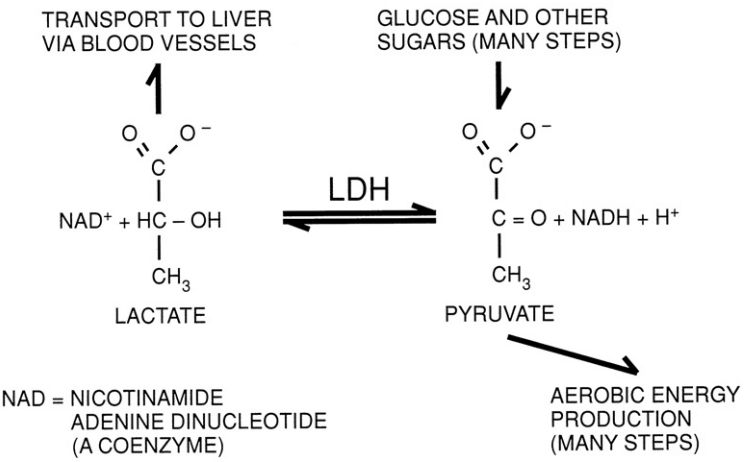


Figure 3-21
Metabolic junction of carbohydrate metabolism between aerobic and anaerobic pathways. ➤ At the top right, glucose and other carbohydrates are converted to pyruvate through many enzymatic reactions (see Chapter 4). If pyruvate is to be converted to cellular energy aerobically, it proceeds along the pathway indicated at the lower right. If pyruvate is to be shunted into the anaerobic pathway, the enzyme lactate dehydrogenase (LDH) catalyzes the reduction (gain of electrons) of pyruvate to form lactate. NADH (reduced nicotinamide adenine dinucleotide, see text) is a cosubstrate for the reaction. The lactate formed is transported ultimately to the liver. The double arrow indicates that the reaction may proceed in both directions and is governed by the amount of pyruvate and lactate present.

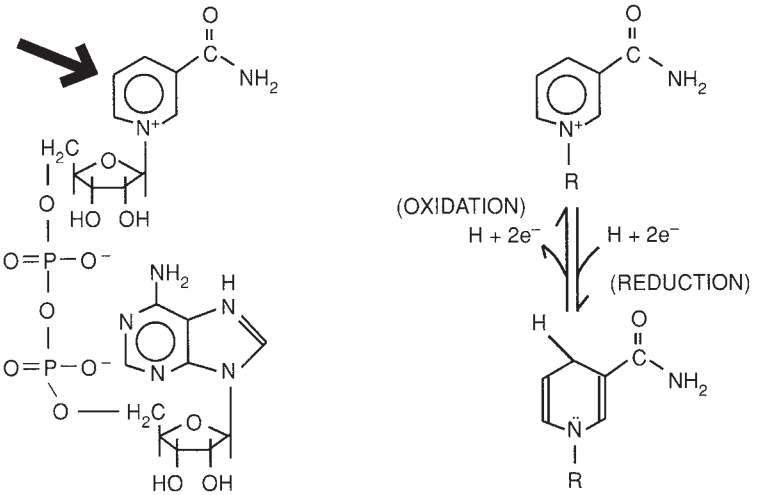


Figure 3–22
Molecule of NAD^+ (nicotinamide adenine dinucleotide) in the oxidized state. ➤ The nicotinamide ring portion is indicated by the arrow and is the site of electron gain (reduction) or loss (oxidation). In fact, both a hydrogen atom (H) and two electrons (e^-) are transferred to and from the nicotinamide ring as shown on the right hand side of the diagram. Oxidation-reduction mechanisms similar to this are common for many of the coenzymes derived from water-soluble vitamins.

Nonetheless, this Michaelis-Menten enzyme has, at least, three different kinds of polypeptides that may occur in various combinations of its tetramers. In this way, the enzyme's kinetics may be "designed" by the cell to carry out specific requirements of the cell's aerobic and anaerobic metabolism. The subunits are designated *H, M,* and *K* where H stands for heart, M represents muscle, and K is a designation first applied to the subunit found in cancer cells (from the Greek: karkinoma, *cancer*). Various combinations of these polypeptides can "customize" the activity of LDH. In H_4, four subunits of the H type are found in heart muscle and tend to support aerobic metabolism. This means that pyruvate formation is favored. In M_4, four subunits of the M type are found in striated muscles and support anaerobic metabolism. This means that lactate formation is favored. The H_3M, H_2M_2, and HM_3 combinations are found in other cell types and support various levels of aerobic or anaerobic metabolism. The K_4 or LDH_K type is found in photoreceptors as well as cancer cells and is characteristic of cells with very high metabolic rates. These different forms (subunits) of the same enzyme are known as *isozymes*. Sometimes the more general term: *isoform* is used to mean the same thing. This distinction of isoforms for LDH is important in ocular tissues inasmuch as the kinetic properties of each enzyme type direct the relative percentage of aerobic and anaerobic pathways to be used by the cells and determine whether the cell will obtain quick energy (anaerobic) or fuel efficient energy (aerobic). One would, therefore, find that kinetically with the H_4 LDH enzyme: (1)

$$(1)\quad \left[\; Lactate \underset{\leftarrow}{\xrightarrow{LDH}} Pyruvate \qquad \frac{[Pyruvate]}{[Lactate]} = Keq > 1 \;\right]$$

In this case, lactate formation is impeded and pyruvate formation is favored.

The M_4LDH form, however, would permit the reaction to proceed equally in either direction. Here the formation of lactate is driven by the concentration of pyruvate so that: (2)

TABLE 3–1 ➤ SOME COENZYMES AND THEIR WATER SOLUBLE VITAMIN SOURCES

Coenzyme	Related Vitamin (and food sources)	Function	Deficiency Causes
Nicotinamide adenine dinucleotide (NAD)	Niacin or Nicotinic acid, Vitamin B_3 (fortified products, bread, pasta, brown rice)	Dehydrogenase coenzyme for electron transfer (glycolysis)	Pellagra (dermatitis, depression, diarrhea)
Nicotinamide adenine dinucleotide phosphate (NADP)	Niacin or Nicotinic acid Vitamin B_3 (as above)	Dehydrogenase coenzyme for electron transfer (pentose shunt)	Pellagra (as above)
Flavin adenine dinucleotide (FAD)	Riboflavin, Vitamin B_2 (eggs, milk, meat, cereals)	Coenzyme for metalloenzymes involved in electron transfer (aerobic glycolysis)	Skin gland malfunction, dryness/ scaling of mouth, photophobia
Thiamin pyrophosphate	Thiamin, Vitamin B_1 (grains, nuts, cereals, rice)	Coenzyme for electron transfer of pentose phosphate shunt enzymes	Berberi (muscle loss, fatigue, depression, loss of eye coordination)
Pyridoxal phosphate	Pyridoxine, Pyridoxal, Pyridoxamine, Vitamin B_6 (cereals, potatoes, nuts, pears, avocados)	Coenzyme for transamination reactions and glycogen lysis	Deficiencies are rare, but may cause similar symptoms to other B vitamin deficiencies
Deoxyadenosyl-cobalamin	Cobalamin, Vitamin B_{12} (fortified foods)	Coenzyme for certain enzymes in amino acid metabolism	Pernicious anemia and brain demyelination
Ascorbic acid	Ascorbic acid, Vitamin C (citrus fruits, vegetables, strawberries)	Reducing agent for many reactions (electron transfer)	Scurvy (incomplete formation of collagen)

(2) $$Lactate \xleftrightarrow{LDH} Pyruvate \qquad \frac{[Pyruvate]}{[Lactate]} = Keq = 1$$

The K form of LDH is one whose catalytic rate increases with the partial pressure of oxygen. One might say that K_{eq} approaches 1 as the pO_2 increases: (3)

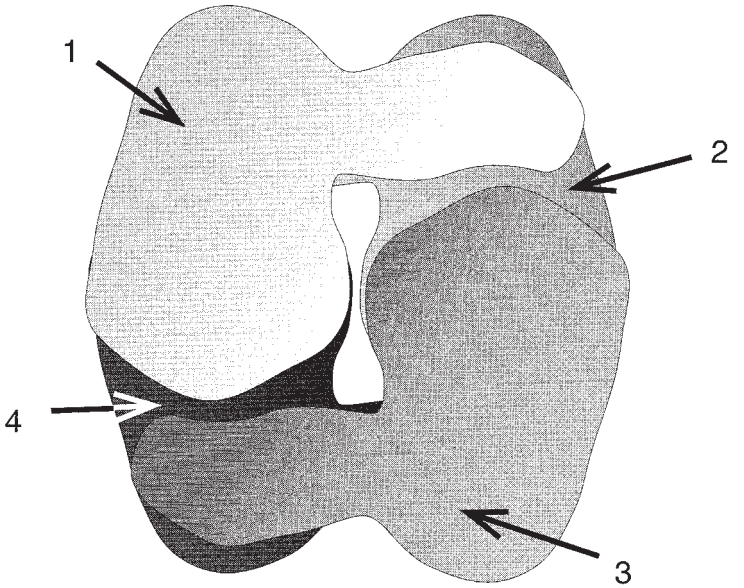


Figure 3–23
Diagram of the quaternary structure of lactate dehydrogenase. ➤ Each subunit, numbered 1 through 4, is indicated by a different shade of grey and each possesses a catalytic site. Although enzymes with multiple polypeptide chains are usually allosteric enzymes, this one is not. The variation in its activity is dependent on the types and combinations of polypeptides that make up its quaternary structure.

(3)
$$\left[\begin{array}{l} Lactate \xleftrightarrow{LDH} Pyruvate \;\; as \; pO_2 \uparrow \\ \dfrac{[Pyruvate]}{[Lactate]} \rightarrow Keq = 1 \;\; as \; pO_2 \uparrow \end{array}\right]$$

In the corneal epithelium, it is important that the cells survive under conditions of relatively low partial pressures of oxygen as occurs during lid closure and, even more so, during contact lens wear when oxygen transmissibility through the contact lens is impeded. Under such conditions, epithelial cells, which normally obtain oxygen from the precorneal tear film, must shunt their carbohydrate metabolism to lactate production via LDH. This mechanism can successfully support epithelial cells down to oxygen partial pressures of about 15 to 20 mm Hg. In the rabbit cornea, it is known that the synthesis of lactate increases at lowered pressures of about 15 to 20 mm Hg. Even in isolated rabbit corneas, the synthesis of lactate increases about 33% when atmospheric air is entirely replaced by nitrogen. Jacq et al (1982) found that the isozyme forms of LDH in the isolated whole human cornea consisted of 25% H_2M_2, 65% HM_3, and 10% M_4 (Figure 3–24). The combination of these isoforms would be favorable to lactate production under conditions of oxygen starvation.

In the whole lens, the dominant population of cells are lens fiber cells whose metabolic rate is low compared to most cell types. When it is considered that the lens is also isolated from a direct blood supply and that the deeper lens fiber cells have no subcellular organelles, it is not surprising that these cells make little use of oxygen for metabolism. Jacq et al (1982) found only HM_3 and M_4 LDH isozymes in whole human lenses (see Figure 3–24).

In the retina, LDH_K acts to open the pathway to lactate when no further pyruvate can be driven through the aerobic pathway. This

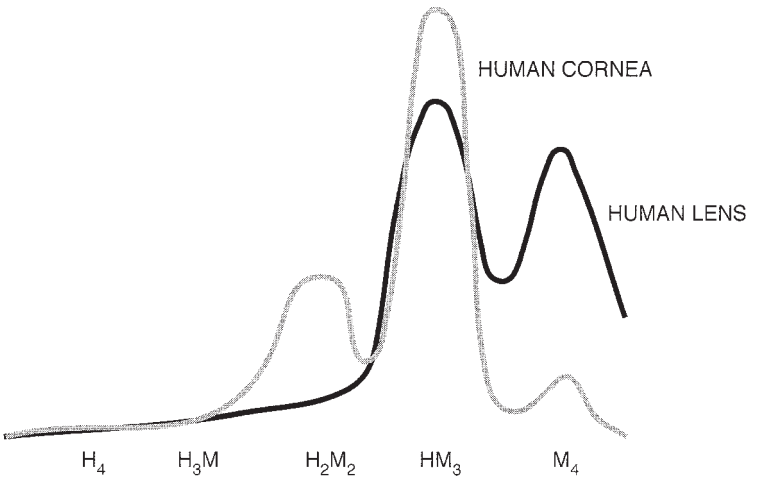
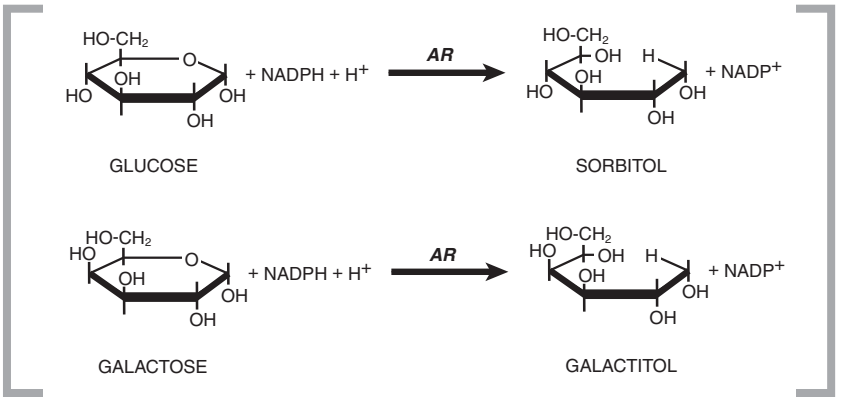


Figure 3–24
Gel density pattern of the isozyme forms of LDH present in human cornea and lens. ➤ The patterns indicate that the cornea is more oriented toward aerobic metabolism than the lens as indicated by the relatively high presence of H_2M_2 in the cornea and relatively high presence of M_4 in the lens.

additional property aids in even more rapid metabolism of glucose since the energy demand in photoreceptors is very high. Saavedra, Cordoba, and Anderson (1985) have described this enzyme form as being quite different from the other commonly found isozymes of LDH. In 1988, Li and coworkers discovered that LDH_K was actually LDH M_4 modified in two ways: (1) phosphate is bound on tyrosine residue #238 near the active site (causing a conformational change) and (2) the enzyme is bound to other proteins. Either or both of these changes may explain its sensitivity to oxygen. The production of lactate in retina is higher than in any other aerobic tissue and the phenomenon of high lactate production coupled with high glucose and oxygen consumption has been termed the *Warburg effect* (Warburg, Posener, Newgalein, 1924).

Aldose Reductase

Another enzyme that is linked to the metabolism of carbohydrates is aldose reductase. The normal role of aldose reductase has been proposed to be an oxidative prevention mechanism (Rittner et al, 1999). As such, it would prevent damage from lipid peroxidation. Another proposal indicates that it is an osmotic regulator (Robinson et al, 1993). Although the catalytic activity of this enzyme is not related to normal carbohydrate metabolism, its activity has been associated with cataract formation in diabetics and in patients having galactosemia. The enzyme belongs to a family of enzymes called aldo-keto reductases (Flynn, 1986). Kador, Kinoshita, and Sharpless (1986) describe the enzyme as having a molecular weight range of 28,000 to 45,000 (depending on the biological source), but consisting of a single polypeptide chain. It is postulated to exist in globular form as with many water-soluble enzymes. The biochemical activity of aldose reductase was discovered from the work of van Heyningen (1959) and Kinoshita (1965, 1974). Aldose reductase catalyzes the following reactions in the lens:



Note that both reactions require the coenzyme NADPH, a phosphorylated form of reduced nicotinamide adenine dinucleotide.

These reactions are the first step of a short two-step pathway known as the *polyol pathway* (This pathway is also discussed in Chapter 4). The intermediate products from glucose and galactose are *sorbitol* and *galactitol* respectively and, chemically, these intermediates are classified as polyols or polyhydroxy-alcohols. Their connection to cataract formation is the fact that polyols can produce an osmotic imbalance in lens fiber cells causing them to swell and eventually burst since they cannot exit the cell. The cataract itself is manifested by the light scattering produced by the cellular debris.

Normally aldose reductase is inactive or nearly so in the lens until the concentration of either glucose or galactose rises to cause its activation. This occurs, of course, in the diseases associated with these carbohydrates as discussed in Chapter 4. Since the K_m for glucose is 100 mM for this enzyme and fasting blood levels are normally about 5 mM, the enzyme's activity is virtually nonexistent in the fasting state. Even after a meal when blood sugar levels may temporarily rise to 10 mM, the enzyme would be minimally activated. In diabetes, however, blood sugar levels may approach and exceed 20 mM for sustained periods if uncontrolled. This can bring about significant activation of aldose reductase.

Much interest has been focused on the development of a therapeutic inhibitor for this enzyme not only as an anticataractogenic agent, but also for the possible prevention of diabetic retinopathy due to aldose reductase activity. Kador, Kinoshita, and Sharpless (1986) explained that there is an inhibitor site on the enzyme that is lipophilic (or hydrophobic) and is, therefore, capable of associating with a wide variety of fat soluble substances that may act as inhibitors. Figure 3–25 indicates that each of the binding sites for the sugar, NADPH, and, an inhibitor is in a separate, but nearby location. The structures of three of the many inhibitors that have been tested are also shown: quercetin, sorbinil, and tolrestat. Quercetin was an earlier studied inhibitor, but was found too weak. Sorbinil has undergone extensive testing, but unfortunately causes an unacceptably high degree of hypersensitivity reactions (Frank, 1990). Tolrestat is a more recently tested compound (Frank, 1990). Unfortunately, no aldose reductase inhibitor has yet been able to meet Federal Drug Administration standards (Harding JJ, 2001).

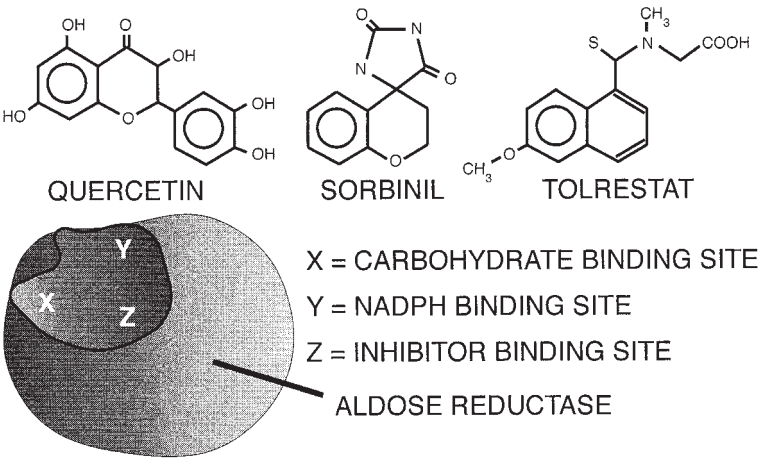


Figure 3–25
Diagram of aldose reductase indicating the relative active site locations for carbohydrates, NADPH and inhibitor binding sites. ➤ On the top are some typical inhibitors that have been tested for this enzyme. Note that all the inhibitors are ring structures with considerable hydrophobicity (a solubility characteristic of a portion of the active site of the enzyme).

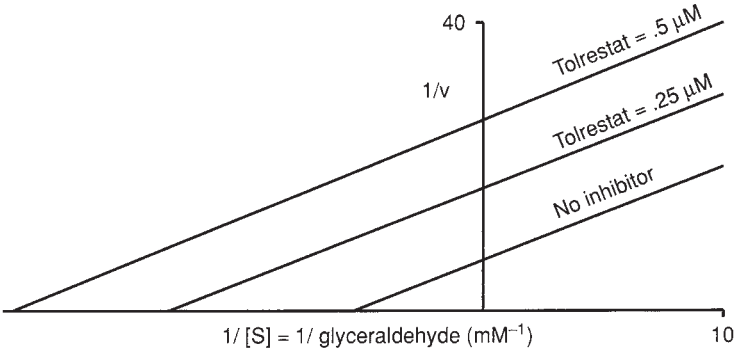


Figure 3–26
Lineweaver-Burk pattern of uncompetitive inhibition of aldose reductase with the inhibitor tolrestat. ➤ Compare this diagram with Figure 3-10 (bottom). The substrate used in this case was glyceraldehyde (a simple sugar). The unit 10 on the *x*-axis stands for the mM concentration of the substrate. The unit 40 on the *y*-axis stands for mmoles/Liter converted to product formed per min. Since this is a Lineweaver-Burk plot, the units on both axes are reciprocal units.

The inhibition of aldose reductase is considered to be either uncompetitive or noncompetitive (Figure 3–26) according to investigations made by Bhatnager et al (1990). The mechanism depends on the inhibitor used.

Matrix Metalloproteinases

More recently, a family of enzymes has been described in the eye that is involved in the breakdown of extracellular matrices or noncellular, tissue architecture (Woessner, 1998). These enzymes are protein hydrolases and are called *matrix metalloproteinases (MMPs.)* They control such functions as: growth and development, tissue maintenance, and tissue reformation and deterioration. The name comes from the metal (zinc) that is required at the catalytic site for the enzyme to carry out its lytic activity. The greater than 17 members of this family include the *collagenases*, *gelatinases,* and *stromolysins*. Although the name collagenase is somewhat descriptive of the enzyme's activity, gelatinase is less so (both forms of gelatinase seem to be specific for types IV and V collagens) while stromolysins are specific for noncollagenous extracellular matrix proteins (Woessner, 1998). In the eye, the great bulk of collagens and other extracellular proteins forces one to consider the importance of these enzymes in such roles as the remodeling of the eye's axial length with the

development of myopia as well as the destruction of corneal collagens following alkali and other chemical burns.

The role of MMPs is to hydrolyze a great variety of extracellular matrix proteins. As with all tissues, the eye also possesses such a variety of matrix proteins. The best, and worse, thing that can be said about MMP matrix protein specificity is that there is a tremendous overlap in what proteins may be broken down. As such, 6 of the 14 well defined MMPs (collagenases 1 and 2, stromelysin 1, matrilysin, as well as gelatinases A and B), for example, will hydrolyze the Gly-Ile bond of the following sequence equally well:

[Gly-Pro-Gln-Gly-*-Ile-Ala-Gly-Gln]

However, minor variations in this sequence, for example substituting Val for Gln on amino acid #3, will cause a considered variation in specificity and activity of individual MMPs. Consequently, in describing and investigating these enzymes, one may be at a loss to say which enzyme acts optimally to bring about corrective or degradative processes in matrix proteins.

The molecular components of these enzymes will include most of the following: signal peptide, propeptide, furin-site insert, catalytic domain,

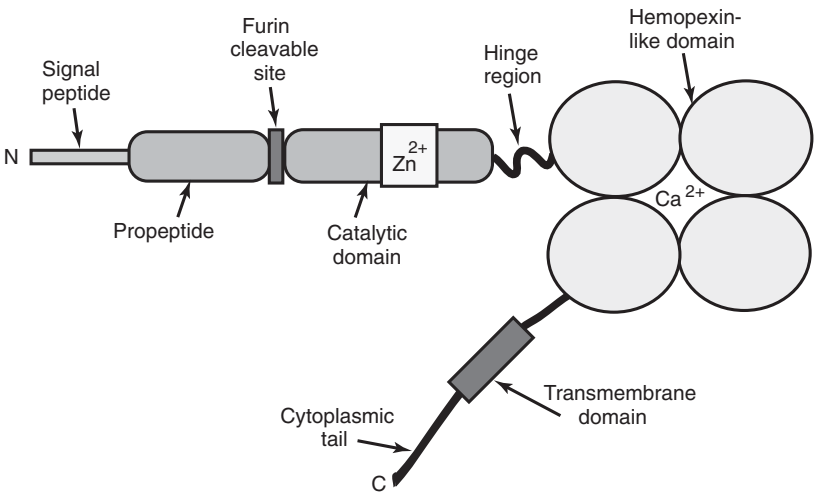


Figure 3–27
Molecular diagram of a *m*atrix *m*etallo*p*roteinase (MMP) in its most complex form. ➤ Usually MMPs do not possess all of these forms. The molecular structure depends on the use of the enzyme in the tissues where it occurs. Characteristics include: signal peptide (ultimate instructions for moving the enzyme from the cell); propeptide (keeps the enzyme in its inactive form until needed); furin cleavable site (involved in enzyme activation); catalytic domain (location of the active site that requires the presence of zinc ions); hinge region (a connecting peptide); hemopexin-like domain (substrate and inhibitor binding sites); transmembrane domain and cytoplasmic tail (needed for insertion into a cell to further extend enzyme activation). (Diagram modified from Knauper V, Murphy G: Membrane-type matrix metalloproteinases and cell surface-associated activation cascades for matrix metalloproteinases, In Parks WC, Mecham RP, editors: *Matrix Metalloproteinases.* San Diego, 1998, Academic Press.)

fibrinlike repeats, hinge region, hemopexin domain, and a membrane insertion extension. The complexity of these components gives the enzyme its capability to perform the varied tasks related to the lysis of collagens and other matrix proteins. Figure 3–27 illustrates a hypothetical enzyme with all of the possible components found in such enzymes. In the figure, the *signal peptide* is an area that "directs" the cell to move the newly synthesized enzyme into the endoplasmic reticulum for transport out of the cell. The *propeptide* maintains the enzyme in its inactive form, until needed, by inhibiting the critical zinc atom that is necessary for catalytic activity. Consequently, activation of the enzyme is dependent upon changes or chemical signals made to the propeptide region. The *furin cleavable site* is one of the highly specialized areas within the propeptide that bring about activation of the enzyme. Furin is a Golgi associated proteinase that cleaves the propeptide region to cause activation. The *catalytic domain,* containing the zinc ion, also binds to calcium ions and actually carries out the catalytic activity of the enzyme. The *hinge* region connects the catalytic domain with the hemopexin domain. The *hemopexin domain* (so named because of its similarity to the protein hemopexin, a heme binding protein) carries out at least two functions: (1) substrate specificity binding; and (2) binding to a natural inhibitor, a tissue inhibitor of metalloproteinase or TIMP (Paoli et al, 1999). Last, the *transmembrane domain* and *cytoplasmic tail* are used to physically extend the enzyme into cells that express MMPs and serve as an additional molecular mechanism for activation of the enzyme (Woessner, 1998).

It is emphasized that control (activation and inhibition) of MMPs is very important and this, therefore, explains the complexity of the enzyme structure. It is also pointed out that there are considerable variations in the presence of specific domains of individual MMPs to reflect the roles of each enzyme in particular tissues. This is also true of variations in the amino acid sequences of the domains as well.

An example of the role played by MMPs in ocular tissues has been shown in a study conducted by Rada et al (1999). In myopia or nearsightedness, the ocular globe is lengthened along its anterior-posterior axis. This condition is associated with remodeling or reformation of the extracellular matrix proteins in the posterior sclera. Rada's group studied levels of MMP-2: gelatinase A and its known inhibitor: TIMP-2 when chick eyes were *form deprived* to induce myopia. As shown in Figure 3–28, the mRNA, used to synthesize gelatinase A, was increased by 125% over its control tissue while the mRNA, used to synthesize TIMP–2, was decreased by 75% of its control tissue. When form deprivation was discontinued for 24 hours, the mRNA for gelatinase A decreased by 50% of control while the mRNA for TIMP–2 increased by 12%. Similarly, in the tree shrew (a mammalian species that serves as a model of human myopia), it was found by Guggenheim and McBrien (1996) that MMP-2 increased threefold after 5 days of form deprivation. When form deprivation was halted after 3 days, the levels of MMP-2 were fivefold lower than the deprived eyes. These results indicate that controlled gelatinase A activity plays a role in the process of lengthening the axis of the globe, when myopia is induced, by partially digesting scleral proteins so that new proteins can be formed in the sclera to establish a new scleral length.

Whenever a cornea becomes damaged as a result of exposure to strong chemicals such as acid or alkali, the damaged proteins are often removed from the cornea by the action of MMPs. Unfortunately, the process may also weaken the cornea, to the extent that it perforates or

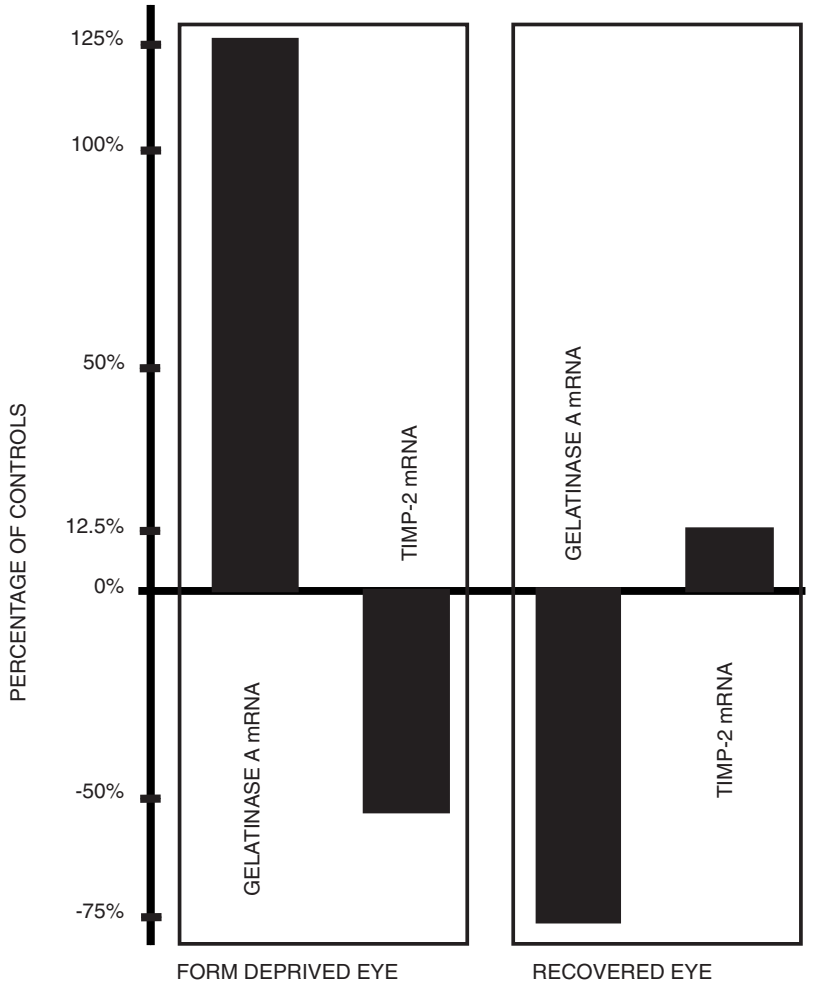


Figure 3–28
Messenger RNA (mRNA) of gelatinase A and TIMP-2 in form deprived vs. recovered eyes. ➤ Levels of specific mRNAs are an indication of the availability (synthesis) of a given protein. Compare the high level of mRNA for gelatinase A in the form deprived eye, left, with the low level of mRNA for gelatinase A in the recovered eye, right. Similarly, note how low mRNA for the tissue inhibitor of metalloproteinase-2 (TIMP-2) is in the form deprived eye (left) vs. the higher level in the recovered eye, right. These data suggest that extracellular matrix proteins are being broken down during form deprivation. For more information on mRNAs, see Chapter 7. (Figure redrawn from Rada JA, et al: Gelatinase A and TIMP-2 expression in the fibrous sclera of myopic and recovering chick eyes, *Invest Ophthalmol Vis Sci* 40:3091–3099, 1999.)

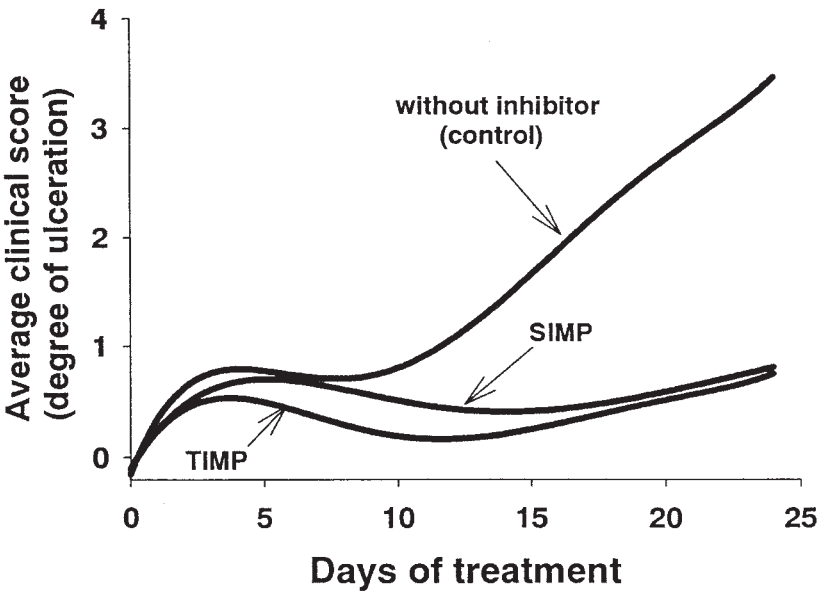


Figure 3–29
The effect of MMP inhibitors on corneal ulcer development. ➤ The graph shows the progress of corneal ulceration on rabbits after alkali injury with 2 N NaOH. After 24 days, ulceration in the controls extended to almost complete destruction of the stroma (descmetocele formation or Descemet's membrane bulging forward), clinical score of four. However, with the use of either a synthetic inhibitor (SIMP) or a natural, tissue inhibitor (TIMP) of MMP the destruction was kept within the first third of the cornea, clinical score of one. (Redrawn from Patterson CA, et al. Recombinant tissue inhibitor of metalloproteinases type 1 suppresses alkali-burn–induced corneal ulceration in rabbits, *Invest Ophthalmol Vis Sci* 35:677–684, 1994.)

breaks structurally, a process that could result in the loss of the eye (Parrish, Chandler, 1998). It has been shown that MMPs also have a role in this degratory process. This was accomplished by studying the effects of both artificial and natural inhibitors of MMPs, on limiting the process of corneal ulceration, following alkali burns of the cornea in rabbits (Wentworth, Paterson, Gray, 1992; Paterson et al, 1994). In Figure 3–29, it can be easily seen that alkali burned corneas that had been exposed to β-mercaptomethyl-Leu-Phe-Ala (a SIMP or *s*ynthetic *i*nhibitor of *m*etallo*p*roteinase) maintained the burned tissue at a clinical score of approximately 1 for 15 days (where 1= a superficial ulcer at a depth of no more than one-third of the anterior tissue). Tissues not exposed to the SIMP achieved a score of 3 or worse after 4 days (where a score of 3 = a deep ulcer to the posterior one-third of the tissue). When a similar test was run with a TIMP inhibitor (*t*issue *i*nhibitor of *m*etallo*p*roteinase) a natural polypeptide usually secreted with the enzyme ulcer formation was equally inhibited (Nigase, 1998).

SUMMARY

Enzymes are proteins that act as biological catalysts for a variety of cellular and extracellular reactions. In the eye, enzymes promote many of the same reactions found in other parts of the body. However, some enzymes have specialized activities related to ocular function and repair. For example, lysozyme acts in the precorneal tear film to destroy gram positive bacteria by the hydrolysis of their peptidoglycan coats. The enzyme may also be used to diagnose normal tear production. Sodium, potassium activated ATPase acts in the corneal endothelium to maintain a normal rate of deturgescence. The same enzyme also generates the intraocular pressure that originates in the ciliary body. Both functions occur as a result of enzymatic cation pumping and the generation of osmotic flow. Lactate dehydrogenase is an enzyme involved in shunting carbohydrate metabolites between aerobic and anaerobic metabolism. This is accomplished by having the enzyme exist in several forms (isozymes). In the retina, a special isozyme exists to promote maximal production of cellular energy. Aldose reductase is a lens enzyme that is normally inactive. In diabetes and galactosemia, however, it catalyzes the formation of polyols that bring about the osmotic destruction of lens fiber cells. Considerable effort has been made to develop an inhibitor for this enzyme. Matrix metalloproteinases act to remodel the shape of the ocular globe, a function that if misdirected, can lead to myopia. Because of chemical burns to the eye, matrix metalloproteinases can also cause excessive degradation of matrix proteins to affect corneal ulceration.

PROBLEMS

1. Explain why a substrate with a high K_m value does not make a good substrate for an enzyme.

2. The following data pairs were collected for an enzyme catalyzed reaction: 0.19 mM (0.19 units); 0.22 mM (0.22 units); 0.26 mM (0.25 units); 0.40 mM (0.31 units); and 1 mM (0.44 units). The first number of each set is the substrate concentration while the second number in parentheses is the enzyme activity in units. Construct a Lineweaver-Burk plot from the data and determine the K_m of the substrate.

3. If the $V_{max}/2$ of an allosteric enzyme is always the same, why is it possible for the K_{app} to have different values?

4. In the case of a noncompetitive inhibitor, the V_{max} for a given enzyme is known to be 35 units (where 1 unit = 2 mmoles of product formed per sec). If 0.5 mM of inhibitor is used in the reaction, what would the K_i of the inhibitor be if the y-intercept of the Lineweaver-Burk plot = 0.02/mmoles/sec?

5. If the V_{max} of aldose reductase (the enzyme that contributes to diabetic cataracts) is 32 mmoles/sec for a given substrate, what concentration of an inhibitor with a K_i of 0.6 mM would be needed for the enzyme to have 50% of V_{max} activity? Here the y-intercept at V_{max} = 0.0625/mmoles/sec.

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CHAPTER 4

Carbohydrates

General Characteristics of Carbohydrates

Sweet tasting substances have been known since ancient times. However, sugars were only isolated as chemical substances in the 18th and 19th centuries (Roehrig, 1984). For example, one of the most common sugars, glucose, was isolated by Dumas in 1838 from the hydrolysis of starch. The structures of glucose and other sugars were determined by Emil Fischer just after the turn of the century. The name sugar (which comes from the Sanskrit word shakará) is generally replaced in biochemistry by the term carbohydrate, which literally refers to a compound made of carbon and water with the general formula: $C_n(H_20)_n$. However, the term carbohydrate is now used more widely to include polyhydroxy compounds, which may also contain aldehydes, ketones, alcohols, acids, and amines as well as their derivatives. The simple carbohydrates that are used for cellular foods (fuels) are either aldehydes or ketones, whereas the other types often serve to support tissue morphology (form) (Figure 4–1). The notable exceptions are the polymer storage forms of glucose: glycogen (in animals) and starch (in plants).

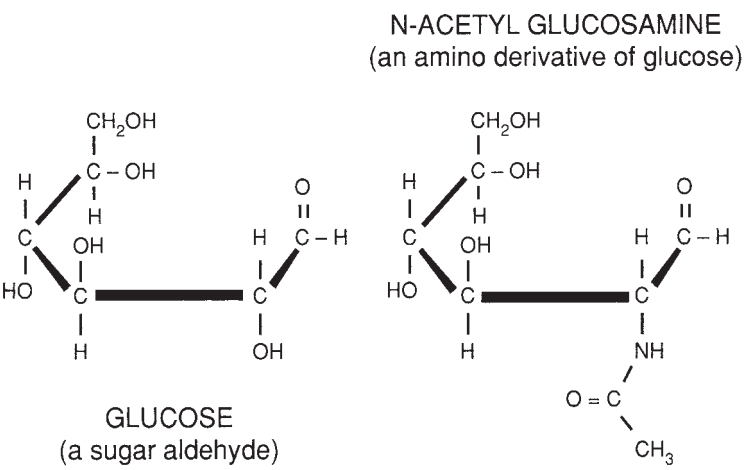


Figure 4–1
Two examples of carbohydrates: glucose and N-acetyl glucosamine. ➤ Note the aldehyde group on both, and that both have multiple hydroxyl groups. The latter characteristic increases their water solubility properties. N-acetyl glucosamine is metabolically derived from glucose.

Carbohydrate structure may be represented in three ways called: *Fischer*, *Haworth*, and *conformational* as shown in Figure 4–2. The Haworth structure represents a reasonable compromise between the misleading Fischer structure and the somewhat confusing (but accurate) conformational structure. The Haworth structure will be used in the remainder of this book. In these figures, it should be understood that the heavier lines are the closest to the reader. Hydrogen atoms are implied at the end of each vertical line where no element or functional group is given and the carbons within the ring are not written.

Carbohydrates are capable of extensive *isomerization* either on their own or by the action of cellular enzymes. In the case of the simple carbohydrates (e.g., glucose or fructose), C-1 can form an oxygen bridge by itself with C-5 to construct a six-membered, closed ring known as a *pyran* ring or C-2 can form an oxygen bridge with C-5 to form a five-membered closed ring known as a *furan* ring. These simple carbohydrates form rings about 99% of the time while in solution (Figure 4–3). C-1 in a six-membered closed ring is known as the *anomeric carbon* since it is the center of the two configurational isomers (α and β). Although the presence of the aldehyde form is minimal, C-1 of the aldehyde form is quite reactive (C-1 is also known as a *reducing carbon*) since it will donate electrons to other substances. This property of the anomeric carbon has served as the basis for determining the concentra-

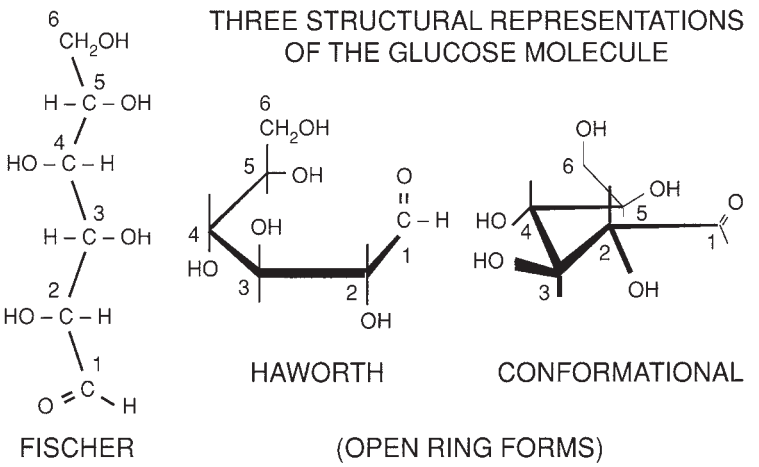


Figure 4–2
The Haworth structure is a commonly used representation for carbohydrates although the conformational structure is more accurate. ➤ The use of the latter structure makes it difficult to distinguish individual carbohydrate types in three-dimensional representation. The Fischer structure is the least accurate of the three representations.

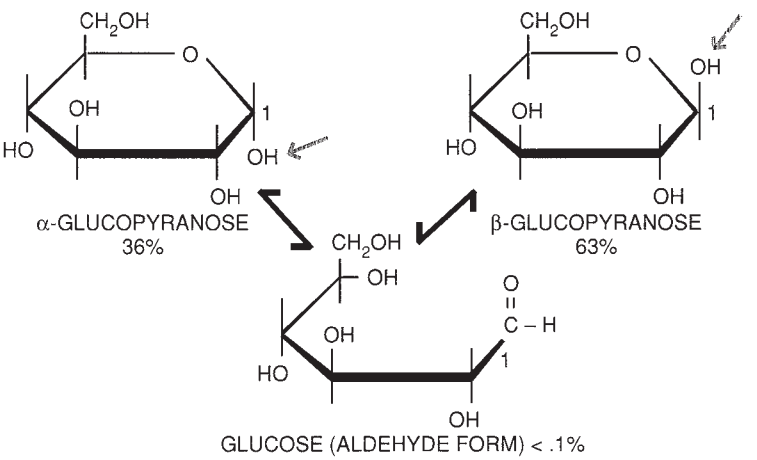


Figure 4–3
In solution, monosaccharides constantly alternate their isomeric forms by opening and closing their ring structures. ➤ The percentages indicate which forms are present at any given time. By convention, when the hydroxy group on carbon number one is down (in the figure), the isomer is designated as the α form, and when it is up, it is the β form.

tion of glucose in blood and urine. For example, the aldehyde of C-1 reacts with o-toluidine to form a colored covalent complex giving a reliable estimate of glucose concentrations (Caraway, Watts, 1986) as shown in Figure 4–4. It has also been discovered that C-1 will react with proteins forming a permanent bond when present in high concentrations, as occurs in diabetes (Cohen, 1986). This will be discussed in some detail later in this chapter.

The most common five- and six-member ringed carbohydrates of biological importance are *glucose*, *galactose*, *mannose,* and *fructose*. These carbohydrates are called *monosaccharides* [from the Greek *monos* (single) and *saccharon* (sugar)]. Each sugar consists of a single ring as shown in Figure 4–5. Although glucose may be considered the most important sugar, all four carbohydrates have nutritional value for cells and serve as metabolic building blocks for more complex carbohydrates. This statement applies equally to ocular tissues.

Two-sugar units are also quite common. They are called disaccharides (which means, literally, two sugars). Some of these are *maltose, sucrose, and lactose*. Maltose is a disaccharide, which occurs as the result of the hydrolysis of *starch,* and can be found in germinating cereals and grains. Starch consists of two glucose units held together by an oxygen bridge (Figure 4–6). Sucrose, or common table sugar, comes from a variety of plants such as cane, beets, pineapple, and carrots.

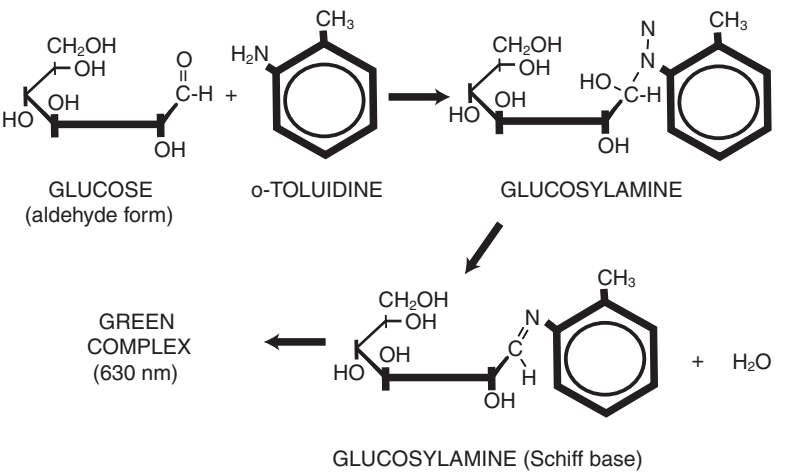


Figure 4–4
A common laboratory procedure (there are several) for determining the concentration of glucose in blood (and other body fluids) is the o-toluidine method. ➤ Ortho-toluidine forms a Schiff base with glucose (as a glucosylamine). Subsequently, the glucosylamine reacts to produce a green complex of undetermined structure (Carraway and Watts, 1986) that is proportional to the glucose concentration and may be read in a spectrophotometer.

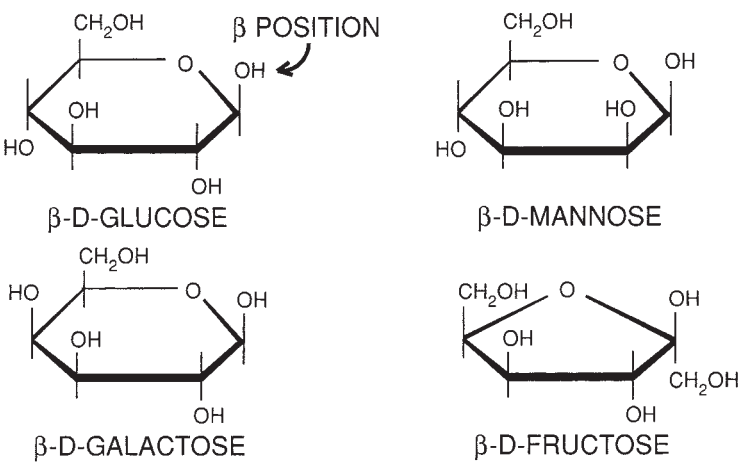


Figure 4–5
The four common monosaccharides that are used as sources of ATP as well as for synthesizing building blocks of extracellular polymers after metabolic processing.

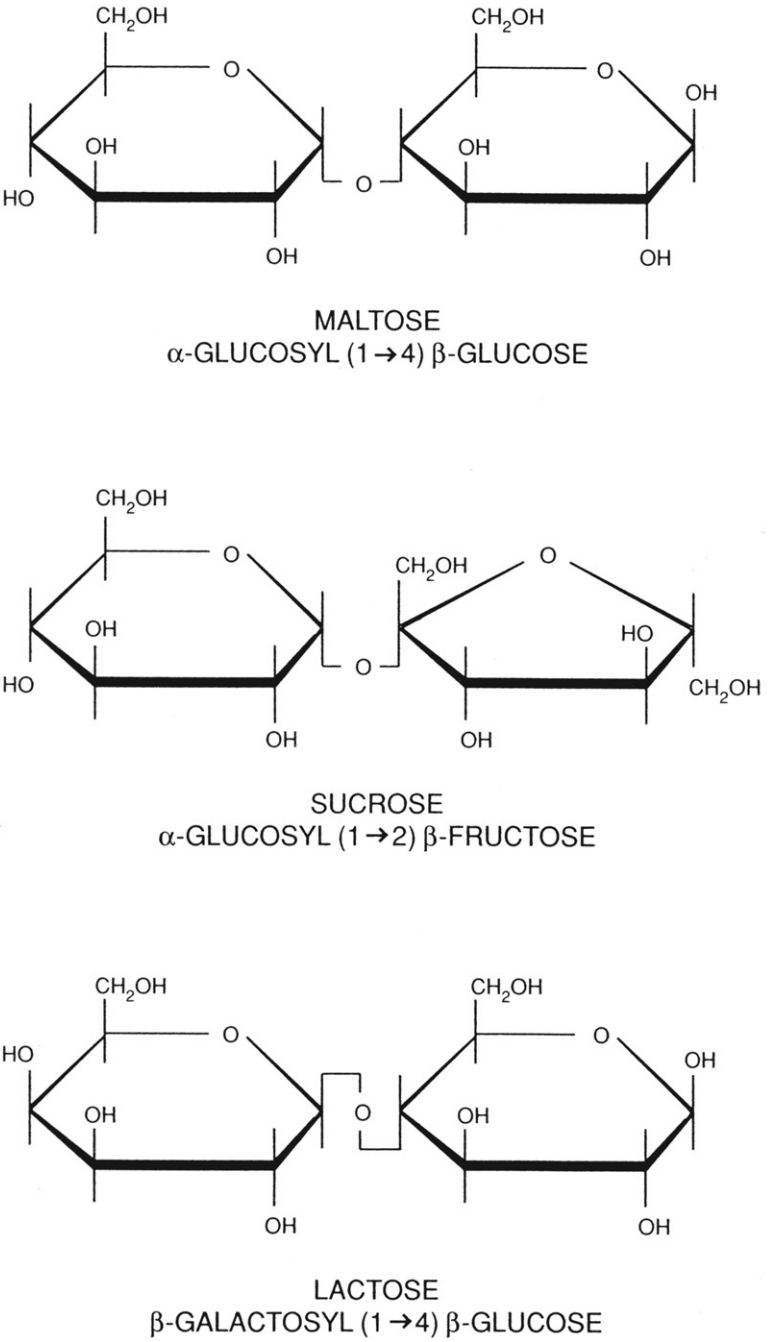


Figure 4–6
Three common disaccharides that occur in the diet. ➤ Maltose is a hydrolysis product of starch, where sucrose is common table sugar. Lactose is milk sugar. See text for further description.

Sucrose is composed of one glucose and one fructose molecule also joined by an oxygen bridge as shown in the same figure. Lactose is the disaccharide found in milk and is made up of galactose and glucose joined by an oxygen bridge as shown. The nature of the oxygen bridge varies in disaccharides. In maltose, it is known as an $\alpha(1\rightarrow4)$ linkage, which means that the oxygen bridge joins C-1 of the left hand unit to C-4 of the right hand unit. In the bridge, the position of the oxygen joining C-1 is α (or "down") relative to the carbon in the Haworth conventional usage discussed previously. In sucrose, the linkage is an $\alpha(1\rightarrow2)$ type. Here, however, in the convention used, the fructose unit

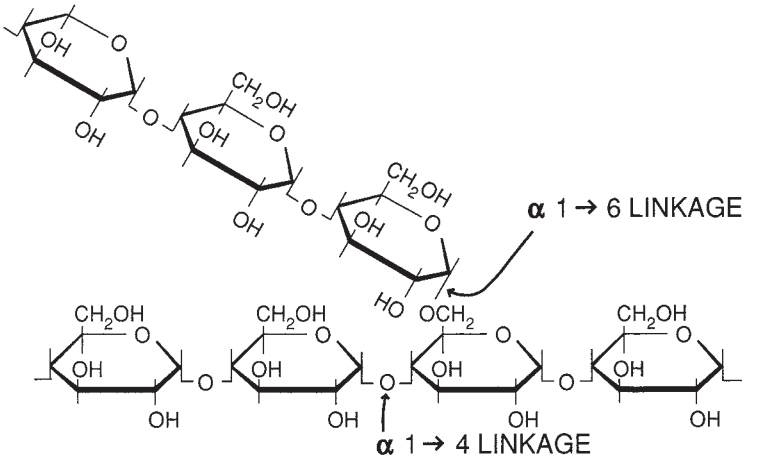


Figure 4–7
Partial structure and bonds of storage forms of carbohydrates in animal and plant cells. ➤ All forms have glucose molecules linked in α1→4 bonds. Glycogen (in animals) also has α 1→6 bonds (branches), which may occur as frequently as every sixth carbohydrate. Amylopectin (in plants) has α 1→6 branches less frequently than glycogen. Amylose (also in plants) has no α 1 →6 branches.

(the right hand, five-membered ring) is flipped over so that what appears to be C-5 is actually C-2. In lactose, the linkage is a β(1→4) type. That is, the oxygen linkage is β (or "up" in the convention used) relative to C-1 on the galactose unit. In disaccharides, only the right hand six-membered ring carbohydrate (as shown in the figure) may have a reactive or reducing carbon.

Carbohydrates with more than two units are known as either *oligosaccharides* (a few sugars) or *polysaccharides* (many sugars) depending on their chain length. The division is arbitrary. However, when the number of saccharide units or rings is greater than 10, polysaccharide or *glycan* are the preferred terms (Roehrig, 1984; Hecht, 1999). Polysaccharides serve two principal functions in all biological tissues, a storage function and a structural (morphological) function. Storage polysaccharides in animal tissues have a greater diversity of branching linkages compared to those in plants. The storage form of polysaccharide in animals is known as glycogen and the two most common forms in plants are *amylose* and *amylopectin* (collectively known as starch). The straight and branching linkages are shown in Figure 4–7. Glycogen may approach a molecular weight of approximately 10^5 to 10^7 D with α(1→6) branching occurring anywhere from every 6 to 21 glucose units as shown by animal studies (Hecht, 1999). The extensive branching of glycogen causes the molecule to be very compact, which is highly desirable for storage purposes (Figure 4–8). Glycogen is kept in the cytoplasm of, primarily, liver and muscle cell (Stryer, 1988). In the eye, some tissues have cells that are known to maintain glycogen stores such as the corneal epithelial cells and the retinal Müller cells in particular. Some ocular cells do not contain glycogen, such as corneal endothelial cells and retinal photoreceptors. The latter use glucose at such a high rate that they cannot store it. Structural polysaccharides will be considered later in this chapter.

Energy Metabolism

Carbohydrates play two principal roles in ocular and nonocular tissues: (1) they contribute to metabolism by acting as fuels and (2) they are significant constituents of biological structures. Here we consider the first role. However, it is necessary to consider first the nature of metabolism

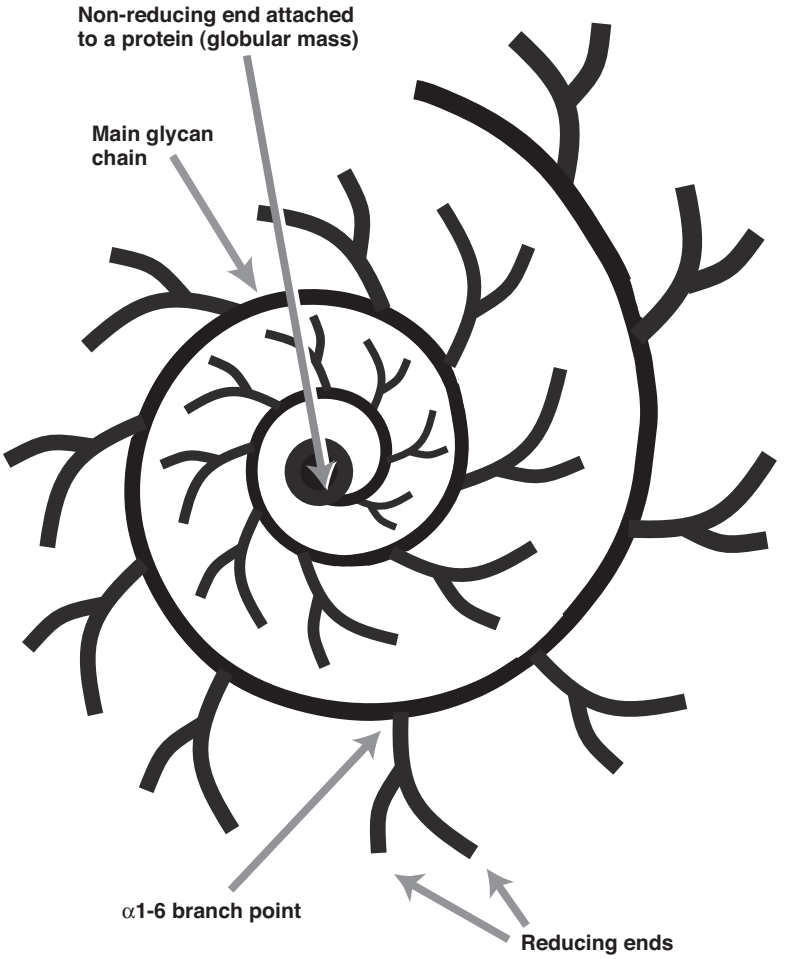


Figure 4–8
A diagrammatic and simplified representation of a glycogen molecule based on the structure of amylopectin, a glycan found in plants. ➤ Glycogen, however, has more branch points than amylopectin. The structure (at the nonreducing end) is anchored into a protein known as glycogenin. The molecule spirals outward toward the viewer from the protein. (Redrawn from Lehninger AL, Nelson DL, Cox MM: Principles of Biochemistry, ed 2, New York, 1993, Worth Publishers, p. 309.)

itself. In order to exist and carry on the business of living, all cells must have a system for producing and using energy while following two laws of thermodynamics: the first law states that *energy can be transferred* (into or out of a cell), *but cannot be made or destroyed*, and the second law states that *any system* (e.g., a cell*) tends to lose energy* or go to a state of maximum disorder *if left by itself*.

This seems simple enough. It means that cellular tissues must obtain an energy source, such as glucose, from outside its boundaries and convert the energy within glucose into a useful form that will run cell processes, which are constantly running down (i.e., burning energy). That, in a nutshell, is what the physical biochemistry of metabolism is all about.

How does a cell handle this? It carries out a series of chemical reactions involving energy production and energy consumption that are respectively called: *anabolic* and *catabolic processes*. Anabolic processes in cells are those reactions that synthesize cellular components and maintain its functions (i.e., they use energy). These reactions are coupled to catabolic processes, which extract energy from compounds such as glucose and allow the cell to carry out its anabolic reactions, for example, making proteins, building cell walls, or causing visual transduction. Instead of requiring heat energy (the same kind of energy that a car needs to run), cells use high-energy compounds to drive their synthetic (anabolic) reactions. The high-energy compounds, therefore,

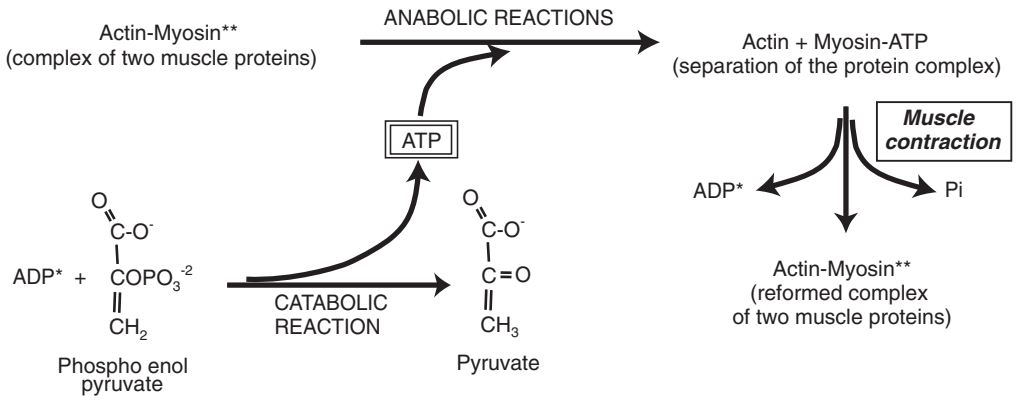
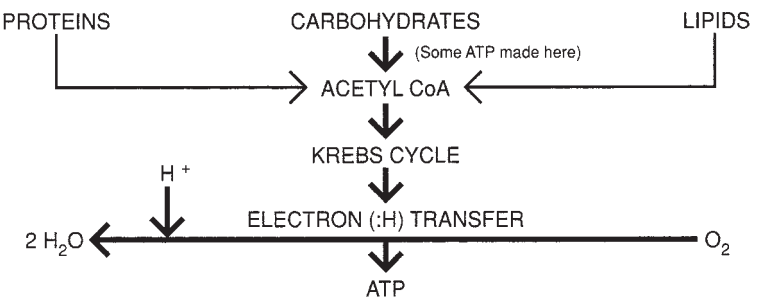


Figure 4–9
An example of coupled anabolic and catabolic reactions. ➤ The figure shows how high energy adenosine triphosphate, initially generated by glucose via the Embden-Meyerhof pathway, is used to cause muscle contraction.

provide the connection between anabolic and catabolic reactions. An example of a coupled series of anabolic and catabolic reactions is shown in Figure 4–9. Here phosphoenol pyruvate is used in a catabolic reaction to form high-energy adenosine triphosphate (ATP) from adenosine diphosphate (ADP). The ATP bridge compound transfers its energy to the muscle protein myosin to initiate muscle contraction in a series of two anabolic reactions. The reformed ADP may then be used in the next catabolic reaction.

The most important of these high-energy compounds is *adenosine triphosphate* or ATP. This compound, shown in Figure 4–10, has three different kinds of molecular components: adenine, ribose, and three phosphate groups. The adenine and ribose portions act as "handles" to position the molecule at enzyme or protein reactive sites so that it may release its potential energy by breaking the outermost phosphate bond. This action either "powers up" an enzyme for catalytic work or increases the potential energy of a protein for some biological task. The latter is exemplified by Figure 4–9. Bridger and Henderson (1983) have stated that the potential energy in ATP stems from the crowded negative charges of the phosphate groups and the somewhat constricted ability of the electrons to move about (delocalize) within the phosphate groups. Carbohydrates act on cells to return relatively lower energy adenosine diphosphate (ADP) to its higher energy ATP form and maintain this cellular energy system. In fact, not only carbohydrates but also lipids and proteins are capable of "making" ATP according to the following general metabolic scheme:



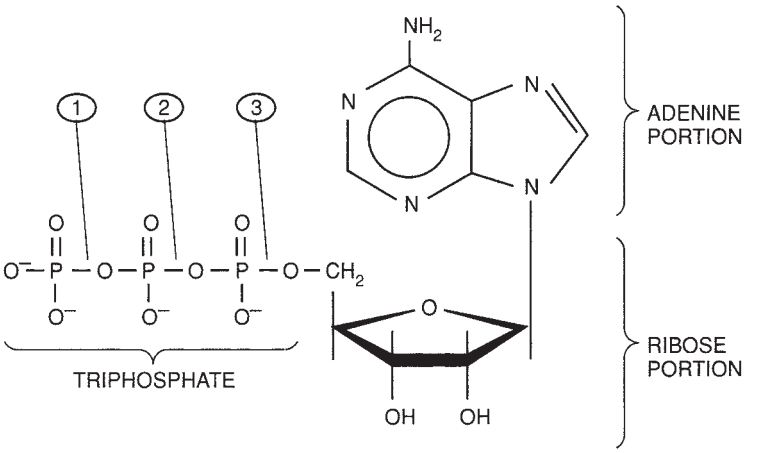


Figure 4–10
Adenosine triphosphate (ATP). ➤ Energy is released from this compound with the hydrolysis of each phosphate group. Usually, only the outermost phosphate is released to form adenosine diphosphate (ADP). The release of the outermost phosphate group will transfer 31 kJ of energy under standardized conditions (pH 7, 25°C). However, under actual conditions in cells that energy transfer may be estimated to be in the range of 50 kJ (Mathews, van Holde, 1990). For calorie counters, the equivalent amounts are 7.41 and 11.95 kilocalories, respectively.

In this scheme acetyl CoA acts as an intermediate two-carbon unit (acetate) coupled to a carrier molecule (coenzyme A). Although the two-carbon unit can be made from proteins and lipids as well as carbohydrates, carbohydrates are the most common and immediate sources of acetyl-CoA for ATP production. In fact, the use of proteins and lipids to make ATP can be pathological (or destructive) to tissues when that usage becomes excessive. This can occur in either diabetes mellitus or in starvation.

Metabolic pathways, from carbohydrates to acetyl CoA through the Krebs cycle to ATP production, consist of a series of biochemical reactions, which are all enzyme catalyzed. The electron transfer, shown in the previous scheme, is caused by the Krebs cycle and is an important source of efficient ATP production. This will be explained further on. These metabolic reactions may occur in the presence (*aerobic metabolism*) or absence (*anaerobic metabolism*) of oxygen. Up to the generation of acetyl CoA, all reactions take place in the cytoplasm of the cell. Beyond that stage, they occur in the mitochondria. The storage of carbohydrates (as glycogen) as well as the synthesis of lipids (as fatty acids) and nucleic acids (as pentoses) and cell detoxification are also linked to these pathways. The pathways are outlined in Figure 4–11. All ocular cells, as well as other animal cells, make use of these pathways in varying degrees.

GLYCOLYSIS

When carbohydrates are consumed, either as monosaccharides or in some chain form (such as starch), they enter the circulation (after being converted enzymatically to monosaccharides as necessary) and travel from the gut to individual cells, which they enter. How they enter cells will be considered further on. In the cellular cytoplasm, carbohydrates are immediately phosphorylated to prevent their escape from the cell. This is so, inasmuch as the negative charges on the phosphate group will not allow the carbohydrate to pass through the hydrophobic interior of the plasma membrane of the cell. This is the first step of the *Embden-Meyerhof (E-M) glycolytic pathway* and, ironically, requires a molecule of ATP (Figure 4–12) to prime the reaction. Glucose 6-phosphate represents a metabolic junction between either the continuation of glycolysis,

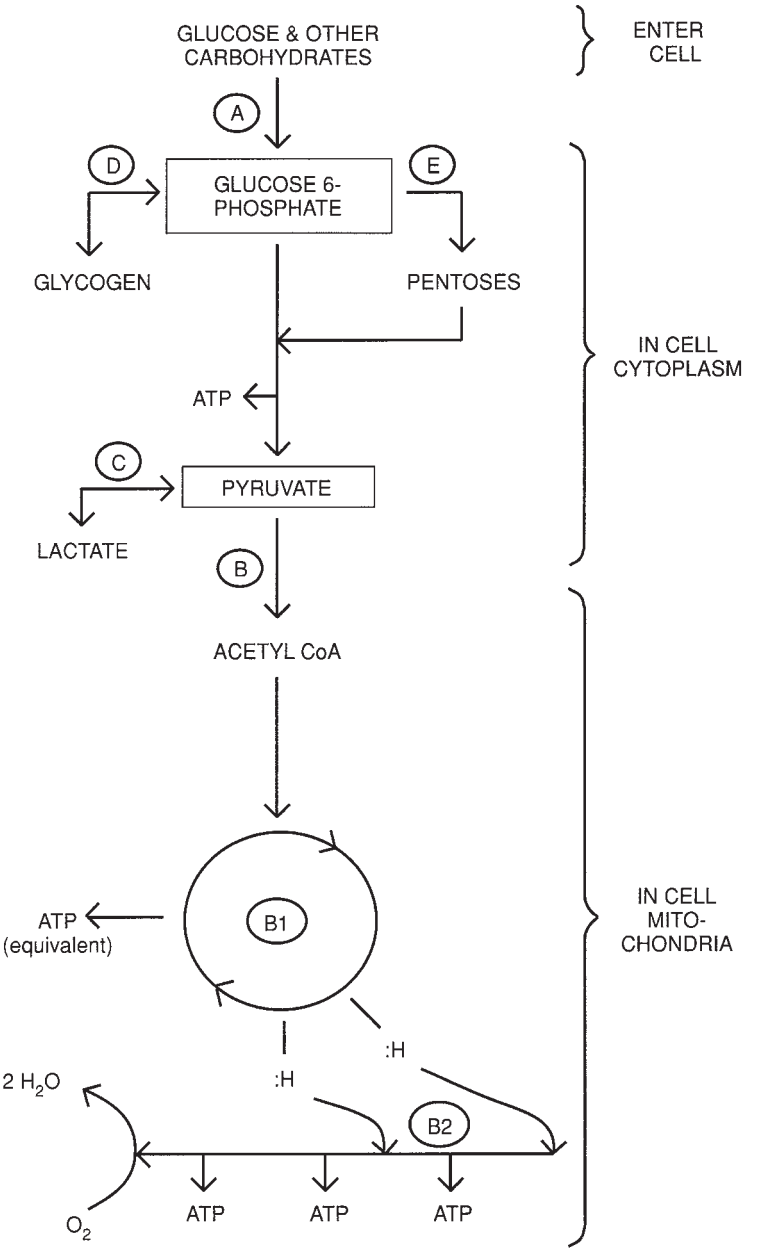


Figure 4–11
Diagrammed outline of carbohydrate metabolism. ➤ Glucose and other monosaccharides enter the pathways initially at the Embden-Meyerhof (E-M) pathway. (A) Glucose and galactose enter as glucose 6-phosphate while fructose and mannose enter as fructose 6-phosphate (the latter two enter later in the pathway—not shown here). All carbohydrates are enzymatically converted to the three-carbon triose: pyruvate at the end of the E-M pathway. The process is also known as glycolysis. A small amount of ATP is produced in the formation of pyruvate. Some pyruvate is converted to acetyl CoA (B), and in so doing the triose has entered the aerobic phase of glycolysis. This takes place in cellular mitochondria. At (B1), acetyl CoA is incorporated into the Krebs cycle (also known as the tricarboxylic acid cycle or citric acid cycle). In the cycle, ATP equivalents (guanosine triphosphate, GTP) and electron-bearing compounds (NADH, $FADH_2$) are released. The electron-bearing compounds are shuttled along the mitochondrial, inner membrane (B2) in a process known as oxidative phosphorylation, which results indirectly in the production of substantial quantities of ATP. The electron shuttle (or transport) eventually ends at the formation of water (by combining hydrogen and oxygen) while the Krebs cycle forms CO_2 (not shown) both as by products. Some pyruvate is converted to lactate (C), in which case the triose enters the anaerobic phase of glycolysis. Near the top of the scheme, it can be seen that glucose 6-phosphate can also be converted to glycogen (D) for storage purposes or can be converted to pentoses (E) for other metabolic requirements of the cell (see text under the Pentose Shunt, p. 103). The boxed carbohydrate intermediates are at metabolic crossroads.

storage (as glycogen), or the pentose shunt as shown previously in Figure 4–11. In the continuation of glycolysis, the carbohydrate is prepared for fractionation into three-carbon units, split apart, and then further rearranged in order to generate four new molecules of ATP. Figures 4–13 and 4–14 show the additional steps of this pathway. In Figure 4–13, glucose 6-phosphate (in its open chain, reactive form) is isomerized to fructose 6-phosphate. The movement of the carbonyl group (*slanted arrows*) allows the molecule to be phosphorylated at C-1 in the following step. This requires a second molecule of ATP, which

Figure 4–12
The first step in glycolysis has the purpose of retaining carbohydrates within cells by adding a phosphate group. ➤ The double negative charge at C-6 of glucose prevents any diffusion through the cell membrane. This is the first reaction of the E-M pathway.

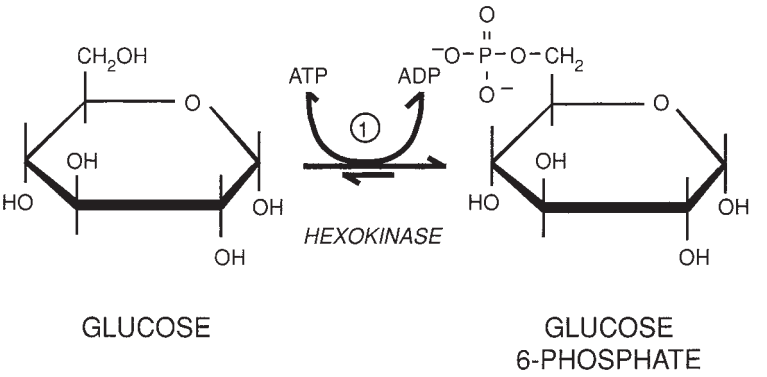


Figure 4–13
Reactions 2 through 5 of the E-M pathway. ➤ Each reaction is numbered. The names of the enzymes are given in italics. In reaction 4, the substrate and products have their carbons numbered to show their positions before and after the reaction. See text for explanation.

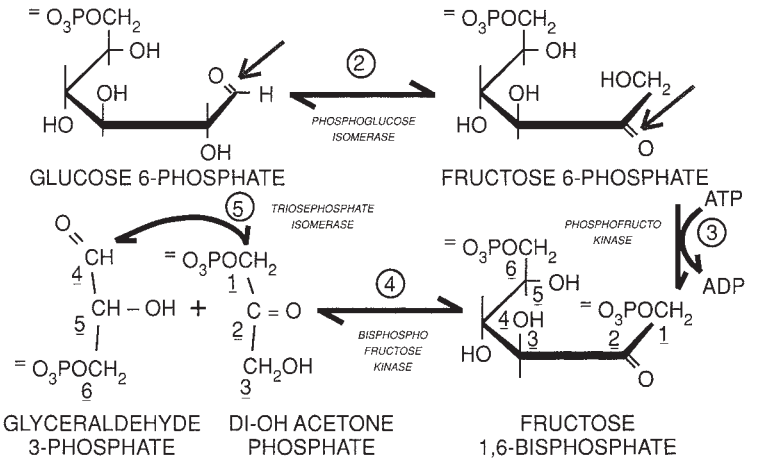
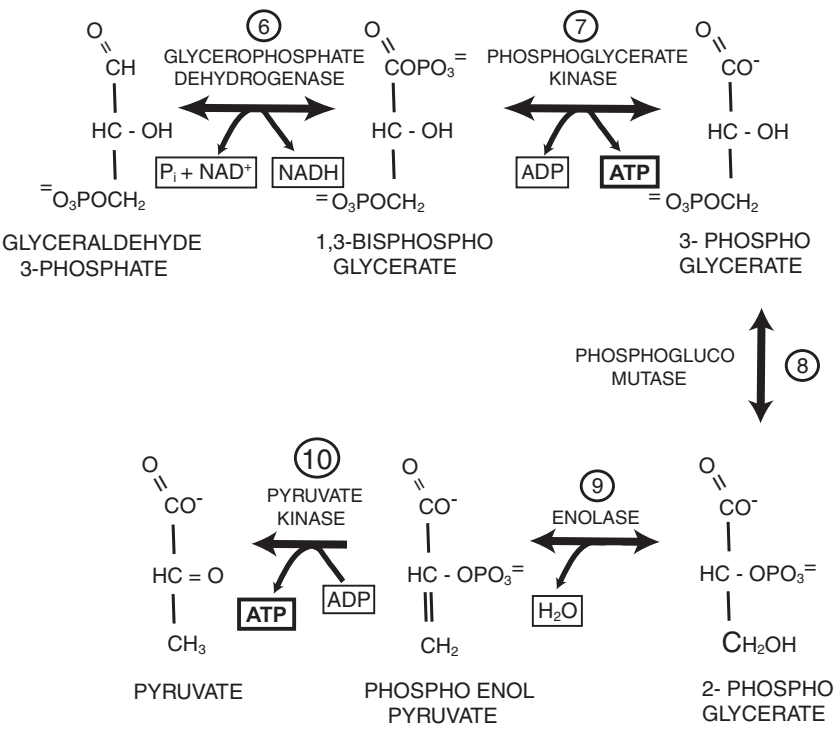


Figure 4–14
Reactions 6 through 10 of the E-M pathway. ➤ ATPs produced in reactions 7 and 10 are shown in boxes. See text and Figure 4–13 for other explanations.



energetically primes the molecule for splitting (i.e., the actual step of glycolysis or sugar splitting). In reaction four the phosphorylated carbohydrate is broken into two three-carbon fragments that are isomers of each other. The presence of phosphate groups on each fragment assures that they will remain within the cell cytoplasm. These isomers can be interconverted because of the catalytic action of an isomerase enzyme. In fact, most of the dihydroxyacetone phosphate is converted to glyceraldehyde 3-phosphate, as that compound is funneled off into the remainder of the E-M pathway.

In this manner, the cell acquires two metabolic intermediates from each carbohydrate it sends through the pathway. Note that conversion of fructose 6-phosphate to fructose 1,6-bisphosphate is catalyzed by the enzyme *phosphofructokinase* (see Figure 4–13, reaction 3). This allosteric enzyme dominates by controlling the rate of the entire pathway. It is unidirectional and its rate is, in turn, influenced by the varying energy requirements of individual cells as determined by factors such as the level of available ATP (low levels stimulate it), H^+ concentration (high levels inhibit it), and the amount of fructose 6-phosphate present (high levels indirectly stimulate it) (Stryer, 1988). In the remainder of the pathway (see Figure 4–14) there is a shuffling of phosphate groups, which are ultimately transferred onto adenosine diphosphate (ADP) to form ATP (reactions 7 and 10). In step 6 glyceraldehyde 3-phosphate acquires a second phosphate group. This time, however, it comes from inorganic phosphate in the cytoplasm rather than ATP. This reaction requires the coenzyme NAD^+, which one may recall from the previous chapter, is derived from the vitamin niacin. In the following reaction (reaction 7), the high-energy phosphate group on C-1 is transferred to ADP to form ATP. The reaction is termed *substrate-level phosphorylation* in which the substrate is the immediate source of phosphate. This kind of a reaction is distinguished from *oxidative phosphorylation,* which is a very efficient formation of ATP by electron flow to be shown later. In the subsequent reactions, the remaining phosphate group on 3-phosphoglycerate is prepared for transfer to ADP by isomerization (reaction 8) and dehydration (reaction 9) of the glycerate molecule. These reactions increase the potential energy for transfer of the phosphate group by about fourfold so that, in reaction 10, ATP is easily formed from ADP. By the conclusion of the tenth reaction of this pathway, two molecules of ATP have been consumed and four molecules of ATP have been formed for a net gain of two ATPs per glucose (or other carbohydrate) molecule.

THE ANAEROBIC EXIT OF GLYCOLYSIS

Pyruvate is the last intermediate of the E-M glycolytic pathway. This intermediate is at a junction point between anaerobic and aerobic metabolism. In anaerobic metabolism, there is only a single reaction beyond the E-M pathway that involves the formation of lactate (Figure 4–15). This reaction has been discussed previously in Chapter 3. However, some additional discussion will clarify the abruptness of this pathway. This exit combined with the E-M pathway represents a quick and relatively uncomplicated means for cells to obtain ATP in the absence of oxygen, although the yield is quite small (2 ATPs per glucose molecule). If the cell obtains its ATP from the breakdown of stored glycogen (see pathway D, Figure 4–11) it will usually realize a net gain of 3 ATPs anaerobically because no ATP is required to form glucose 6-phosphate from glycogen

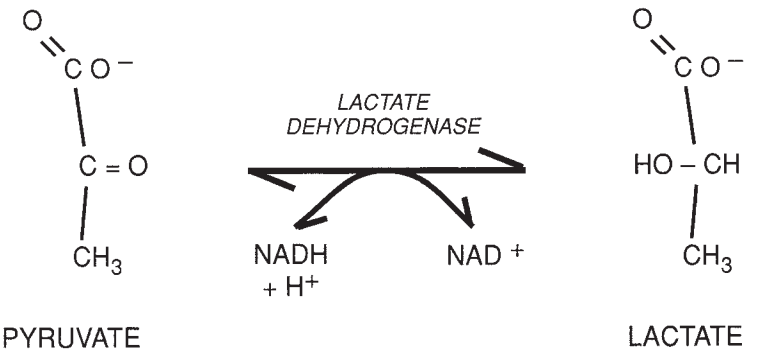


Figure 4–15
The single reaction of the anaerobic extension of glycolysis. ➤ See text for details.

(as will be explained later). Moreover, as anaerobic glycolysis is relatively simple, the pathway can be run at a faster rate. In energetic terms, this means that the cell may be able to obtain a relatively high-energy supply in a short period. Surprisingly, many cells process a relatively significant percentage of glucose (or glycogen) *via* this pathway, including ocular tissues. One reason for this is that a sufficient amount of NAD^+ must be regenerated from NADH (see Figure 4–15) to be reused in reaction 6 of the E-M pathway in order for the pathway to operate (see Figure 4–14). Muscle tissues, in particular, will increase their rate of glycolysis with an anaerobic exit since muscles quickly exceed their capacity for aerobic glycolysis when heavily used.

In the eye, corneal epithelia have a decreased amount of available oxygen during contact lens wear and this causes epithelial cells to increase their percentage of anaerobic glycolysis. This was once a major problem in the use of hard contact lenses as nearly 80% of the available glycogen would be used in just over 8 hours of lens wear compared to soft lenses (Figure 4–16). The resultant metabolic strain on the epithelial cells caused significant swelling of both epithelial and anterior stromal corneal tissues. This is due to the increase in lactate that occurs in epithelial tissues and causes an osmotic strain and consequent swelling (Klyce, 1981). The increase in total corneal swelling could be as much as 20% of the tissue volume (Hamano, Kaufman, 1987) especially when the oxygen levels fall below 54 mm Hg (Mizutani et al, 1983). The recent use of rigid gas-permeable lenses has largely eliminated this problem as these lenses allow the passage of oxygen more efficiently than even soft

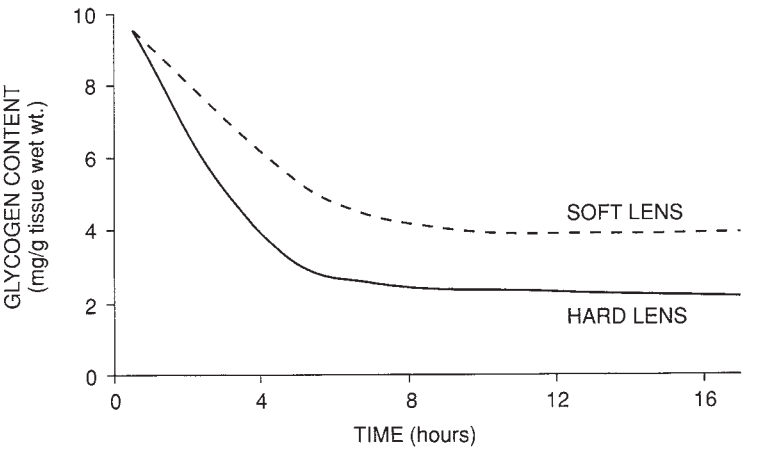


Figure 4–16
Decrease of glycogen concentration in the corneal epithelium with contact lens wear. ➤ (Adapted from Hamano et al: The effects of hard and soft contact lenses on rabbit cornea, J Jpn Cl Soc 14:29–37, 1972.)

lenses (Swarbrick, Holden, 1997). Although this is good news for extended contact lens wearers, the understanding of all parameters involved is still incomplete. For example, not all swelling is avoided as some swelling (approximately 3%) may normally occur overnight with closed lids when the oxygen partial pressure falls from 155 mm Hg to 60 mm Hg (Swarbrick, Holden, 1997). In addition, there is always the problem of the build-up of protein and other debris on the lens that, though comparatively reduced in the newer lenses, still occurs with time and can cause other problems not related to oxygen uptake and carbohydrate metabolism.

THE AEROBIC EXIT OF GLYCOLYSIS

The alternative of conversion to lactate at the end of the E-M pathway for pyruvate entails its diffusion into cellular *mitochondria* to begin the aerobic phase of ATP production. In order to understand this process, it is helpful to digress into a short explanation of mitochondrial function and anatomy. Cellular mitochondria are organelles that have two principal functions: the manufacture of ATP aerobically and the enzymatic processing of other reactions, both of which require a separate inner compartment. Mitochondria exist in various spherical and oblong shapes, but all have a double membrane (Figure 4–17). The inner membrane is impermeable to virtually all molecules and ions without a transport mechanism. The inner membrane often has a very large surface area represented by infoldings known as *cristae*. The innermost *matrix* (compartment) contains numerous soluble enzymes not found in the cellular cytoplasm. Contained on the inner membrane are a number of insoluble electron transferring proteins and an enzyme known as ATP synthase. Many of the matrix enzymes as well as the inner membrane proteins, including ATP synthase, are essential to aerobic ATP production. As shown back in Figure 4–11, the first metabolite to be produced from pyruvate in the aerobic exit of the E-M pathway is acetyl CoA. The reaction takes place in the mitochondrial matrix and this reaction is shown, in simplified form, in Figure 4–18.

The reaction is actually a series of reactions owing to the activity of three enzymes and five coenzymes held together in a molecular particle known as the *pyruvate dehydrogenase complex*. Two of the five coenzymes, CoA and NAD^+ are given in the figure. The other three coenzymes

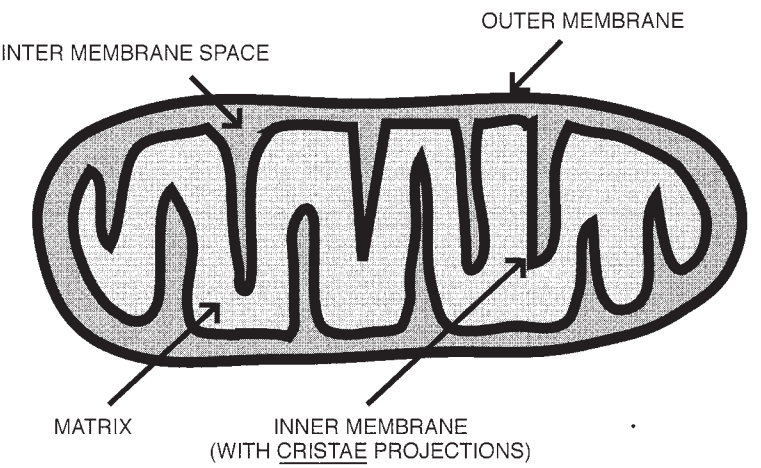


Figure 4–17
Typical cross-sectional diagram of a mitochondrion. ➤ This subcellular organelle is divided into two compartments (intermembrane space and matrix) by two membranes (outer membrane and inner membrane). The inner membrane has its surface area enlarged by many infoldings (cristae). The shape and number of cristae vary from cell to cell depending on the metabolic demands of the cell. High energy requiring cells have greater numbers of cristae.

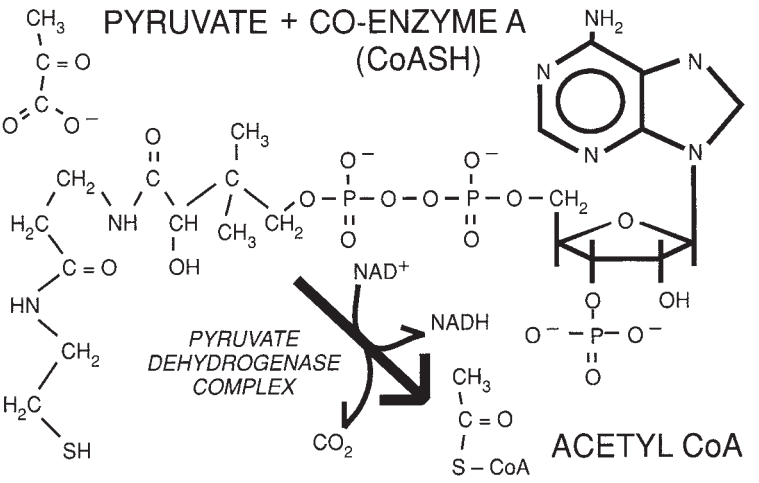


Figure 4–18
Formation of acetyl coenzyme A from pyruvate and coenzyme A. ➤ See text for explanation.

are flavin adenine dinucleotide (FAD), thiamine pyrophosphate (TPP), and lipoate (see Table 3–1 and Chapter 3) and are derived from water-soluble vitamins. An important consideration of this complex reaction is the overall transfer of two electrons from pyruvate to NAD^+ to form NADH. These electrons (as will be explained) are the first source of ATP synthesis in this aerobic pathway. At this stage both two-carbon units (i.e., acetyl CoA), which originated from one molecule of glucose (or other carbohydrate), are now ready to be incorporated into the *Krebs' cycle*.

This cyclic pathway, which was discovered by Hans Krebs in 1937, is multifunctional. Its primary purpose, in energy production, is to supply electrons for the synthesis of ATP. The cycle is diagrammed in Figure 4–19. One turn of the cycle involves nine reactions that are all enzyme catalyzed and occur in the mitochondrial matrix. As each turn of the cycle occurs, two carbons are lost as CO_2, but the carbons are regained as acetyl CoA at the beginning of a new cycle. The important energy deriving reactions are: 4, 5, 6, 7, and 9. In these reactions, electrons are removed with either NADH, $FADH_2$, or a high-energy phosphate trapped in the compound GTP (guanosine triphosphate, a high-energy compound similar to ATP). GTP is readily converted to ATP by the reaction GTP + ADP → GDP + ATP (which is also enzyme catalyzed). The compounds NADH and $FADH_2$, which have been previously shown to be coenzymes, diffuse from the matrix to the inner mitochondrial membrane where they donate their electrons to the electron transferring (or redox) proteins located there.

When electrons arrive at the inner membrane bound to either NADH or $FADH_2$, they are subsequently transported (shuttled) between four protein complexes and, ultimately, combine with oxygen and hydrogen to form water. This process is diagrammed in Figure 4–20. The protein complexes are essentially immobile and are served by coenzyme Q (a type of lipid) and cytochrome C (a protein). Coenzyme Q is a lipid soluble quinone with a long hydrocarbon tail of isoprene units (Figure 4–21 A). It shuttles electrons between protein complex I and complex III and also between complex II and complex III as separate operations. Cytochrome C is a small lipophilic protein of 13,000 D (see Figure 4–21 B) that shuttles electrons between complex III and complex IV. It is NADH that ferries electrons to protein complex I and $FADH_2$

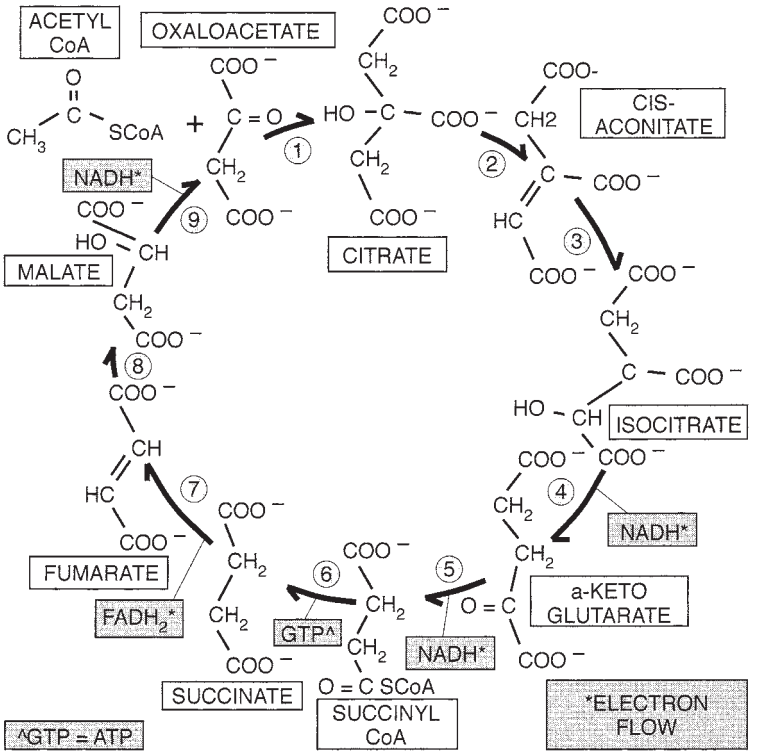


Figure 4–19
The Krebs cycle. ➤ The cycle begins with acetyl CoA and oxaloacetate (reaction 1) and ends with the formation of oxaloacetate from malate (reaction 9). Electron flow or transport exits the cycle with NADH and $FADH_2$ in reactions 4, 5, 7, and 9. The ATP equivalent, GTP, is generated in reaction 6. Each reaction is enzyme catalyzed (not shown).

that carries electrons to protein complex II. The unique feature of this electron transport is the *electromotive force* that is generated in the inner mitochondrial membrane as the transport occurs. This force is analogous to electricity flowing through a wire. It generates useful energy equivalent to approximately 53 kcal per mol of oxygen consumed (Stryer, 1988). In more practical terms this is roughly enough energy to heat a milliliter of water 28°C (about 50°F) for each 1.12 liters of oxygen used. This energy is used to pump hydrogen ions from the mitochondrial matrix to the intermembrane space creating a relatively acidic environment outside the matrix. The hydrogen ions or protons flow back into

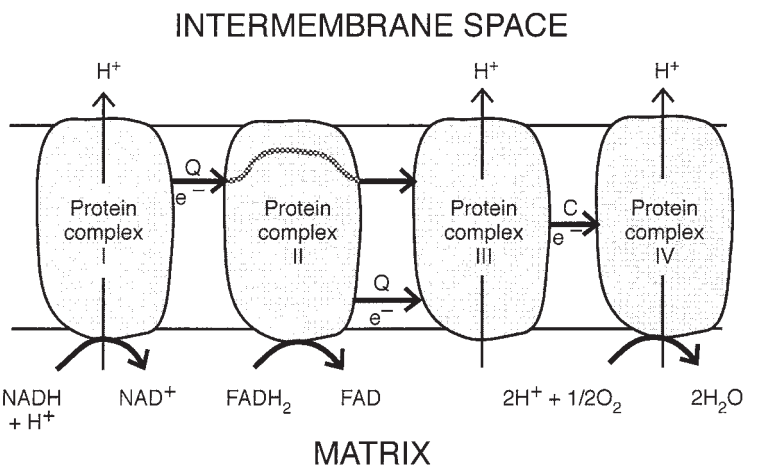


Figure 4–20
Electron transfer in the inner mitochondrial membrane. ➤ Electrons from NADH are transferred to protein complex I (25 polypeptides) in the inner membrane. Coenzyme Q transfers electrons from complex I to complex III (10 polypeptides), which also receives electrons from complex II (4 polypeptides) via coenzyme Q. Complex II receives electrons from $FADH_2$. From complex III, electrons are transported to complex IV via cytochrome C and from there to form water from oxygen and hydrogen. Each transfer is an oxidation/reduction reaction. In complexes I, III, and IV, hydrogen ions are transported from the matrix to the intermembrane space.

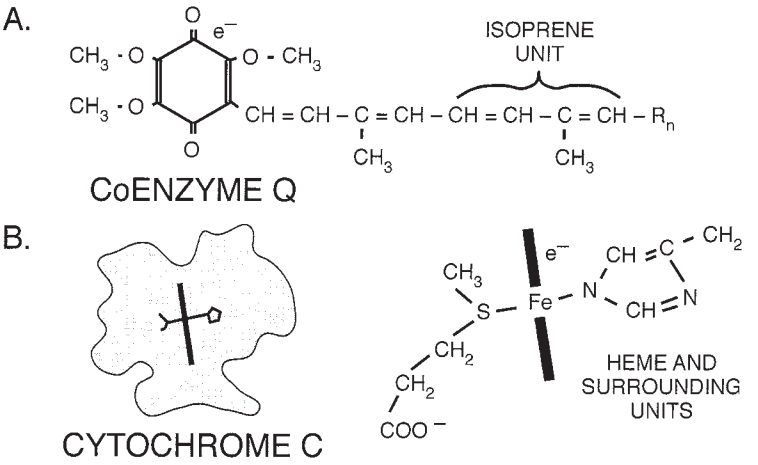


Figure 4–21
Coenzyme Q and cytochrome C are lipid soluble molecules in the mitochondrial membrane. ➤ Coenzyme Q is a quinone derivative with a long tail of isoprene units. Electrons are incorporated into the oxygen on the quinone ring. Cytochrome C is a globular protein with a molecular weight of 13,000. The electron carrying moiety is a prosthetic (see Chapter 2) heme group with iron at its center. The protein complexes in the membrane (mentioned in Figure 4–20) also contain heme groups.

the matrix through the pores of a fifth protein complex known as *ATP synthase*. The flow provides the energy to phosphorylate ADP to form ATP on this enzyme. In fact, the flow simply causes the release of ATP from ATP synthase. The entire process is shown for NADH-originated electron transport in Figure 4–22. It is thought that the passage of three protons back through the ATP synthase (protein complex V) will generate one molecule of ATP. In general, the movement of two electrons from NADH to water ultimately produces three molecules of ATP while the passage of two electrons from $FADH_2$ to water produces two molecules

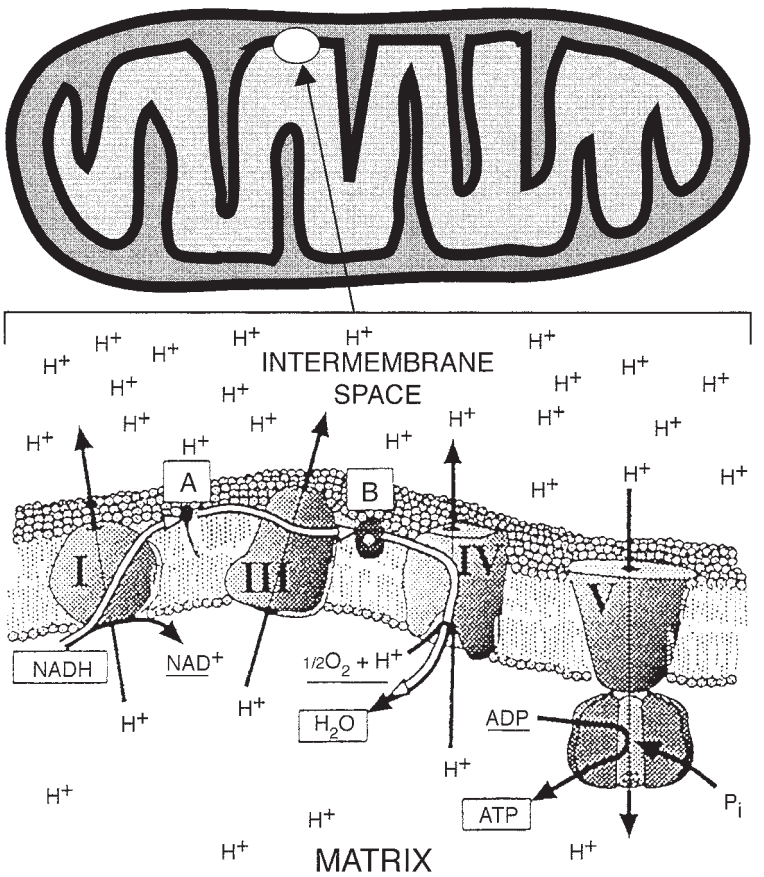


Figure 4–22
A complete overview of oxidative phosphorylation to produce ATP. ➤ ATP is formed (actually released) when hydrogen ions (protons) flow through protein complex V (ATP synthase). In order for this to occur protons are pumped into the intermembrane space as a result of electron flow between protein complexes I, III, and IV using coenzyme Q (A) and cytochrome C (B) starting with NADH and ending with water formation. The electron flow starting with $FADH_2$ and protein complex II is not shown. It should be noted also that protons are not pumped through protein complex II (see Figure 4–20), but are pumped subsequently.

TABLE 4–1 ➤ YIELD OF ATP FROM AEROBIC GLYCOLYSIS

Pathway	Yield per Molecule of Glucose
Embden-Meyerhof (E-M)	2
Krebs cycle (as GTP)	2
Oxidative phosphorylation	
From Krebs cycle NADH	18
From Krebs cycle $FADH_2$	4
From CoA formation NADH	6
From E-M NADH[1]	4–6
Total	36–38

[1]Two extra molecules of ATP may be produced by another mechanism to transport E-M pathway NADH into the mitochondria.

of ATP. This suggests that nine protons are pumped out of the inner membrane when $NADH_2$ is the source of electrons and six protons are pumped out of the inner membrane when $FADH_2$ is the electron source. In the case of $FADH_2$, only two ATP molecules are produced as no protons are transported through protein complex II (see Figure 4–20). The total ATP produced by the aerobic exit of glycolysis is 36 to 38 molecules, as summarized in Table 4–1.

At this point, all the sources of ATP have been described except for ATP arising from electrons originating from NADH in the E-M pathway in the cell cytoplasm. The ATP molecules from this source are obtained by the *glycerol phosphate* and *malate-aspartate shuttles*. In the glycerol phosphate shuttle, which commonly occurs in muscle and brain cells, the intermediate dihydroxyacetone phosphate (see Figure 4–13) is "borrowed" from the E-M pathway by reaction with NADH (Figure 4–23) to produce glycerol 3-phosphate. This intermediate (bearing two electrons) diffuses to the outer surface of inner mitochondrial membrane (at the intermembrane space) where it reacts by giving up its two electrons to FAD at the membrane *via* the activity of glycerol 3-phosphate dehydrogenase. The glycerol 3-phosphate is converted back to dihydroxyacetone phosphate and returns (by diffusion) to the E-M pathway in the cytoplasm. The FAD (as $FADH_2$) is now a source for the production of two additional molecules of ATP. The malate-aspartate shuttle, that commonly occurs in liver, kidney, and heart cells; operates by a somewhat similar, but more complex mechanism and has one important difference, the final electron acceptor molecule in the mitochondrial matrix is

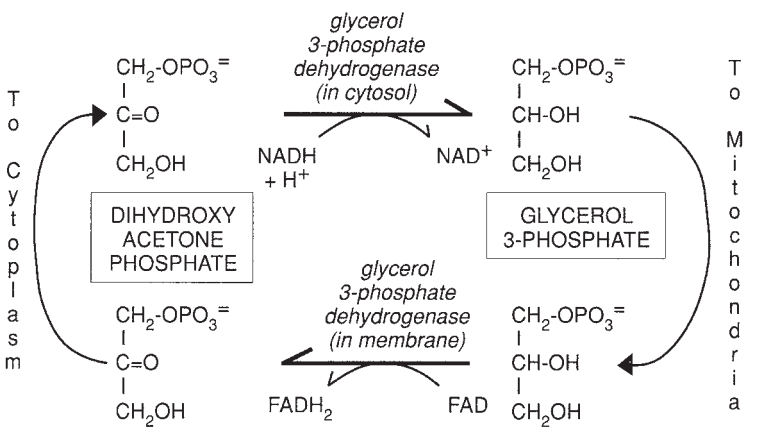


Figure 4–23
The glycerol phosphate shuttle exists to transport cytoplasmic NADH electrons to the electron transport protein complexes in the inner mitochondrial membrane.

NADH rather than $FADH_2$. This difference enables the cell to obtain an additional one molecule of ATP for each two-carbon electron donor. For both shuttles, one obtains a net of four or six molecules of ATP from a single glucose molecule. Information about the types of shuttles that operate in ocular tissues is not currently available.

GLYCOGEN FORMATION AND DEGRADATION

Much of the glucose that enters certain cells can be stored as the polysaccharide glycogen as previously mentioned. Generally, in the eye, glycogen formation seems to be limited to corneal epithelial cells and Müller cells in the retina. However, glycogen is held in the liver for the distribution of glucose to other parts of the body including the eye.

The formation begins at the junction point of glucose 6-phosphate in the E-M pathway and is relatively direct. Glucose 6-phosphate is isomerized to glucose 1-phosphate. Glucose 1-phosphate reacts with an ATP equivalent known as uridine triphosphate (UTP) to form uridine diphosphoglucose and this form is bound to glucose as an energetic mechanism to add glucose to a growing chain of glycogen by the action of the enzyme *glycogen synthase*. Figure 4–24 outlines those reactions. As the molecule grows linearly, a branching enzyme (a transferase) will periodically remove some of the growing terminal chains and bind them onto C-6 of some units as shown in Figure 4–7. This occurs every time the main chain grows by 6 to 21 units as previously mentioned.

When glycogen is broken down to obtain glucose monomers the process is reversed, but it proceeds by a separate pathway. It is important to have a separate pathway so that the cell can have optimal control of its carbohydrate reserves. Glycogen is broken down, one residue or unit at a time, by the enzyme *glycogen phosphorylase*. Each glucose unit is released as glucose 1-phosphate and then re-enters the E-M pathway after subsequent formation of glucose 6-phosphate. During breakdown, debranching of the complex glycogen must also occur. Debranching

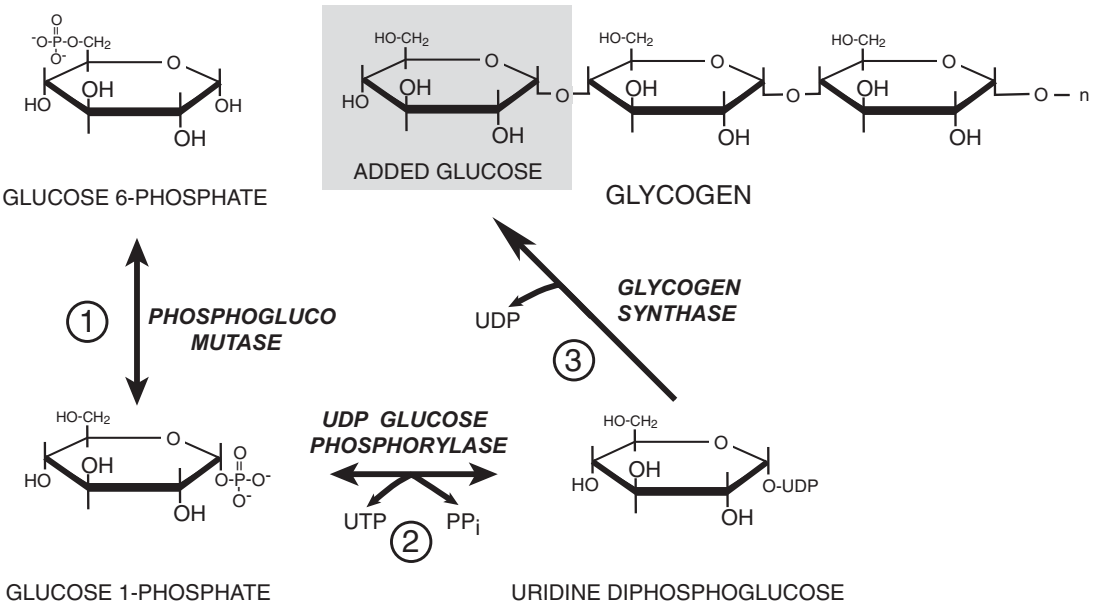


Figure 4–24
The formation of glycogen from glucose 6-phosphate. ➤ Enzymes are named in italics. See text for explanation.

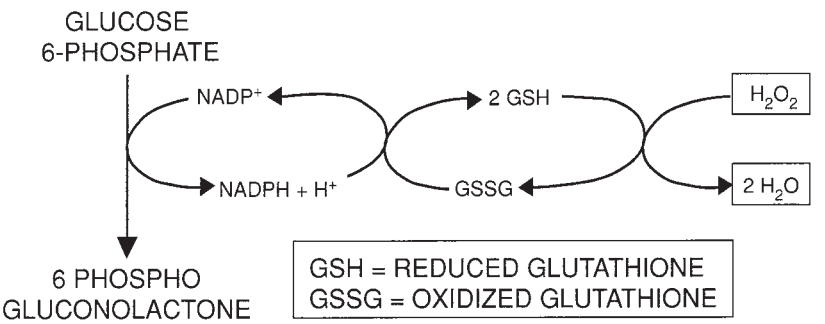
occurs from the activity of a "debranching" enzyme. This enzyme has both glycosyl transferase and glucosidase activities to transfer the branch to the main stem and to remove the terminal sugar that is bound by an $\alpha 1 \rightarrow 6$ linkage to the main stem. It is an allosteric enzyme in which ATP and glucose 6-phosphate maintain it in its T form until needed (see Chapter 3). The activity of glycogen phosphorylase is also controlled by high levels of ATP and glucose 6-phosphate, which inhibit it.

Glycogen synthase and glycogen phosphorylase are reciprocally activated and deactivated by the addition and removal of phosphate groups from each enzyme. When phosphate is added to phosphorylase (with low levels of ATP), the enzyme is activated to break down glycogen while the synthase is inactivated when phosphate is added to its structure. The reverse is true with high levels of ATP for each enzyme as shown in Table 4–2.

THE PENTOSE SHUNT

Another metabolic branch that leads from glucose 6-phosphate is the pentose shunt. This pathway has three principal functions: the *generation of pentoses* (to be used for the synthesis of nucleic acids and nucleotides such as adenosine); the *production of fatty acids* (for membrane synthesis and other functions requiring fatty acids); and *cell detoxification* by the removal of destructive forms of oxygen (e.g., hydrogen peroxide). In addition, some metabolic intermediates can be recovered back into the E-M pathway for the *generation of more ATP*. Here we will consider only the first part of the pathway.

Figure 4–25 shows the fate of glucose 6-phosphate in this pathway. In reaction 1, electrons are removed from C-1, which then forms a carbonyl group. The compound NADPH becomes the second product in the reaction. The electrons that are inserted into the coenzyme $NADP^+$ (a phosphorylated cousin of NADH), serve two principal functions. One function, in this early stage of the pathway, is for the reductive (electron-requiring) synthesis of fatty acids. The second function is for the removal of hydrogen peroxide by a linked redox system. In the redox system, electrons from NADPH are used to reduce the tripeptide *glutathione* as shown in the accompanying schematic diagram. Glutathione, in turn, reduces hydrogen peroxide to water.



Each reaction is enzyme catalyzed. The importance of these coupled reactions lies in the fact that cell membranes (containing lipids and proteins) can be destroyed by excessive amounts of hydrogen peroxide and other forms of active oxygen (e.g., superoxide radicals). Such detoxification mechanisms are known to be present in ocular tissues (Whikehart, 1978) to prevent tissue destruction. Detoxification of

TABLE 4–2 ➤ EFFECTS OF PHOSPHATE LEVELS ON ENZYMATIC GLYCOGEN SYNTHESIS AND DEGRADATION

Enzyme	Function	Effects of High Phosphate	Effects of Low Phosphate
Glycogen synthase	Adds glucose to glycogen	Inhibits	Activates
Glycogen phosphorylase	Removes glucose from glycogen	Activates	Inhibits

reactive forms of oxygen is an important biochemical process in all biological tissues and cells. Molecular oxygen, besides being an important metabolite for the completion of oxidative phosphorylation and other synthetic reactions, can also be reduced into pathological, highly reactive forms that attack both proteins and lipids. Such a reductive pathway is shown below.

$$O_2 \text{ (molecular oxygen)} \rightarrow O\text{-}O^{\cdot} \text{ (superoxide)} \rightarrow H_2O_2 \text{ (hydrogen peroxide)} \rightarrow OH^{\cdot} \text{ (hydroxide radical)} \rightarrow O_2^{*} \text{ (singlet oxygen)}$$

The formation of these reactive forms of oxygen and their actions will be discussed in more detail in Chapter 9 under *inflammation*. Suffice it to state here that these forms usually contain an unpaired electron that is unstable and readily reacts with other compounds.

In the second reaction of the pentose shunt, the gluconolactone (which is an internal ester) is hydrolyzed with a water molecule to open the ring structure and produce a sugar acid (gluconate). In the third reac-

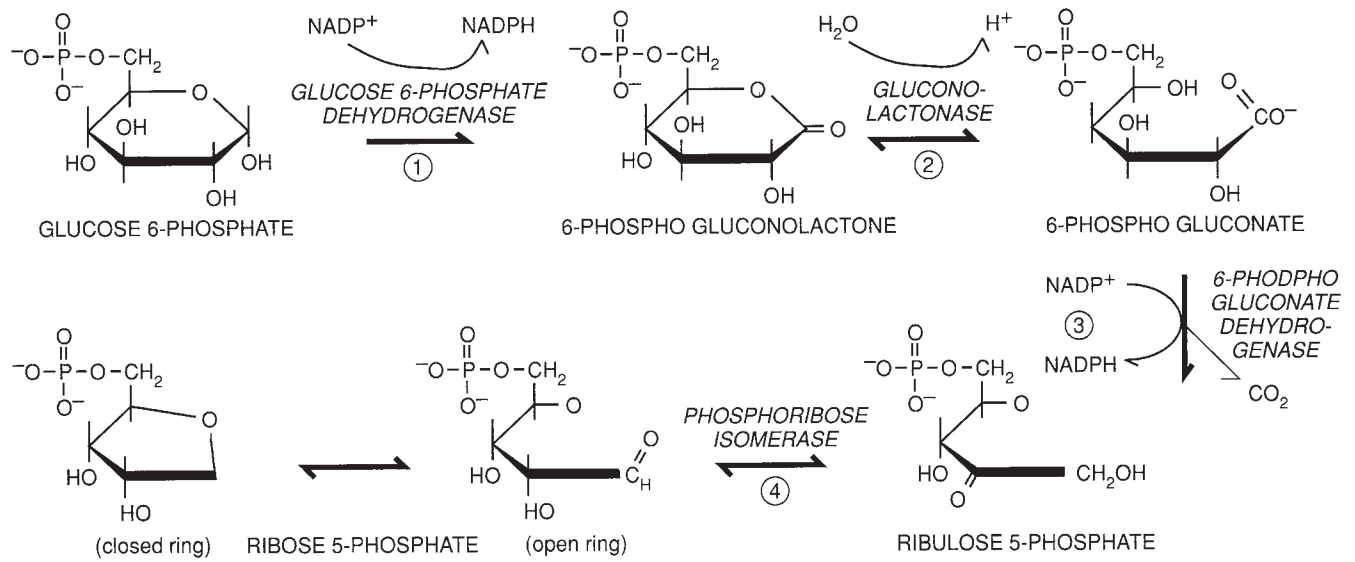


Figure 4–25
The formation of pentoses from glucose 6-phosphate. ➤ This is the initial part of the pentose shunt pathway. See text for explanation.

tion, the acid is oxidized (it loses electrons) and these electrons are again absorbed by another molecule of $NADP^+$. A molecule of CO_2 is also lost. The product, ribulose 5-phosphate, is isomerized in reaction 4 to ribose 5-phosphate by transferring the carbonyl group from carbon 2 to carbon 1 so that a closed-ring pentose can be formed. The pentose can then be incorporated into nucleotides and nucleic acids Ocular tissues undergoing growth make extensive use of this pathway both for the production of nucleic acids (in order to synthesize proteins) and lipids (to be incorporated into cellular membranes). An ocular example of where the pentose shunt occurs would be in the epithelial cells of the cornea.

OTHER ASPECTS OF CARBOHYDRATE METABOLISM

There are two other pathways of normal carbohydrate metabolism that are significant in certain ocular tissues. They are *gluconeogenesis* and the *Warburg effect*. Gluconeogenesis (which means the synthesis of new glucose) proceeds essentially as a reversal of the E-M pathway. However, gluconeogenesis uses four different enzymes, not found in the E-M pathway, to proceed from lactate to glucose (Figure 4–26). The process begins in the mitochondria with two of the enzymes that are specific for the pathway. Here again, the use of separate enzymes is employed to maintain independent control of the gluconeogenesis pathway (carbohydrate formation) vs. the glycolysis pathway (carbohydrate breakdown). Although the liver (and to some extent the kidneys) are the principal sites of gluconeogenesis, it is the retina (along with the brain) that are principal beneficiaries of maintaining adequate levels of glucose in the

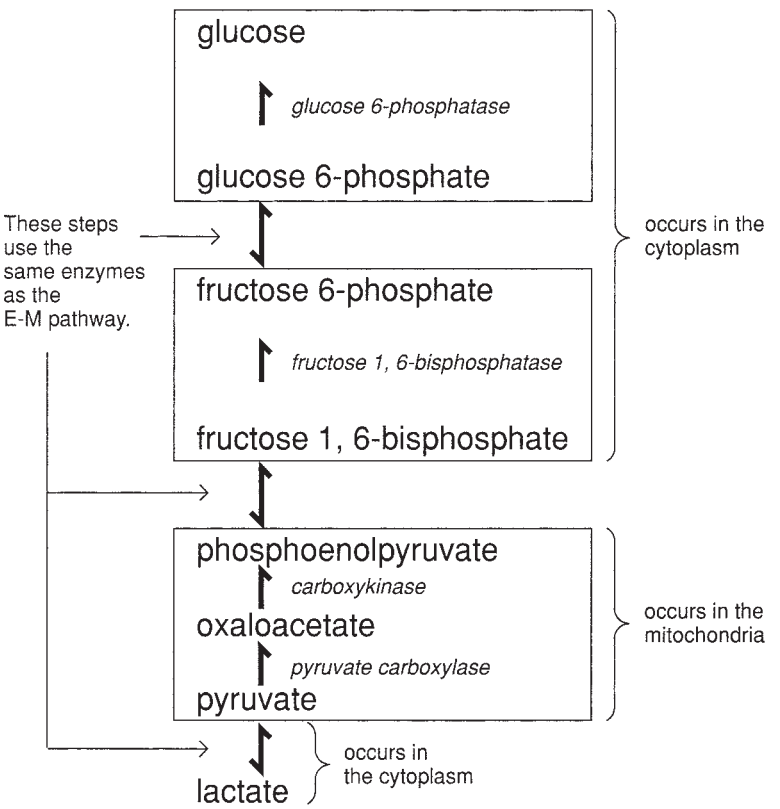


Figure 4–26
Gluconeogenesis. ➤ This pathway uses some of the enzymes of the E-M pathway. The enzymes unique to the pathway are shown in italics.

bloodstream by using this pathway. Gluconeogenesis is especially important to the eye since the photoreceptors have one of the highest demands for a constant supply of glucose and oxygen. Another characteristic of carbohydrate breakdown (known as the Warburg effect) simply "directs" excessive amounts of pyruvate to become metabolized to lactate when there is a relatively high oxygen partial pressure and a heavy demand for cellular energy. This characteristic of the retina was discussed in Chapter 3 under lactate dehydrogenase. Still another pathway, but of abnormal carbohydrate metabolism in the eye, is the polyol pathway. This was explained in Chapter 3 and will be further detailed in the section *Problems of Carbohydrate Transport and Metabolism: Diabetes and Galactosemia* of this chapter.

Ocular Comparative Metabolism

The types of carbohydrate metabolism, and their relatively occurring percentages, that are found in ocular tissues are dependent upon the roles and associated energy demands of each tissue type. At extremely high levels of relative energy demands are photoreceptors whereas lens fiber cells are at the low end of the energy requiring spectrum. Table 4–3 outlines what is presently known about several cell types found in the anterior segment of the eye.

In the cornea, as in most tissues, there is a predominance of glucose funneled through the anaerobic exit of glycolysis (E-M pathway). Although this is the case, it must be remembered that the ATP energy derived from aerobic glycolysis does not require as much glucose as anaerobic glycolysis. From the data in the table, one may calculate that for every 100 molecules of glucose utilized in the corneal epithelial cells and corneal keratocytes (stromal cells) that there are 114 ATP molecules produced anaerobically and 254 ATP molecules produced aerobically. That is, anaerobically 57% or 57 glucose molecules × 2 ATPs produced per glucose = 114 molecules and aerobically 8% or 8 glucose molecules × 36 ATPs produced per glucose = 254 molecules. The

TABLE 4–3 ➤ COMPARATIVE CARBOHYDRATE METABOLISM IN THE ANTERIOR SEGMENT OF THE EYE

Cell type	Anaerobic Glycolysis (%)	Aerobic Glycolysis (%)	Pentose Shunt (%)	Glycogen Storage	Other (%)
Cornea[1]					
Epithelial	57	8	35	Yes	Polyol pathway may be present in diabetes
Stromal	57	8	35	No	
Endothelial	70	23	7	No	
Lens[2]					
Epithelial	81	4	15	No	Polyol pathway in diabetes
Fiber[3]	83	2	15	No	
Ciliary body[4]					
Pigmented, Nonpigmented	85% of EM[5]	15% of EM[5]	Present	No	Unknown

[1]Data from M. Riley. 1983; and D. Whikehart, 1989.
[2]Data from J. Kuck, 1970; R. van Heyningen and Linklater, 1975; and Winkler and Riley, 1991. The pentose shunt % is an assumed minimal value for epithelial cells.
[3]Values are actually for whole lens; lens fiber cells may have a lower proportion of aerobic glycolysis, especially in the nucleus.
[4]Data derived from D. Cole, 1970.
[5]Percentage of that glucose in the Embden-Meyerhof pathway. The actual percentage in glycolysis is unknown.

total yield = 368 ATP molecules/100 glucose molecules. This neglects the possible recovery of some intermediates via the pentose shunt and the fact that some glucose may be temporarily stored as glycogen.

The relatively high percentage of the pentose shunt found in the corneal epithelial and stromal (keratocyte) cells may be related to the physiological roles of these cells. Epithelial cells, which are 5 to 6 layers deep in the human, are in a constant state of division posteriorly. There is, therefore, a heavy and consistent need for protein and lipid production to achieve cell division and growth. It may be recalled that two roles of the pentose shunt are for the synthesis of pentoses (required for nucleic acids) and the generation of electron bearing NADPH (required to synthesize fatty acids). Nucleic acids, in turn, make cellular proteins while fatty acids are used to build up cellular plasma membranes. The cellular proteins have, of course, many roles to maintain, operate, and reproduce these cells.

The role of keratocytes is one of maintenance and repair of the structure that constitutes the stroma. Although these cells occupy only about 5% to 10% of the stromal volume, they too are involved in protein (collagen and proteoglycan) and structural carbohydrate (glycosaminoglycan) production. Therefore, they have to maintain an adequate supply of pentoses for nucleic acids.

The corneal endothelial cells, on the other hand, have a higher energy demand than the other corneal cell types since they must maintain the cornea in a relatively equilibrated state of clarity (deturgescence). This ocular "sump pump" is thought to be constituted by the plasma membrane enzyme Na,K-activated ATPase (discussed in the previous chapter). This enzyme has a high demand for its substrate ATP so that the proportion of both aerobic and anaerobic glycolysis in endothelial cells is increased compared to keratocytes and epithelial cells. Here the energy yield of ATP per 100 moles of glucose is 140 (anaerobic) and 838 (aerobic) molecules to yield 968 molecules, about 2.6 times as many ATP molecules as are produced by the other corneal cell types!

In the lens, although there is a constant increase in the production of lens fiber cells, the rate of production after birth is very slow. Potassium ions are pumped into and through the lens (anteriorly to posteriorly) by the epithelial cells. However, the tissues do not have the tendency to imbibe water and swell as does the cornea. Moreover, the lens fiber cells, as they mature, tend to lose their subcellular organelles. Consequently, energy demands in the lens cells are considerably lower in comparison to the cornea. In the lens epithelium, the ATP yield per 100 glucose molecules is 162 (anaerobically) and 144 (aerobically) molecules to yield 306 molecules. In the lens fiber cells, the ATP yield per 100 glucose molecules is lower still with 166 (anaerobically) and 72 (aerobically) molecules to give 238 molecules. In the lens nucleus, it is highly probable that the ATP yield is considerably lower, that is, approaching a value of 170 molecules of ATP per 100 glucose molecules. The ATP yield of the ciliary body cannot be accurately calculated since the percentage of glucose sent through the pentose shunt is unknown. However, it is perfectly reasonable to assume that the energy requirement is substantial inasmuch as the cells of this organ generate the intraocular pressure and prepare the aqueous as an ultrafiltrate of blood just as the kidney prepares urine as an ultrafiltrate of blood. Consequently, one may postulate that the amount of ATP produced might rival that of the corneal endothelium.

TABLE 4–4 ➤ COMPARATIVE CARBOHYDRATE METABOLISM IN THE RETINA AND BRAIN

Cell Type	Anaerobic Glycolysis (%)	Aerobic Glycolysis (%)	Pentose Shunt (%)	Glycogen Storage	Other
Retina[1]					
Photoreceptors and all others	60	25	15	None[3]	Polyol pathway may occur
Brain[2]					
Whole brain	17	82.7	<0.3	Minimal	None

[1]Data from Graymore, 1970; and Winkler, 1983.
[2]Except Müller cells.
[3]Data from Hawkins, Mann, 1983.

TABLE 4–5 ➤ OXYGEN CONSUMPTION RATES (QO_2)[1] FOR OCULAR AND NONOCULAR TISSUES

Ocular Tissues	Rate	Nonocular Tissues	Rate
Retina	31	Kidney	21
Cornea	2	Cerebral cortex	12
Lens	0.5	Heart	5

[1]μL O_2/mg tissue dry weight/hour.

TABLE 4–6 ➤ BLOOD FLOW[1] THROUGH VARIOUS OCULAR AND NONOCULAR TISSUES

Ocular Tissues[2]	Rate	Nonocular Tissues[3]	Rate
Retina (choroid)	12	Heart	0.6
Ciliary processes	1.5	Kidney	4
Iris	1	Brain (gray matter)	0.5

[1]mL/g tissue/min.
[2]Calculated from Henkind et al, 1979.
[3]Calculated from Folkow, Neil, 1971.

In the retina, the amount of glucose passed through aerobic glycolysis is higher than any other part of the eye in accord with the energy demands of this tissue. This is indicated in Table 4–4 where it is compared with brain tissue. Nine hundred molecules of ATP are produced aerobically versus 120 molecules of ATP produced anaerobically (total of 1020) per 100 glucose molecules. In the brain, the ATP yield is even higher. However, the rate of ATP production, that is, the number of glucose molecules processed through the pathways in the retina is higher than that of brain per unit time. This is so since the flow of glucose to these tissues *via* the choroidal circulation is very high. This means that ATP production is actually higher in the retina! This is known from oxygen consumption rates or QO_2 values (given as μL oxygen consumed/mg tissue dry weight/hour) as shown in Table 4–5 where retina has the highest value. It is further reflected in blood flow rates through these tissues as shown in Table 4–6.

Problems of Carbohydrate Transport and Metabolism: Diabetes and Galactosemia

INTRODUCTION TO DIABETES

Diabetes is, undoubtedly, one of the major disorders affecting humankind. In the United States, about 5% to 6% of the population is afflicted with some form of the disease (Wildman, Medeiros, 2000). The disease has been long known and was first described some 3000 years ago. The name "diabetes" stems from a Greek word meaning *to pass through* and refers to the excessive urination that is common to its untreated sufferers (Williams, Pickup, 1999). Diabetes is a metabolic disorder of cellular carbohydrate uptake that affects not only carbohydrate metabolism itself, but protein and lipid metabolism as well. Primary effects occur to blood vessels of the brain, eyes, kidneys, and external limbs. In the eyes, the retina, lens, and cornea may be pathologically affected. Consequently, blindness (diabetic retinopathy) and visual debilitation (cataract formation) can occur.

Diabetes occurs essentially in two forms: *type 1, formerly known as juvenile-onset diabetes (or type I)*; and *type 2, which was once called mature-onset diabetes (or type II)*. Approximately 10% of the diabetic population has type 1, which is usually the more severe form. Although the mechanisms for each form vary, both forms involve an inability of glucose to enter certain classes of cells in the body that are dependent upon insulin-activated, transport protein systems.

GLUCOSE TRANSPORT INTO CELLS AND ITS RELATIONSHIP TO DIABETES

In general, glucose and other carbohydrates are taken into cells by facilitated diffusion and active transport systems (transport systems are discussed in more detail in Chapter 5). Both mechanisms use *transport proteins* located in the plasma membranes of cells. Generally, carbohydrates either pass through these membrane proteins to enter the cells by themselves (in *facilitated diffusion*) or accompanied by Na^+ ions (as a form of *active transport*). Disorders that involve the inability to transport glucose into cells fall under the general category known as diabetes or *diabetes mellitus* (a name that literally means "passing sugar" from the fact that excess, unused glucose is dumped into the urine from the blood circulation). In higher animals such as humans, glucose is transported into cells only by means of a facilitated diffusion process using a family of related proteins that are classified as *glucose transport proteins* (abbreviated as *GLUT*).

There are at least seven members of this protein family (Wildman, Medeiros, 2000) and their general structure is shown in Figure 4–27. The figure shows that glucose passes into the cell through a pore formed by five of the twelve helices in the plasma membrane. Two important points about this transport are made here. *The first* is that the relative ability of a cell to take up glucose is dependent upon the population of GLUT molecules that are present in the plasma membranes. One should be aware that there are also GLUT molecules stored within the cell, but they have no role in transport when stored. *The second* is that some GLUT molecules (GLUT-4) at the plasma membrane are dependent upon the action of the hormone *insulin* and its receptor protein (*the insulin receptor*) in

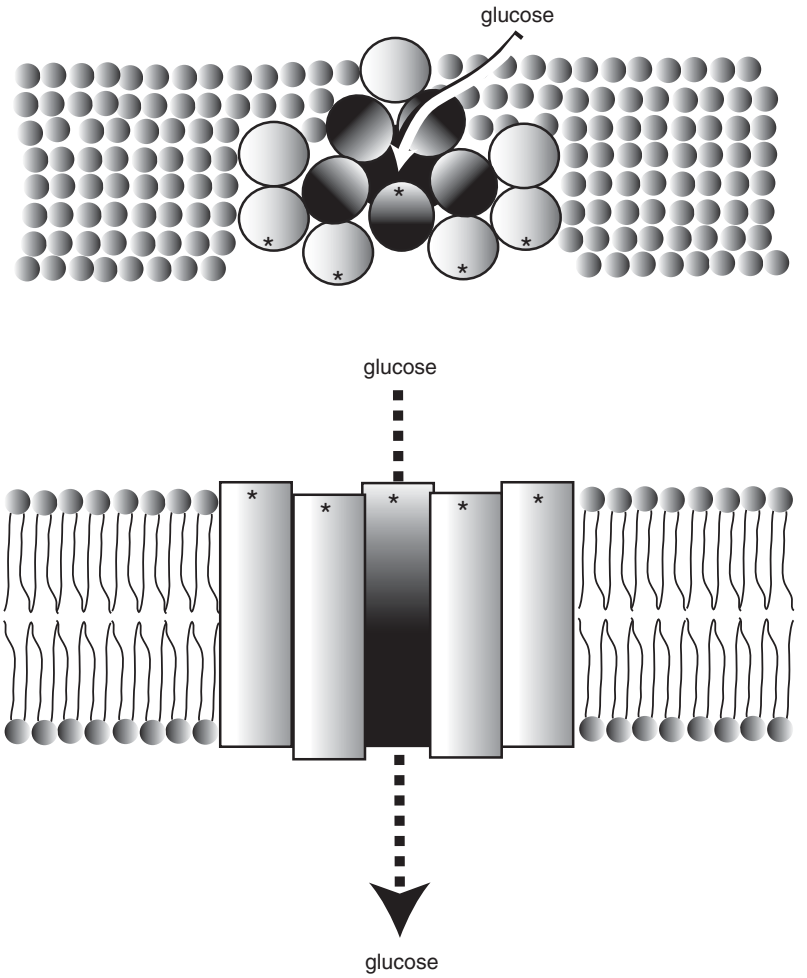


Figure 4–27
A typical glucose transport protein (GLUT). ➤ Upper figure shows a top view of the 12 trans-helical peptides that cross the cell's plasma membrane. In the lower figure, the protein is rotated 90° to show a cross section. Glucose is transported by the pore formed by the innermost peptides (dark colored). Small stars identify the trans-helical peptides seen in both the upper and lower figures. All trans-helical peptides are joined to each other by connecting peptides (not shown). (Based on a diagram in Williams G, Pickup JC: Handbook of diabetes, ed 2, Oxford, 1999, Blackwell Science; p. 34.)

which the latter is also present at the cell plasma membrane. In type 1 diabetes there is a lack of insulin and in type 2 diabetes there is a problem with the insulin receptor protein (a condition known as *insulin resistance*). Insulin and its receptor are shown in Figures 4–28 and 4–29, respectively. In normal operation, insulin binds to its receptor protein to induce a conformational shift in the protein that causes the activation of a tyrosine kinase, which is part of the receptor and is located on the cytoplasmic side of the receptor (Figure 4–30). The activation of the kinase initiates a cascade of phosphorylations in intracellular proteins and enzymes that causes, among other things, the transport of GLUT-4 proteins to the surface of the cell. The presence of more GLUT-4 proteins in the plasma membrane, in turn, increases the ability of the cell to take up glucose (Figure 4–31). This is, of course, a simplification of a process that is still incompletely understood (Pessin, Saltiel, 2000).

TYPE 1 DIABETES

Type 1 diabetes was formerly called "juvenile diabetes" since it typically becomes manifest by age 20. However, it is now known that this form can occur after that age. Type 1 was also known as "insulin dependent diabetes" since its patients require periodic insulin injections. Again,

Figure 4–28
A molecular diagram of insulin. ➤ The molecule consists of two chains joined by disulfide bonds. The grey areas represent portions of the molecule responsible for biological activity (essentially the ability to bind to the insulin receptor). Insulin is synthesized in the pancreas. (Based on that of Martin DW, et al: Harper's review of biochemistry, ed 20, Los Altos, CA, Lange Medical; 1985, p. 588.)

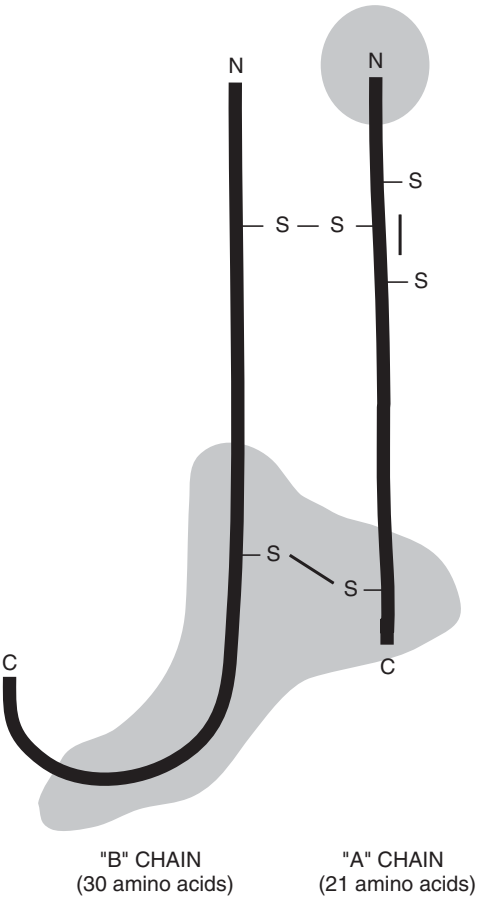
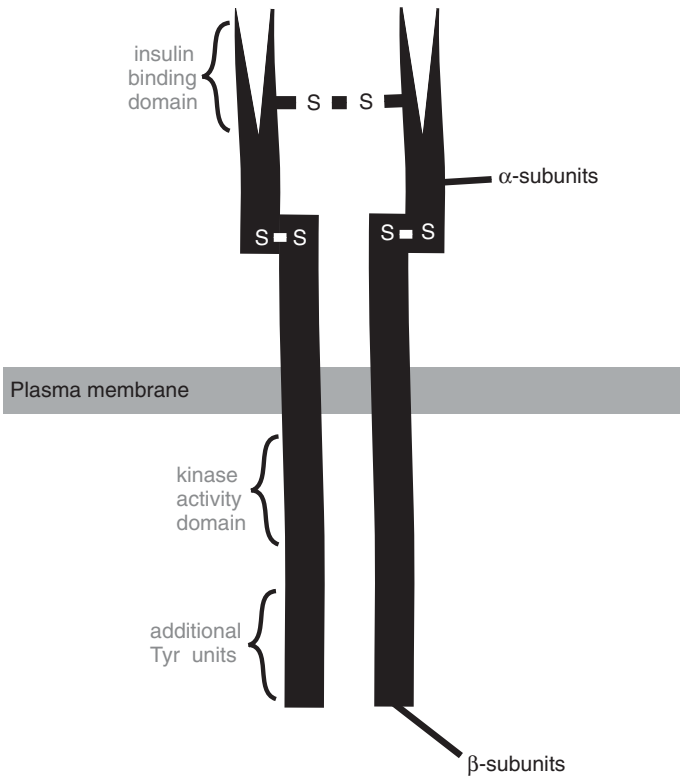


Figure 4–29
Insulin receptor protein at a cell plasma membrane. ➤ The receptor protein consists of four polypeptides held together by disulfide bonds (–S–S–). It incorporates tyrosine kinase enzymes on the cytoplasmic side of the membrane. This enzyme is activated when insulin binds to the receptor and causes phosphorylation of other cytoplasmic proteins as its "message." One message is to move glucose transport proteins to the cell surface.



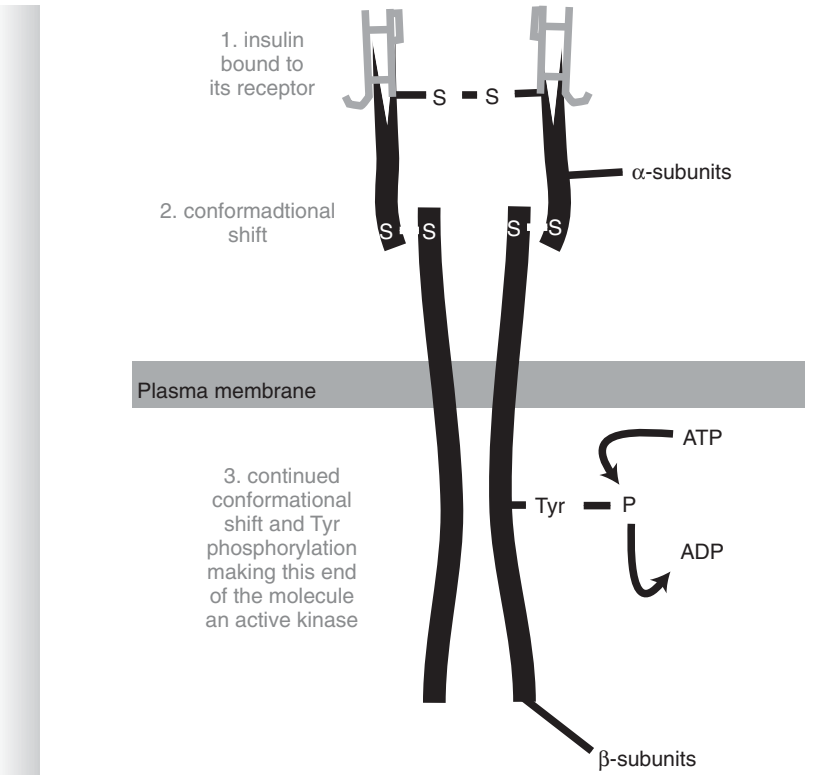


Figure 4–30
Conformational change and activation of the insulin receptor protein upon insulin binding.

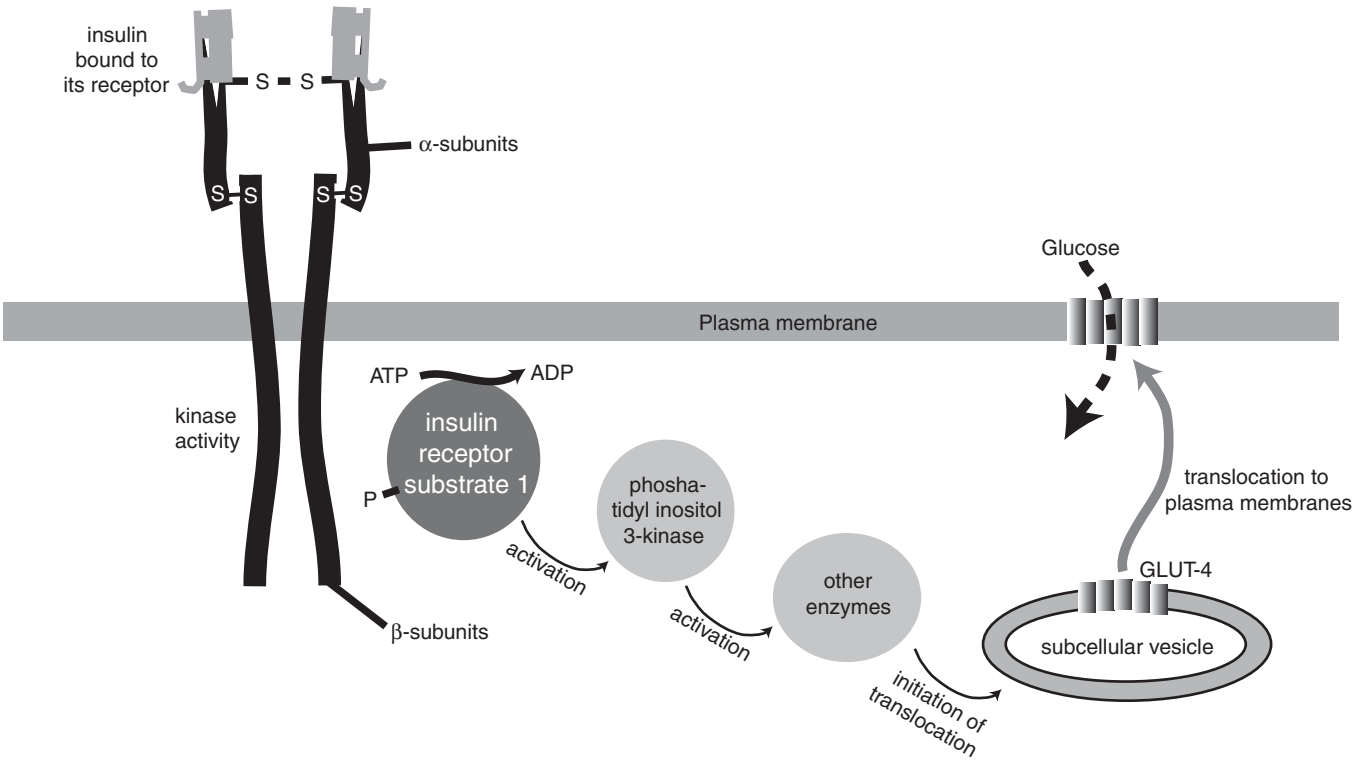


Figure 4–31
Partial mechanism of GLUT-4 transport by insulin. ➤ Upon binding of insulin to its receptor, kinase activity in the receptor is activated at the cytoplasmic side of the receptor. This kinase activity causes the phosphorylation of insulin receptor substrate 1 (IRS-1), a 131 kDa protein. This action brings about noncovalent binding to and activation of phosphatidylinositol 3-kinase whose activity, in turn, initiates GLUT-4 transport to the plasma membrane surface by way of the activation of still other enzymes. (Based on a diagram in Williams G, Pickup JC: Handbook of diabetes, ed 2, Oxford, 1999, Blackwell Science; 32.)

however, patients with type 2 diabetes sometimes also require insulin injections (Wildman, Medeiros, 2000). The immediate cause of type 1 is usually an autoimmune destruction of the β-cells of the pancreas that synthesize insulin (Figure 4–32). In addition to its role in glucose uptake, insulin signals the initiation of many other cell metabolic functions: amino acid uptake, glycolysis, formation of glycogen, and lipid synthesis as well as the synthesis of proteins, DNA, and RNA. One can say that insulin is vital hormone since it communicates the continuation of cell nutrition and growth overall. However, the uptake of glucose remains as one of its most important functions.

The destruction of the β-cells of the pancreas, as mentioned above, appears to be an autoimmune phenomenon. There is a strong genetic component to the appearance of this form of diabetes, which is associated with the chromosome 6 genes for the *human leukocyte antigens* (HLA). Wildman and Medeiros (2000), describe these proteins, made by their respective genes, as critical for distinguishing between host and foreign cells. This will be taken up in more detail in Chapter 9. Because of the loss of insulin, high levels of glucose remain in the circulating blood long after

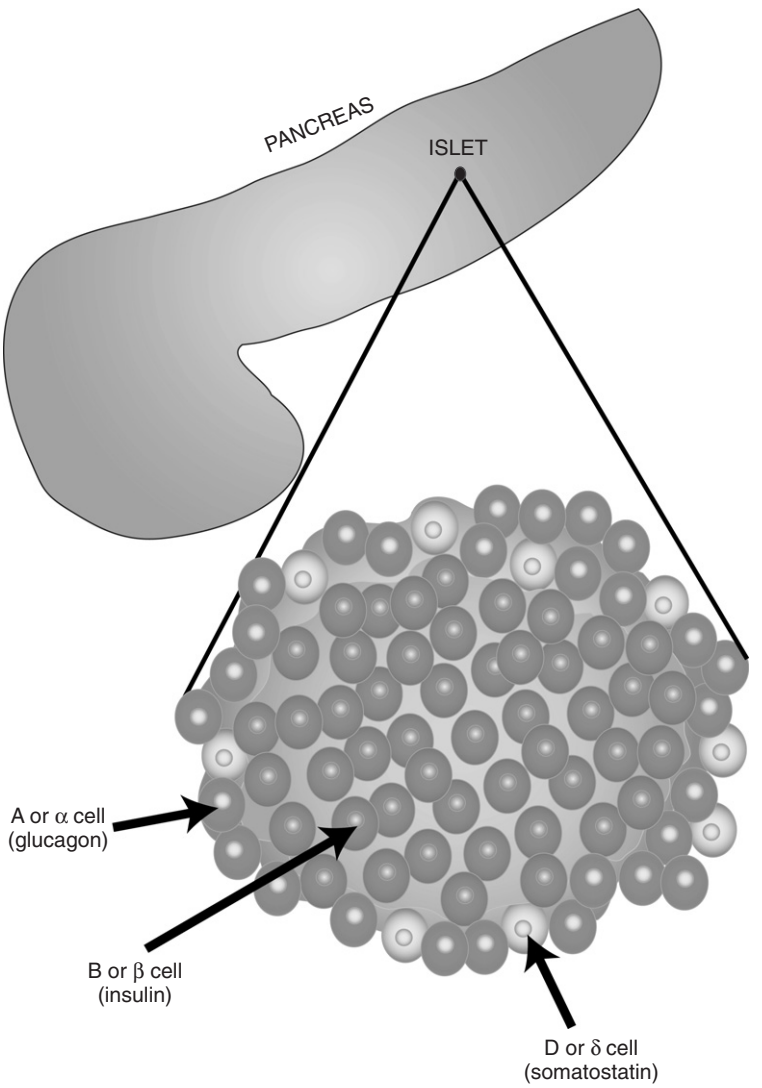


Figure 4–32
Diagram of islet cells in the human pancreas. ➤ Essentially, three types of cells produce insulin (β cells), glucagon (α cells), and somatostatin (δ cells). A fourth type of cell (F) produces pancreatic polypeptide. All cell types secrete hormones that deal with carbohydrate (and other intermediate) metabolic control. (Based on a drawing in Williams G, Pickup JC: Handbook of diabetes, ed 2, Oxford, 1999, Blackwell Science; 27.)

the consumption of a meal. For this reason, type I diabetes has also been known as insulin dependent diabetes mellitus (IDDM).

It is important to understand that those cells that depend on insulin (those that have GLUT-4 transport proteins) become starved for nourishment while other cells, not dependent on insulin, become exposed to higher than normal cytoplasmic levels of glucose. This is to say that some cells have decreased nutrition while other cells are actually exposed to toxic levels of glucose. Cells that are particularly insulin dependent are muscle cells (cardiac, skeletal, and smooth) as well as adipose (fat) cells (McGilvery, Goldstein, 1983) and *cells of blood vessel walls* (Koschinsky, 1988). Among the cells which are not insulin dependent are liver cells, nerve cells, red blood cells, bone cells, and *lens fiber cells* of the eye. This is why the enzyme aldose reductase is activated within lens fiber cells in the diabetic state (see Chapter 3).

In diabetes, particularly type 1, the insulin dependent cells alter their metabolism in pathological (i.e., abnormal) ways in order to compensate for their lack of glucose. In muscle cells, for example, it is common in the diabetic state to accelerate the breakdown of amino acids (Martin et al, 1985) in order to obtain acetyl CoA as a precursor for ATP production (see Figure 4–11). This occurs at the expense of muscle proteins in the body. In fat cells, on the other hand, there is an acceleration of lipid (fat) oxidation or breakdown in order to release fatty acids back into the bloodstream (Martin et al, 1985). These fatty acids are taken up by the liver to produce acetyl CoA for the same purpose of synthesizing ATP. However, in this case, not only are the stores of lipid reduced, but there is also a production of toxic intermediates from excessive fatty acid breakdown. These toxic intermediates are known as *ketone bodies* (Figure 4–33). Most ketone bodies (acetoacetate and β-hydroxybutyrate) are sufficiently acidic to lower blood pH to dangerously low levels. This could occur to the point at which the patient might become comatose and, if untreated, could expire due to complications such as severe dehydration, respiratory distress, cerebral edema, and thromboembolism (Williams, Pickup, 1999). Of course, this represents an extreme of untreated type 1 diabetes or, in some cases, where insulin therapy management is very difficult.

High circulating levels of glucose can also bind to proteins both extracellularly and intracellularly (in those cells that do not have

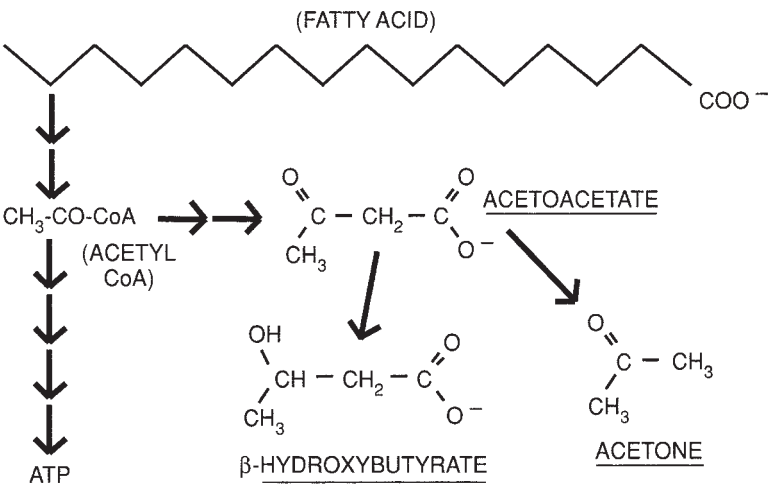


Figure 4–33
The formation of ketone bodies. ➤ Acetoacetate, acetone, and β-hydroxybutyrate are formed from acetyl CoA because of the excessive catabolism of fatty acids. Multiple arrows indicate several enzyme-catalyzed steps.

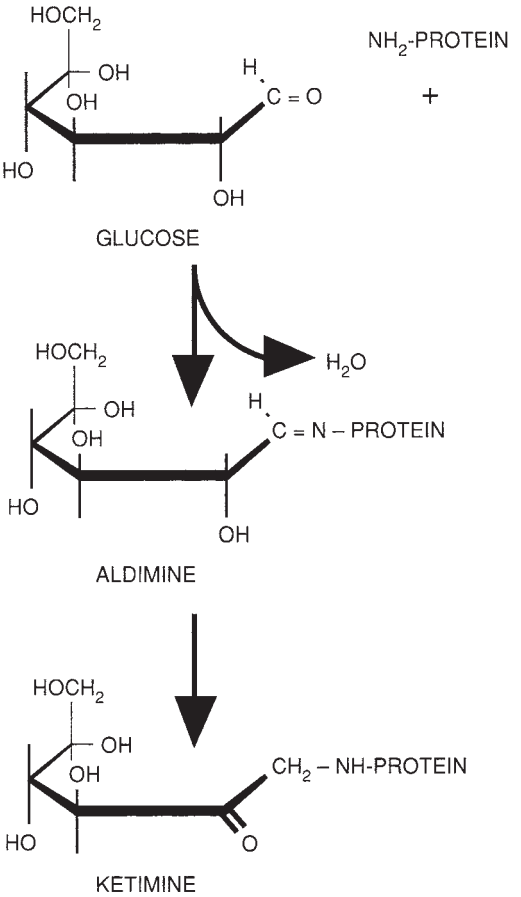


Figure 4–34
Glycation reaction. ➤ Binding of glucose to protein to form an aldimine with subsequent Amadori rearrangement to form a more permanent ketimine.

GLUT-4 transport proteins). The binding of glucose to proteins is known as *glycation*. Glycation is a slow reaction that proceeds through many steps. The initial reactions are shown and described in Figure 4–34. It is important to note that the reaction is permanent, meaning that when glucose levels are lowered the binding of glucose to the protein does not dissociate. Rather the reaction continues and produces more complex forms that result in the denaturation of the protein in what is known as the *Maillard reaction* (Berman, 1991). The role of protein glycation in ocular diabetes is discussed further on.

Finally, it should be noted that glucose in high concentrations has been proposed to cause DNA damage (Morita et al, 1985). However, Schleicher et al, (2001), have indicated that only 1×10^{-5} % of the guanine nucleotides of DNA would be expected to react with a glucose derivative in the diabetic state. This is a negligible amount.

TYPE 2 DIABETES

In type 2 diabetes, formerly know as noninsulin dependent diabetes mellitus (NIDDM), there is *either* an insufficient number of functional insulin receptors on the plasma membranes of insulin dependent cells *or* the receptors, in normal amounts, fail to promote sufficient glucose uptake (Mathews, van Holde, 1990; Martin et al, 1985). Refer again to the insulin receptor molecule in Figure 4–29 and the reactions shown in Figures 4–30 and 4–31. The phenomenon of insufficient response to

normal amounts of insulin is called *insulin resistance*. In addition, patients with this disease also exhibit a somewhat decreased level of secreted insulin for unknown reasons. This insulin deficiency, however, does not often require insulin therapy. The cause(s) of type 2 diabetes remain vague (Wildman, Medeiros, 2000).

One causative attribute has been the association of this disease with obesity. How this occurs is not certain, but some evidence is available. For example, enlarged adipocytes (fat cells) secrete a protein known as *tumor necrosis factor-α* that has been shown to inhibit insulin receptor autophosphorylation.

A characteristic of type 2 diabetes is that the associated hyperglycemia tends to develop more slowly than with type 1 and is of a milder degree. Ketoacidosis, in untreated type 2 diabetics, occurs much less often and then it is usually associated with physiological stress such as an infection. Returning to the problem of insulin resistance, one may point to not only the insulin receptor protein, but also to any of the proteins associated with the cascade from the receptor to the placement of GLUT-4 molecules at the cell plasma membrane (Pessin, Saltiel, 2000). This means that any number of proteins, such as those shown in Figure 4–31 may contribute to the resistance. Numerous data support this to indicate many possible causes of the disease and emphasizes the difficulties of trying to comprehend how this disease can manifest itself. In addition to the effect of fat cells on the insulin receptor (mentioned above), investigations have shown that knocking out the gene for the *insulin substrate proteins* (insulin receptor substrates) in mice can produce insulin resistance as well as cause a decreased β-cell production of insulin in the pancreas (Kulkarni et al, 1999). The next protein in the cascade, as shown in Figure 4–31, is phosphatidylinositol 3-kinase (PI3-K). Although PI3-K activity is necessary for the ultimate transport of GLUT-4 to the plasma membranes of insulin dependent cells (Czech, Corvera, 1999), its activity is not sufficient to carry out the transport. This means that additional mechanisms are involved (Pessin, Saltiel, 2000) and, clearly, more work will be necessary to unravel the causes of insulin resistance. In spite of these uncertainties, a controlled diet coupled with exercise is often sufficient to manage type 2 diabetes.

OCULAR PATHOLOGICAL EFFECTS PRODUCED BY DIABETES

Although all areas of the eye seem to be adversely affected by diabetes, three anatomical parts of the eye deserve special attention: the lens, the retina, and the cornea.

The Diabetic Lens

The lens is being considered first since initial ocular biochemical studies of diabetes have their roots in descriptions of *diabetic cataracts*. The classic osmotic hypothesis of such cataract formation was first proposed by Kinoshita (1963, 1974). The hypothesis states that toxic levels of glucose enter lens cells and activate aldose reductase. This enzyme converts glucose to sorbitol, a polyol intermediate that is not able to escape from the cell and which can generate high intracellular osmotic pressure that is sufficient to burst lens cells. Although a second enzyme in the pathway (polyol dehydrogenase) can convert sorbitol to metabolizable

fructose, the reaction is too slow to prevent osmotic destruction of lens cells. Data that supports this hypothesis is seen in Table 4–7 from studies on the lens of the South American degu as reported by Varma, Mizuno, and Kinoshita (1977). It can be seen from the table that the level of sorbitol is maintained at more than twice that of fructose and is about 12 times that of glucose. The cellular debris generated by this damage becomes the manifestation of a cataract. The polyol pathway is outlined in Figure 4–35 and aldose reductase was discussed in Chapter 3. There is considerable data to promote acceptance of this explanation for diabetic cataract formation (e.g., see Kinoshita et al, 1990). However, others (Bron et al, 1993) have suggested recently that separate mechanisms such as the protein denaturing effects of glycation and oxidative stress (considered later) may also play important roles in diabetic cataract formation. Bron et al state that their reason for this is that aldose reductase is largely limited to the lens epithelium and that any generated sorbitol might not spread to lens fiber cells to produce an equivalent osmotic stress (see Robison et al, 1990). Recently, Kador et al (2000), have given evidence that glycated proteins probably do not have as significant a role for diabetic cataracts as do polyols. In the lens, therefore, these newer proposals remain unsettled.

The Diabetic Retina

The retina is vulnerable to diabetes due to deterioration that occurs to its blood vessels in a condition known as *diabetic retinopathy.* This is similar to diabetic blood vessel damage that also occurs to other parts of

TABLE 4–7 ➤ CARBOHYDRATE CONCENTRATIONS FOUND IN THE LENS OF SOUTH AMERICAN DEGUS[1]

Concentration (μmoles/g tissue wet weight)		
Glucose	Sorbitol	Fructose
1.7	18.7	8.4

[1]The β-cells in the pancreas were destroyed with streptozotocin.
Data from Varma et al., 1977.

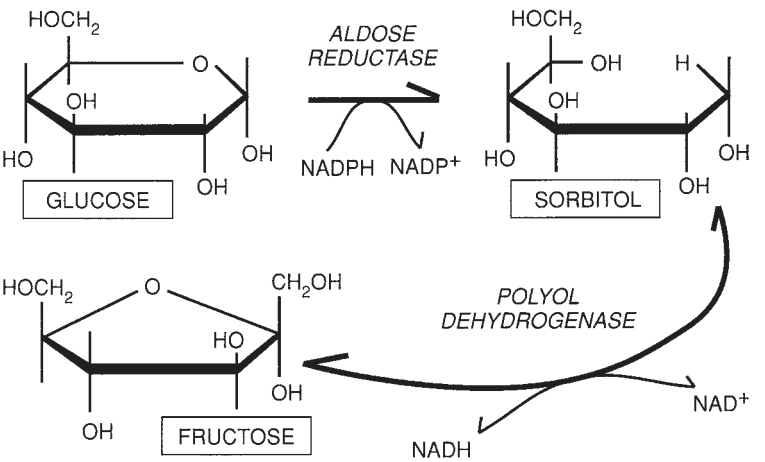


Figure 4–35
The polyol pathway. ➤ Figure shows the formation of sorbitol and fructose in the pathway from glucose. When the substrate is galactose (rather than glucose as shown) the polyol product galactitol serves as a very poor substrate for polyol dehydrogenase.

the body such as the kidneys, brain, and limbs. Essentially, in the retina pericytes are destroyed while the lumen of the vessel becomes blocked due to vessel swelling as the basement membranes thicken (Apple et al, 1988). The diagram in Figure 4–36 shows such a typical blood vessel in cross-section. As vessel pathology develops, hemorrhages and retinal detachment follow. The terminal result of these events is loss of vision in parts or all of the retina. Shedding light on a biochemical mechanism by which this damage occurs has proven to be a Pandora's box. As the box is opened, out pops the familiar enzyme *aldose reductase* and its role of removing excessively toxic amounts of glucose from within cells such as blood vessel endothelial cells (see Figure 4–36). Again, the problem of the generation of osmotic sorbitol arises. The pathway is shown in Figure 4–35 and is the same as discussed previously. From the figure, ostensibly, the problem (the presence of large amounts of high osmotic, cell destroying sorbitol) and the solution (development of an effective inhibitor for aldose reductase) are seen as a logical exercise. However, other previously unknown participants emerge from the Pandora's box: *protein kinase C (PKC)*; *mitogen-activated protein kinase (MAPK)*; *protein glycation*; and *oxidative stress* (Tomlinson, 2000). The complications to understanding that are generated by these other substances/mechanisms are, nonetheless, now seen as more important in the retina compared to the lens osmotic hypothesis. This is so since aldose reductase inhibitors do not seem to have any significant effect on the prevention of blood vessel degeneration in the retina. What roles might these other substances and mechanisms play?

In 1996, King reported that high levels of glucose cause an increase in *diacylglycerol (DAG)*, a phospholipid precursor (discussed in Chapter 6) that is synthesized from glyceraldehyde 3-phosphate and dihydroxyacetone phosphate. The latter two substances are generated in the E-M pathway from glucose (see Figure 4–13). DAG stimulates the activity of protein kinase C (PKC), an enzyme that acts as part of a hormone cascade system at cell surfaces (see Chapter 6). PKC, as part of its many physiological functions, increases both blood vessel permeability and the excessive synthesis of blood vessel membranes. One of the features of diabetic blood vessels is their "leakiness" (permeability) and their thickening with the synthesis of basement membranes as

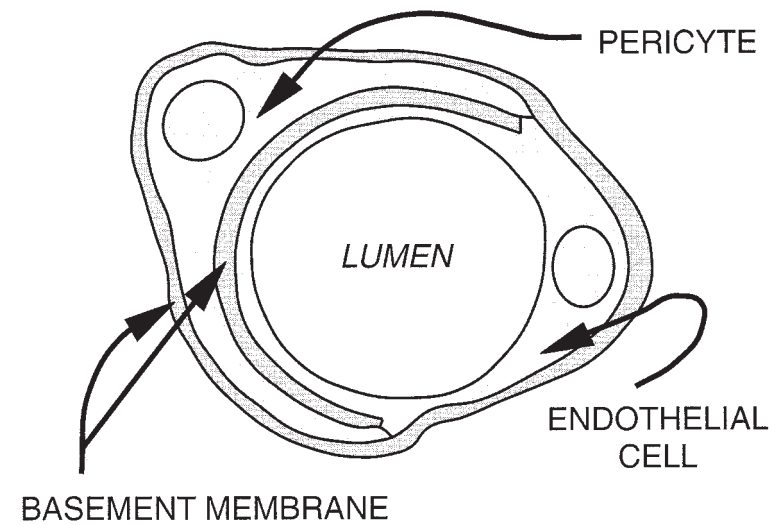


Figure 4–36
Cross-section of a blood vessel typical of those found in capillary beds from the central retinal artery. ➤ (Based on photomicrograph from Frank et al: Pericyte coverage of capillaries, Invest Ophthalmol Vis Sci 31:999–1007, 1990.)

shown in Figure 4–37. In a more recent publication, Park et al (2000), indicate that PKC causes the induction of the synthesis of the protein *endothelin-1 (ET-1)*. This protein is responsible for increased blood vessel permeability as well as its thickening. Similar effects may also be made through the activation of *mitogen activated protein (MAP) kinase*, an enzyme that translocates into the nucleus of the cell and affects many different cell functions *via* phosphorylation (Lodish et al, 2000).

Another effect of diabetes that arises from the Pandora's box is the binding of glucose to proteins (glycation). In higher glucose concentrations, this occurs initially by what is known as the "Amadori rearrangement" of an initial Schiff base (aldimine) formation and is shown in Figure 4–34. The rearranged bond at this early stage is termed a *ketimine*. These protein-carbohydrate conjugates seem to occur in a number of extracellular proteins as well as the intracellular proteins of noninsulin dependent cells (Cohen, 1986). Such early stage complexes are exemplified by the existence of glycated hemoglobin (HbA_{1c}) in the blood. HbA_{1c} can actually be used to assay how well blood glucose control is being maintained in a diabetic (Williams, Pickup, 1999). Among those proteins known to be bound by glucose are collagen, crystallins, and enzymes such as NaK-ATPase, as well as hemoglobin.

Unfortunately, the initially formed protein-glucose ketimines may continue to react with time to form somewhat complex, permanent and generally undefined, *advanced glycation end-products (AGEs)* as reported by Brownlee (1996). Among these AGEs, one has recently been described as an imidazole-crosslinked protein shown in Figure 4–38. AGEs produce a variety of effects including: (1) binding to receptors on vascular endothelial cells to produce a blockage in the vessel; (2) a leakage of the vessel itself; (3) vessel dilation; (4) vessel thickening (Rudnicka, Birch, 2000) and cell death by *apoptosis* (Kern et al, 2000).

The process of oxidation has been proposed as an adjunct to the mechanistic formation of AGEs from glucose and protein-glucose ketimines. Weiss et al (2000), describe how molecular oxygen, in conjunction with metal catalysts, may bring about the formation of glyoxal and other intermediates prior to the crosslinking of proteins as AGEs.

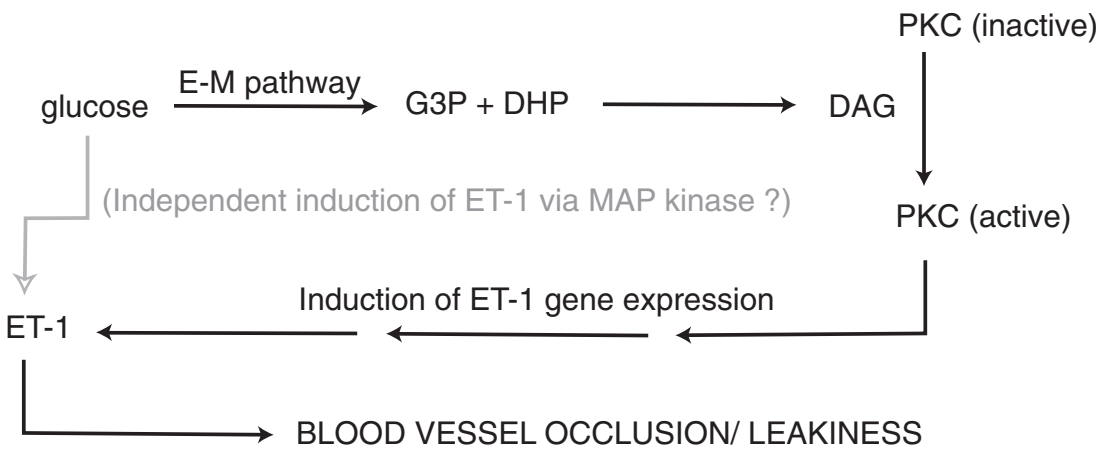


Figure 4–37
Diagram showing the relationship of carbohydrate metabolism, activation of protein kinase C, and the induction of endothelin-1 (ET-1) synthesis in the development of blood vessel pathology in diabetes. ➤ The possible contribution of MAP kinase is also indicated. See text.

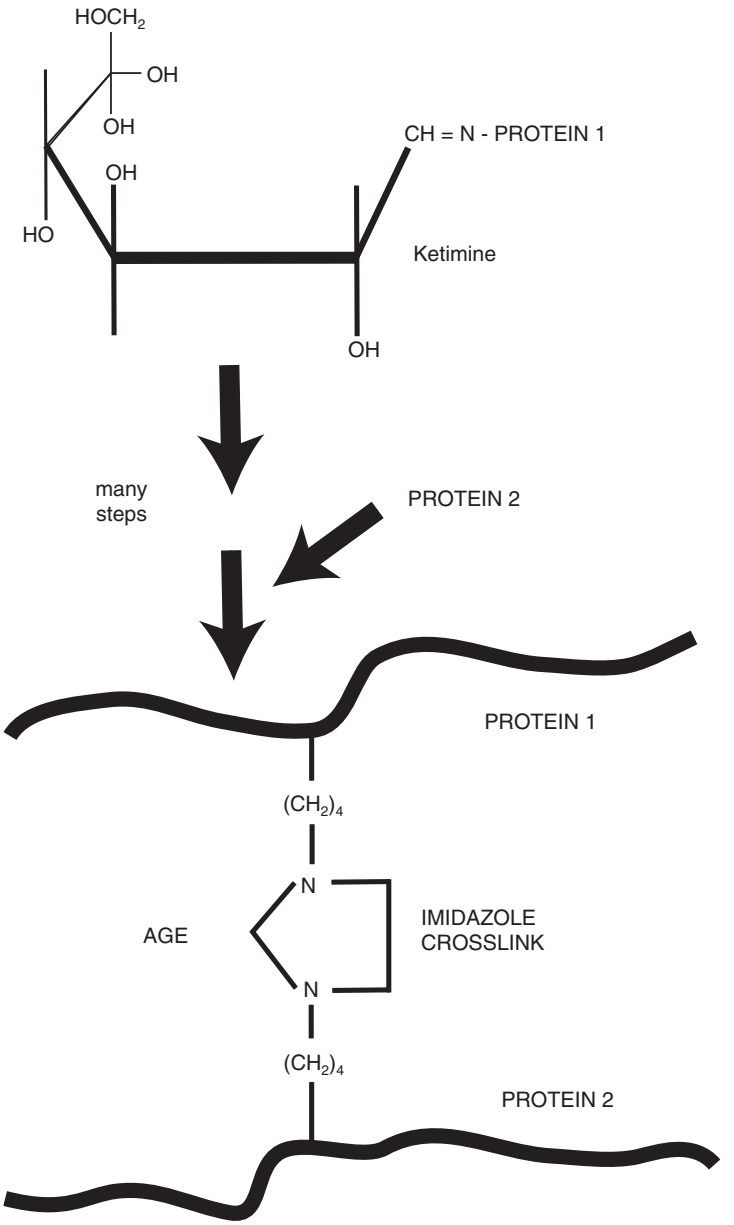


Figure 4–38
The formation of one advanced glycation end product by means of an imidazole crosslinkage of two proteins. ➤ (Based on work of Nagaraj RH, Shipanova IN, Faust FM: Protein cross-linking by the Maillard reaction J Biol Chem 271: 19338–193445, 1996.)

[(HC=O)–(O=CH)]
glyoxal

Detailed discussion of these mechanisms is beyond the scope of this text. The mechanisms, in fact, remain somewhat speculative.

The Diabetic Cornea

Diabetes produces two and, perhaps three, pathological effects on the cornea: (1) decreased epithelial adhesion to the corneal stroma, (2) loss of corneal neural sensitivity, and (3) possibly increased corneal thickness

principally in the stroma (van Bijsterveld, Klaassen-Broekema, 2000). See the diagram in Figure 4–39. The first effect impairs the ability of the corneal epithelium to repair itself especially after corneal surgery. Kenyon et al (1978) reported that about 50% of diabetic patients lose the ability to anchor their epithelial cells to the anterior stroma. Benson, Brown, Tasman (1988), stated that the defective collagen anchoring fibrils (see Figure 2–38), that are located at the anterior surface of the cornea, can lead to tissue erosions. The second effect impedes one's ability to sense corneal contact such that patients may become unaware of bacterial infections and, therefore, undetected corneal ulcerations may occur. The third effect may cause corneal swelling (Herse, 1990; Olsen, Busted, 1981) although some investigators have not concurred (Schultz et al, 1984; Lass et al, 1985). A recent review of this subject (Sanchez-Thorin, 1998), however, indicates that the observed swelling has not been further challenged. Corneal swelling can affect the ability to wear contact lenses. Both Herse (1990), and Whikehart et al (1993), have shown evidence that such swelling is associated with a decrease in the catalytic activity of endothelial Na,K-ATPase, the osmotic pump that controls corneal hydration. The proposed causes of these effects to the cornea, as in the retina, have been multiple. The osmotic hypothesis, as described previously, has been suggested for this tissue (Ohguro et al, 1995). As evidence for this, aldose reductase-like immunoreactivity has been found in corneal endothelial cells of diabetic rats (Chakrabarti et al, 1987) and Neuenschwander et al (1995) have found evidence of polyol pathway activity in galactose-fed dogs. However, there is also evidence of the presence of AGE products, due to protein glycation, in human corneal epithelial cells obtained at autopsy (Kaji et al, 2000). The latter investigators also found that when *laminin* is glycated then the growth of cultured epithelial cells across its matrix is inhibited. Laminin is one of the proteins that forms the extracellular matrix that is needed for the spreading and attachment of epithelial cells during healing. All this evidence indicates that at least the polyol pathway is involved in the endothelium in diabetes leading to abnormal endothelial morphology and, possibly swelling, while glycation is an important cause of epithelial defects.

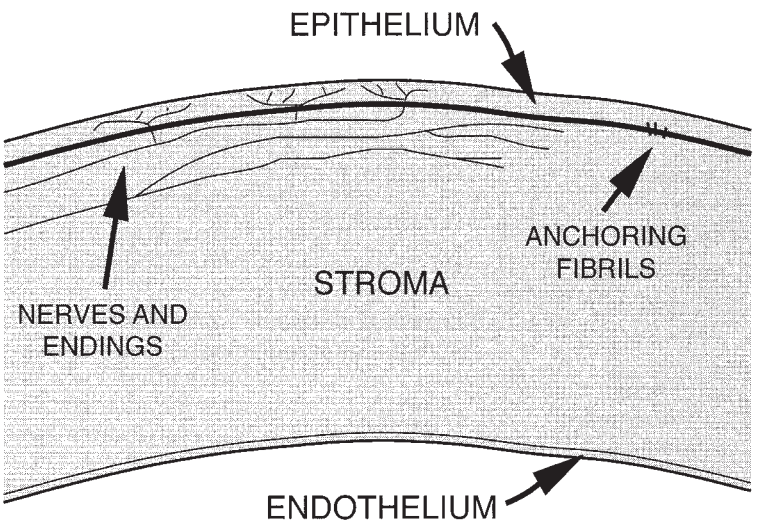


Figure 4–39
Cross-section of the cornea showing the partial distribution of nerves, anchoring fibrils of the epithelium and endothelium. ➤ All these tissues of the cornea are affected in diabetes.

Corneal neuropathy occurs with the development of irregularities in the basal lamina of the Schwann cells at the anterior cornea (Ishida et al, 1984). The biochemical cause of this neuropathy is unknown, but Qian and Ealon (2000) have indicated that the formation of protein AGEs may play a role in all forms of peripheral neuropathy.

INTRODUCTION TO GALACTOSEMIA

Galactose is incorporated into the E-M pathway *via* glucose 1-phosphate by the activity of three enzymes: galactokinase, galactose-phosphate uridyl transferase, and UDP-galactose epimerase. Note that glucose 1-phosphate itself is converted to glucose 6-phosphate in the E-M pathway as has been shown for the breakdown of glycogen in Figure 4–24. However, that portion of the pathway is not involved in galactosemia. The main metabolic pathway for galactose conversion is shown in Figure 4–40. A deficiency in activity of any of the enzymes in the conversion pathway results in the accumulation of either galactose or galactose 1-phosphate in tissues. The resulting disease, due to any of these deficiencies, is known as galactosemia.

In so called classical galactosemia, the primary deficient enzyme is galactose-phosphate uridyl transferase (also called *GALT*). This enzyme shows less than 5% of its normal activity in affected individuals and is due to a genetic defect on chromosome 9. Elsas et al (1994) reported these characteristics and those of some genetic variants. The enzymes galactokinase and epimerase are less frequently deficient in other forms of galactokinase (Segal, Berry, 1995).

Characteristically, newborn patients with the classical form of galactosemia develop vomiting and diarrhea soon after birth. This can then be accompanied by jaundice, abnormal liver function, and galactosemic sepsis (poisoning). If untreated, the patient may die. Patients who are treated for this disease are withheld milk and milk products since milk sugar (lactose) contains galactose. Other galactose containing foods are also withheld. In general, the symptoms of the disease are reversible except for mental retardation which may also occur (Waggoner, Buist, Donnell, 1990). The disease occurs in about 1:20,000 to 80,000 individuals.

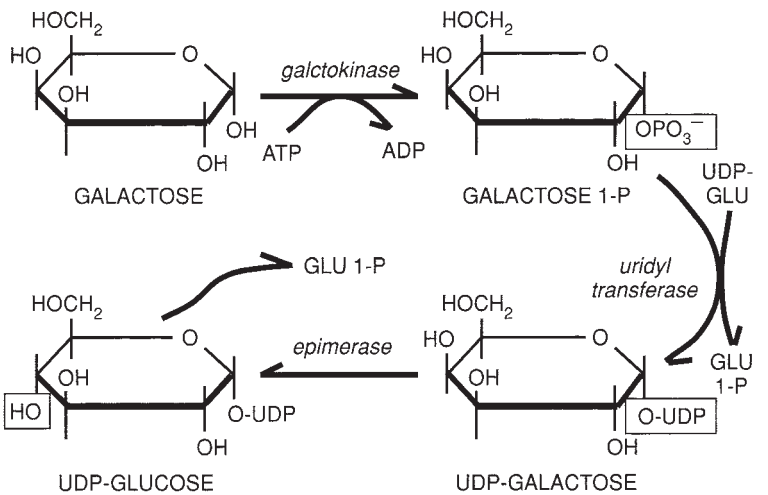


Figure 4–40
The incorporation of galactose into the E-M pathway. ➤ Several reactions are involved. Glucose 1-phosphate is finally converted to glucose 6-phosphate by phosphoglucomutase (not shown). Any enzyme deficiency in this incorporation pathway may cause galactosemia, however, the enzyme uridylyl transferase is deficient in most cases.

OCULAR EFFECTS OF GALACTOSEMIA

Zonular or nuclear cataract development occurs in about 30% of patients with classical galactosemia and is probably due to the rapid accumulation of galactitol in the polyol pathway (see Figure 4–35). In this instance, galactose replaces glucose as a substrate for aldose reductase. The product, galactitol, is an extremely poor substrate for polyol dehydrogenase. Therefore, accumulation of the osmotic galactitol is much faster than it would be for sorbitol, which can be catalyzed by polyol dehydrogenase. In the less severe galactosemia involving a deficiency of galactokinase, cataract development can still occur in the first year of life. In those forms of galactosemia involving epimerase deficiency, cataract formation does not take place (Nelson, 1998).

Structural Carbohydrates

SUGAR OLIGOMERS AND POLYMERS

Oligomers (Oligosaccharides)

Earlier in this chapter the subject of oligosaccharides was touched on as a class of carbohydrates in which 10 or fewer sugars were held together by oxygen bridges. Oligosaccharides are commonly found as covalent appendages to many proteins (known as *glycoproteins*) where they have a variety of roles including: increasing protein hydrophilic solubility, stabilizing protein conformation, and the proper orientation of a protein in a membrane. Oligosaccharides even act as recognition markers for cell sorting prior to protein transport and they may act as immunological identifiers for "friend or foe" reactions. However, the purposes of attaching oligosaccharides to proteins are not understood in all cases.

A specific known example is found in the oligosaccharides that are bound to rhodopsin. In that case, the hydrophilic sugars prevent the molecule from flip-flopping in the outer segment membrane disc. If the protein were able to flip back and forth, the process of visual transduction would be seriously impaired. A typical example of an oligosaccharide, bound to rhodopsin, is shown in Figure 4–41. Oligosaccharides are bound to proteins by either oxygen bridges (O-linked through Ser/Thr) or nitrogen bridges (N-linked through Asn). This is discussed in some detail by Voet and Voet (1995). Some oligosaccharides are also bound to lipids in cell surface membranes.

Polymers (Glycosaminoglycans)

In addition to the sugar polymers that are used to store glucose molecules for energy production as glycogen, there are other sugar polymers that have structural roles. These polymers are found throughout all bodily tissues including those of the eye. They exist predominately in the extracellular matrix, a complex of acellular tissue that shapes and maintains the form of multicellular organisms. These polymers are also involved in cushioning, lubricating and attaching the matrix to various kinds of cells. In the latter role, they can act as a type of biological glue. Such polymers are known as glycosaminoglycans or GAGs (to be described later in this section) and they have important roles in the eye.

For example, as part of the vitreous, GAGs help to support the retina in its concave configuration and they prevent the detachment of

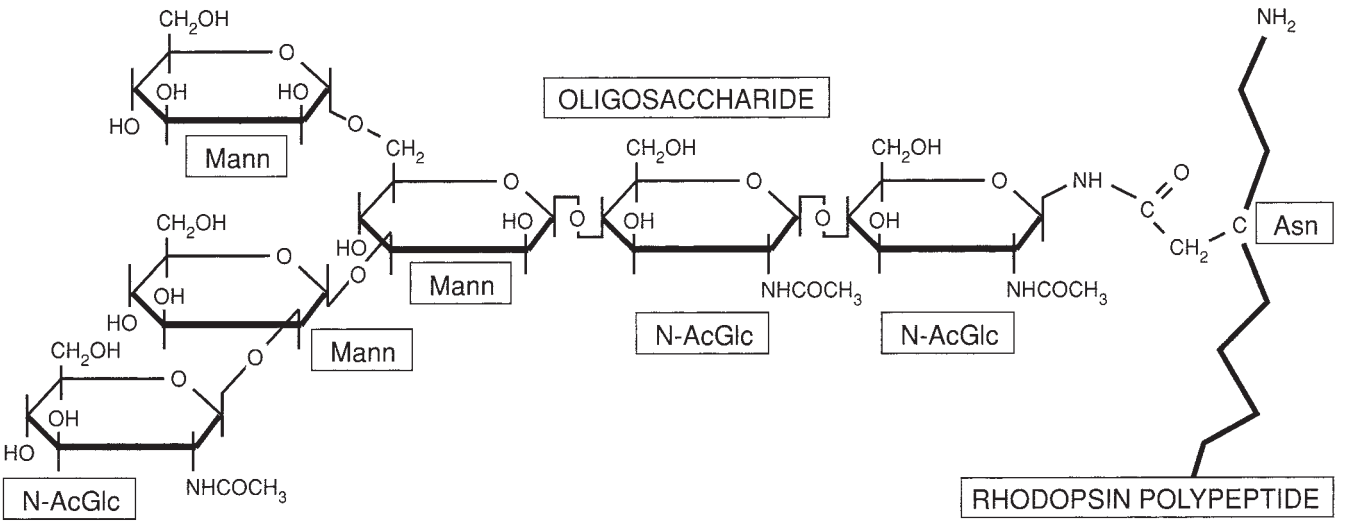


Figure 4–41
An oligosaccharide typically found on rhodopsin molecules. ➤ The oligosaccharide is bound to asparagine (Asn) by an amide bond.

the retinal neurons from the photoreceptors. At the same time, they are involved in absorbing mechanical blows to the eye and even cushioning the force of ocular *sacchades* (i.e., abrupt shifts in fixation from one point to another, as occurs in reading). While doing this, the vitreous polymers must also allow the unhindered passage of light from the lens to the retina.

The term glycosaminoglycan (GAG) refers to each of the repeating units of a sugar bound to an aminosugar that make up the polymer: $(\text{sugar-amino sugar})_n$. As with other sugar units bound together, an oxygen bridge is used to bind all of the sugars to each other. Formerly, GAGs were called *mucopolysaccharides (MPS)* since they are abundant in mucous tissues and were first found there. Most GAGs occur linked to core proteins and the entire assembly is known as a *proteoglycan*. Proteoglycans and GAGs are often associated with collagen in extracellular matrices. The common GAG structural units that are found in ocular tissues are shown in Figures 4–42 and 4–43. Although these struc-

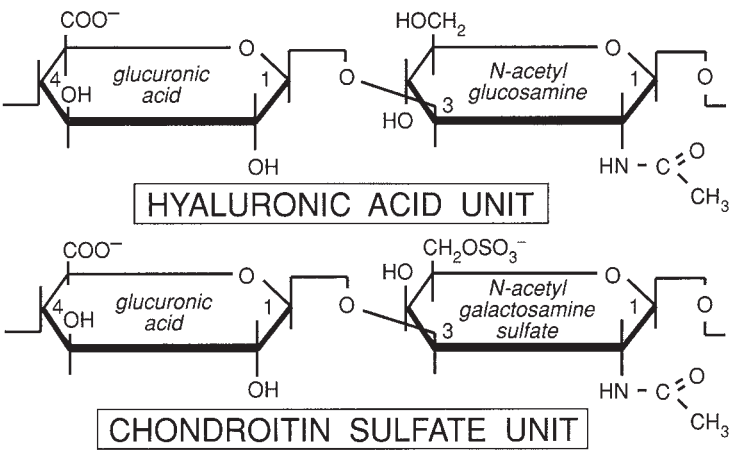


Figure 4–42
Repeating units of the glycosaminoglycans (GAGs) of hyaluronic acid and chondroitin sulfate. ➤ See text for explanation.

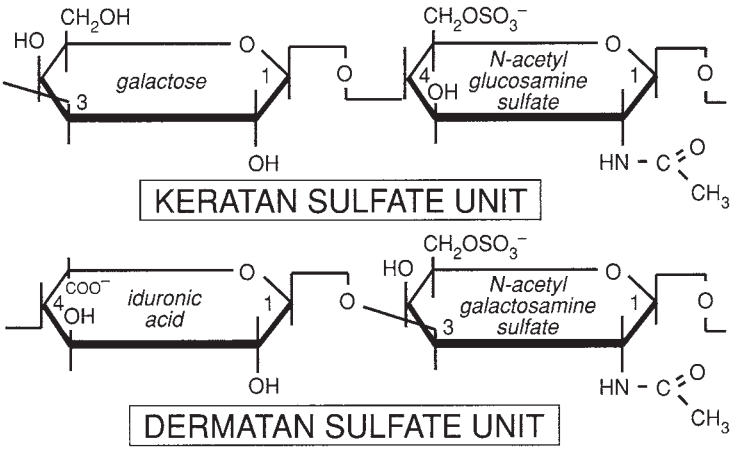


Figure 4–43
Repeating units of the GAGs of keratan sulfate and dermatan sulfate. ➤ See text for explanation.

tures may seem complex, inspection reveals that each unit is a simple derivative of the monosaccharides glucose and galactose described earlier in the chapter.

Glucuronic acid (also known as *glucuronate*) is glucose with the sixth carbon converted to a carboxylic acid while *N*-acetylated glucosamine and *N*-acetylated galactosamine are glucose and galactose to which nitrogens have been added onto carbon two and in which the nitrogens (in turn) have had acetyl (acetate) groups added to them. *Iduronic acid* is an isomer of glucuronic acid (the acidic group is attached downward in the figure). The individual members of each unit are linked $\beta 1 \rightarrow 3$ within the unit while units themselves are linked to other units $\beta 1 \rightarrow 4$ except for keratan sulfate where the order is reversed. *Sulfation* (the addition of sulfate groups) to the right hand unit adds considerable acidity and charge density to the entire unit. The sulfate groups can vary in amount. The negative charge density is an important characteristic of GAGs that attracts counter ions (principally Na^+) and water. This explains why the corneal stroma tends to swell, even uncontrollably in the absence of an active deturgescing enzyme (Na,K-ATPase), with the high osmotic pressure that the GAGs generate there.

Figure 4–44 shows the appearance of a segment of the GAG hyaluronic acid and its structural participation interposed between the collagen fibrils of the secondary vitreous of the eye (compare with Figure 2–36 in Chapter 2). *Hyaluronic acid*, as it exists in the vitreous, is a GAG that is not linked to a core protein. It is also known as *hyaluronan* or *hyaluronate*. The exact molecular form of hyaluronate is not known with certainty. However, it may form a twofold helix in solution as well as be stabilized by noncollagenous proteins there (Brewton, Mayne, 1992). The latter possibility is supported by the studies of Bishop, McLeod, and Reardon (1999) who showed that hyaluronidase is not able to completely break down hyaluronan implying that an unknown protein protects part of its structure.

In the corneal stroma, two types of GAGs are found that are linked to core proteins: keratan sulfate and dermatan sulfate. The core proteins themselves had initially been difficult to isolate and describe. Jost et al (1991) described proteins, which bind to keratan sulfate, one of which is called *lumican* (Figure 4–45). Another core protein which binds to dermatan sulfate is known as *decorin* and is present in cornea (Fisher, Termine, Young, 1989). The exact conformation of these proteins is

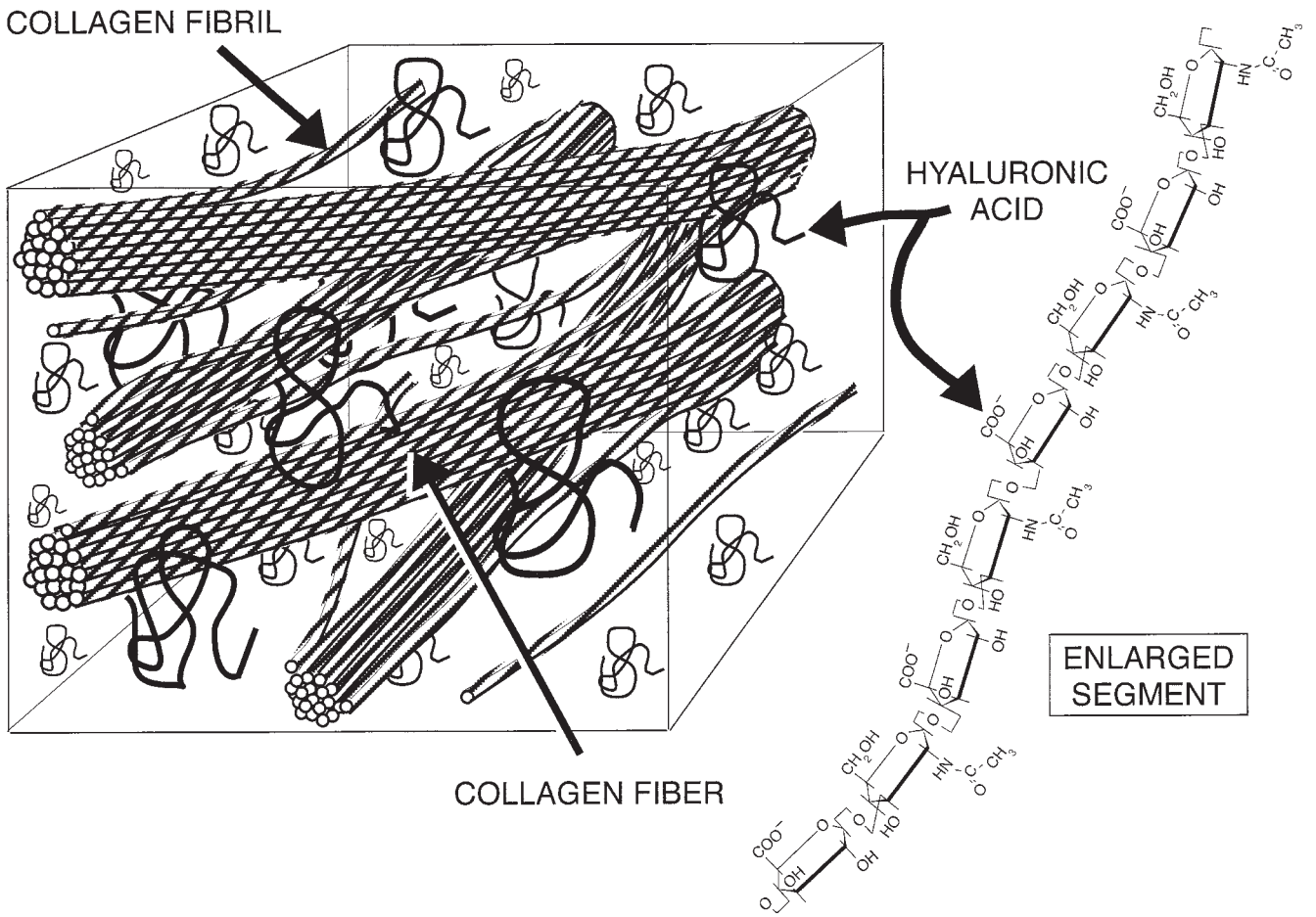
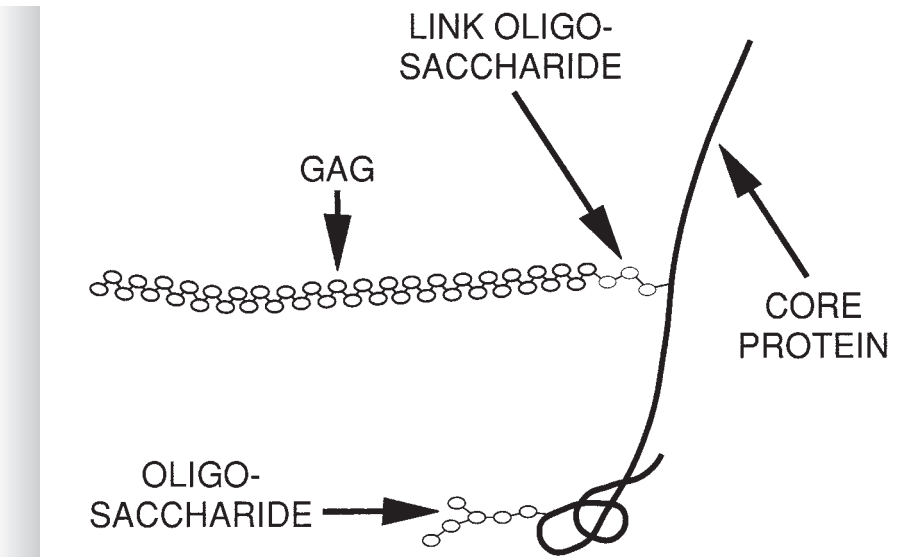


Figure 4–44
Cuboidal section of the vitreous showing GAGs and collagen components. ➤ The hyaluronic acid (HA) and other GAG molecules position themselves in between the collagen fibrils somewhat like packing material that is used to cushion the contents of a package. An enlarged segment of HA is shown to the right of the figure.

Figure 4–45
A proteoglycan typical of those found in the cornea. ➤ One to three GAGs are linked to their core protein (e.g., lumican) by an oligosaccharide. Another oligosaccharide (shown below the GAG) is not linked to a GAG. (Drawing adapted from Hassell J et al: Proteoglycan core protein families, Ann Rev Biochem 55:539–567, 1986.)



unknown, but the core proteins of the proteoglycans in the cornea bind between one and three GAGs. These proteoglycans function as spacer molecules between the collagen fibers of the stromal lamellae (Balazs, 1965). Such a characteristic is important to maintain corneal clarity since the regular distance array causes the mutual destructive interference of light and prevents scatter or cloudiness within the cornea (Maurice, 1957).

In certain diseases (e.g., *corneal dystrophies*) in which the corneal endothelial pump (i.e., Na,K-ATPase) is negatively affected (McCartney, Wood, McLaughlin, 1989), water enters the space where the proteoglycans are located and increases the distance between collagen fibers sufficiently so that the corneal stroma may become cloudy as a result of unequally scattered light. Midura et al (1990) have also demonstrated that there is abnormal proteoglycan synthesis in some of these dystrophies and that may also contribute to the cloudiness.

Much less common are a series of diseases known as *mucopolysaccharidoses*, a name applied when the term mucopolysaccharide (MPS) was used rather than GAG. These diseases result from a deficiency of one of several enzymes that normally breakdown the GAGs (Grayson, 1979). Because of the deficiency, partially degraded GAGs deposit in bodily tissues. In the eye, this occurs in the cornea to cause opacification or cloudiness and in the retina to bring about retinal degeneration and optic atrophy. The effects vary with the enzyme defect. All MPS diseases are inherited, but rare (Neufeld, Muenzer, 1995). An example of an MPS disease is called *Hurler syndrome*. In Hurler syndrome the enzyme α-iduronidase is deficient (Sugar, 1998). Accordingly, GAGs containing iduronic acid (e.g., dermatan sulfate and heparan sulfate) are not broken down past the initial removal of sulfate. Because of this, these GAGs start to accumulate in cell lysosomes where they spill over and then are deposited in all parts of the body including brain tissue, joints and even the cornea. This disease then affects not only the eyes, but also brings about mental retardation, skeletal deformation, and cardiac deficiency. Other forms of MPS diseases produce similar effects on the eyes and other parts of the body due to the pile up of incompletely catabolized GAGs (Sugar, 1998). MPS diseases belong to an even more diverse classification of inherited diseases known as lysosome storage diseases. These diseases also include the sphingolipidoses and mucolipidoses, which will be dealt with in the next chapter.

SUMMARY

Carbohydrates are polyhydroxy compounds containing aldehydes, ketones, and other functional groups. In solution they are capable of forming closed ring structures and most contain at least one reactive carbon (C-1 when free). These compounds may form short or long chain polymers. Ocular tissues use carbohydrates in monosaccharide form as sources of cellular ATP (a high-energy compound that drives many reactions in the cell). The glycolytic reactions that are involved can occur in the presence and absence of oxygen although some oxygen is always required for cell survival. Ocular cells use varying proportions of aerobic

and anaerobic metabolism in glycolysis to achieve their particular energy demands. Photoreceptors require the highest levels of ATP and lens fiber cells require the least. Glycogen is a long branched polymer of glucose, which exists as a cellular storage form of glucose. The metabolic pathway known as the pentose shunt is useful in the production of pentoses (for nucleic acids) and lipids (for cell membranes). It also is coupled to reactions that detoxify cells from intracellular hydrogen peroxide.

When carbohydrates are unable to enter insulin-dependent cells of the body, the body suffers in a diabetic state. This can occur either due to a lack of insulin (type 1 diabetes) or from a malfunction of insulin receptor proteins (type 2 diabetes). In the diabetic state, those cells not receiving sufficient amounts of glucose develop pathological metabolic substitutions: increased protein and lipid catabolism. Those cells and extracellular areas exposed to excessive levels of glucose in diabetes are subject to the toxicity of protein glycation, the osmotic stress of polyol formation, and other biochemical pathology. In the eye, the retina can develop degenerative blood vessels with a loss in vision. In the lens, cataract formation may take place. Furthermore, corneal epithelial cells can fail to reattach to their basement membrane while the whole cornea may swell.

Some carbohydrate derivatives are useful in forming polymers as part of the tissue structures that occur in the extracellular matrix. These derivatives are classified as glycosaminoglycans (GAGs). Many GAGs combine with core proteins to form proteoglycans. In the eye, these polymers are found in the vitreous, cornea, lens capsule, sclera, and blood vessels. When the catabolism of these polymers is incomplete, due to an enzyme defect, the resulting storage disease, a mucopolysaccharidosis, can adversely affect many bodily functions as well as those of the cornea and retina.

PROBLEMS

1. The Embden-Meyerhof pathway produces only two net molecules of ATP per molecule of glucose used when lactate is the final metabolite (anaerobic exit). The aerobic exit of this pathway, however, produces 36 to 38 molecules of ATP. In a given period of time, how would it be possible for the anaerobic exit to produce an amount of ATP equivalent to the aerobic exit?

2. Cyanide, a well-known poison, blocks the transport of electrons between protein complex IV and oxygen in mitochondria. Explain how that affects the production of cellular ATP.

3. If an individual inherits insulin receptor proteins whose molecular structure allows only 65% activation of tyrosine kinase activity,

what type of diabetes might the individual develop? What therapeutic measures might be used to treat the diabetes? Why would those measures be used?

4. Why is diabetes in the retina considered to be biochemically complex?

5. What is the principal damaging problem associated with lysosomal storage diseases?

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CHAPTER 5

Lipids

The terms *lipids* and *fats* have been used interchangeably to refer to groups of compounds that are water insoluble, but soluble in nonpolar solvents such as benzene, chloroform, and hexane. Strictly speaking, however, the term fat refers to lipid esters of fatty acids and glycerol. Lipid compounds are termed *hydrophobic,* which means that they have an aversion to water. When present in an aqueous or polar environment, lipids associate together. A practical example of lipids in a water environment may be seen as droplets of oil in a puddle of water. It turns out, however, that many lipids are also partially polar or *hydrophilic* (compatible with water) at one end or region of their structure. The dual property of hydrophobicity, the dominant characteristic, and hydrophilicity, the minor characteristic, is called an *amphipathic* property. These characteristics make lipids very useful in cell membranes where both solubility properties are needed and where many lipids are found. There are several classifications of lipids and, in fact, some lipids are included in more than one lipid class. The classifications are based on the chemistry and chemical properties of the lipids themselves. Although not exhaustive, the most important lipid classes are included here as *fatty acids, triacylglycerols, phospholipids, isoprenoids, esters, eicosanoids*, and *glycolipids*. Examples of each class are discussed and illustrated below.

Lipid Classifications

FATTY ACIDS

Fatty acids consist of varying chain lengths (approximately 3 to 30 carbons) of hydrocarbons (the hydrophobic portion) with a carboxylic acid group at one end of the chain (the hydrophilic portion). The characteristics of each fatty acid are determined by its chain length (longer chains are more hydrophobic and have higher melting points) and the degree of *unsaturation,* that is, the number of double bonds. The

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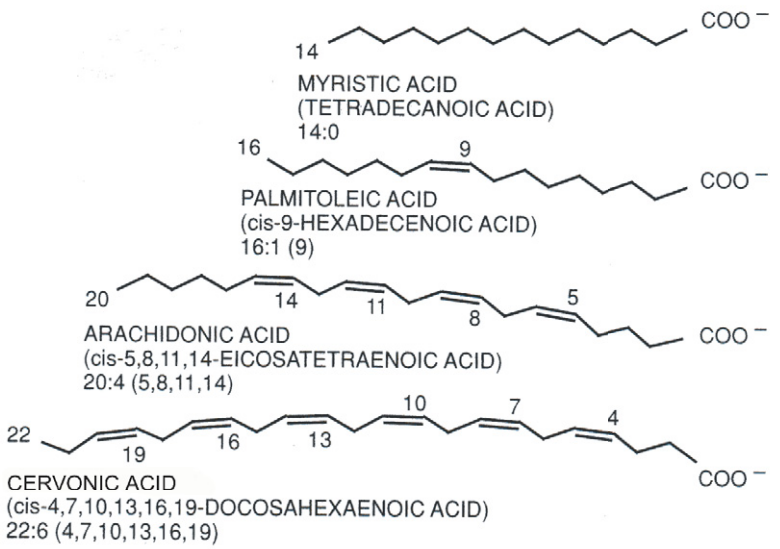


Figure 5–1
Four examples of fatty acids. ➤ For each acid the trivial name is given first, followed by the IUPAC name. Beneath that is the abbreviation for the IUPAC name. In the abbreviation, the first number gives the number of carbons. The number after the colon gives the number of double bonds. The numbers in parentheses designate the carbon number, closest to the carboxylate (COOH) group that shares the double bond. An alternate system (see Figure 5–18) designates the carbon number, starting from the noncarboxylate group (omega number) for the double bond position.

more double bonds present in a fatty acid, the lower its melting point and the greater the degree of fluidity it imparts to a cell membrane.

Figure 5–1 shows four examples of fatty acids found in animals and humans. For each example the first name given is the trivial or nonsystematic name. There is, in fact, nothing trivial about these names. They have been in use for many generations and have their origins in either the material in which the fatty acids were originally found or some characteristic associated with them. For example, myristic acid was originally isolated from nutmeg trees and takes its name from the Greek word "muristikos," a "sweet odor" associated with these trees. The second name is a systematic or structural name as determined by the International Union of Pure and Applied Chemistry (IUPAC system). The third "name" or abbreviation is a shorthand version of the systematic name (see the description to Figure 5–1). The figure shows an example of (1) a saturated fatty acid (myristic acid); (2) a fatty acid with one double bond (palmitoleic acid); (3) a fatty acid with four double bonds (arachidonic acid); and (4) a fattly acid with six double bonds (*cervonic acid*). The last fatty acid is one that is found in significant quantities in membranes of retinal photoreceptors. Examples of important fatty acids are included in Table 5–1.

As mentioned, the carbon length and degree of unsaturation are important constituents in establishing the melting point and fluidity of biological membranes. Figure 5–2 illustrates this point. Although increasing the carbon length in fatty acids can thicken a membrane and raise its melting point, the inclusion of several double bonds lowers the melting point sufficiently to keep the membrane fluid or flexible. This is accomplished by the existence of kinks or twists in the molecules at each double bond (see Figure 5–1). In this way the membrane is less compact and the fatty acids are free to slide by one another. Fatty acids are important building blocks of membrane lipids.

TRIACYLGLYCEROLS

Triacylglycerols (triglycerides) are lipids that represent a storage form of fatty acids. One molecule consists of three fatty acids covalently bonded

TABLE 5–1 ➤ PARTIAL LIST OF FATTY ACIDS IN OCULAR AND NONOCULAR TISSUES

Trivial Name	IUPAC Name	Abbreviation
Myristic acid	Tetradecanoic acid	14:0
Palmitic acid	Hexadecanoic acid	16:0
Stearic acid	Octadecanoic acid	18:0
Lignoceric acid	Tetracosanoic acid	24:0
Palmitoleic acid	*cis*-9-Hexadecenoic acid	16:1(9)
Oleic acid	*cis*-9-Octadecenoic acid	18:1(9)[1]
No name	*cis*-10-Hexadecenoic acid	16:1(10)[1]
No name	*cis*-12-Octadecenoic acid	18:1(12)[1]
Linoleic acid	*cis*-9,12-Octadecadienoic acid	18:2(9,12)[1]
Linolenic acid	*cis*-9,12,15-Octadecatrienoic acid	18:3(9,12,15)
Arachidonic acid	*cis*-5,8,11,14-Eicosatetraenoic acid	20:4(5,8,11,14)
Cervonic acid	*cis*-4,7,10,13,16,19-Docosahexaenoic acid	22:6(4,7,10,13,16,19)[2]

[1]Significant amounts occur as esters in precorneal tear film lipids (Nicolaides et al, 1981).
[2]A significant fatty acid in photoreceptor membrane phospholipids (Anderson, 1983).

to a glycerol molecule using ester bonds (Figure 5–3). The fatty acids on each glycerol molecule are often mixed types of both chain length and saturation in order that the lipid may exist in liquid form. Most triacylglycerols are kept in fat cells and represent a large depot of stored energy, as well as a source of heat insulation, for humans and animals.

Triacylglycerols are an important source of stored energy. In the last chapter, it was stated that lipids may be broken down to form acetyl CoA in order to obtain ATP. Triacylglyerols are the main source of this acetyl CoA from lipids and the process can normally occur without excessive ketone body production (in opposition to what may occur in diabetes).

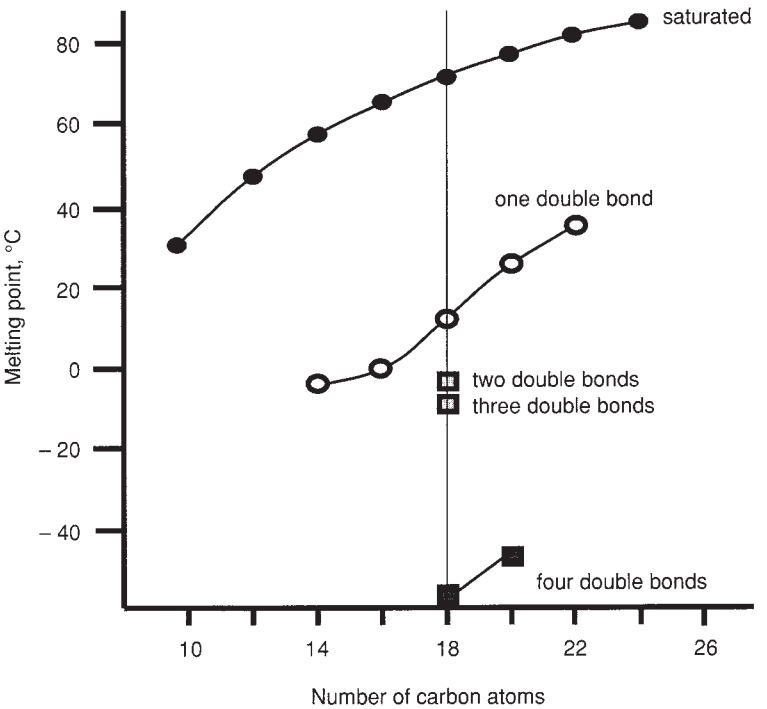


Figure 5–2
The melting point of a fatty acid is an index of how fluid a membrane will be. ➤ Lower melting points generate higher fluidity. An increase in carbon numbers raises the melting point while an increase in unsaturation, or number of double bonds, lowers the melting point. This is most easily seen in the C-18 fatty acids designated by a vertical line.

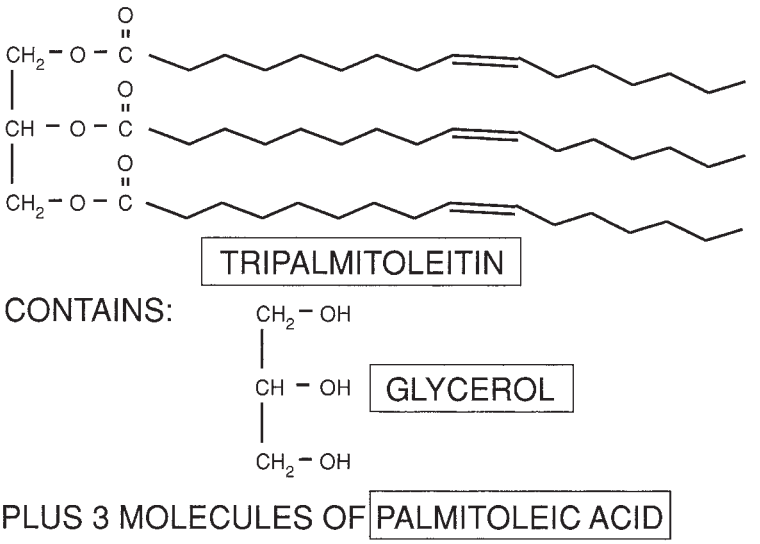


Figure 5–3
Triacylglycerols are composed of glycerol and three fatty acids esterified together. ➤ Tripalmitoleitin is the example here. The fatty acids esterified to glycerol *in vivo*, however, are usually mixed types to maintain fluidity.

In this way, an individual may be sustained when food is not consumed for longer than normal periods of time. An individual who weighs approximately 155 lbs (e.g., the 70 kg individual often quoted in nutrition tables of the National Research Council, 1989) can store 100,000 kcalories of energy in triacylglycerols, but only 600 kcalories in total glycogen (Stryer, 1988). The eye does not maintain any substantial reserves of lipids in the form of triacylglycerols for energy production, but does maintain a limited amount to maintain cellular membranes.

PHOSPHOLIPIDS

This class represents the most important lipid class for the formation and maintenance of all forms of cellular membranes. The structure of phospholipids is similar to that of triacylglycerols (see Figure 5–3 and compare it with Figure 5–4). Both forms use glycerol as the "frame" on which esters are attached. In phospholipids, however, a phosphate ester is used to bond the glycerol to one of four kinds of polar groups: *ethanolamine*; *choline* (a trimethylamine); s*erine* (the amino acid); and *inositol* (a polyhydroxy ring structure derived from glucose). These groups bond to C-3 of the glycerol molecule *via* a phosphate bridge. Phospholipids can be made by cells in a variety of mix and match combinations. In these lipids, the fatty acid composition on each C-1 and C-2 of the glycerol differs in both chain length and degree of saturation. Given the fact that the polar head groups also vary, the phospholipids are capable of existing in a great variety of both their polar and nonpolar regions.

In its configuration, as Figure 5–4 indicates, the charged or polar head regions of the molecule protrude into the aqueous regions inside and outside of a cell while the nonpolar fatty acid regions bury themselves into the interior of a cell membrane. Mitochondrial membranes contain a variation of a phospholipid known as a *cardiolipin*. A cardiolipin has three glycerols esterified together on its polar region in which the two outer glycerols are bound to four fatty acids (nonpolar region). The structure of a membrane with phospholipids can be seen in Figure 5–5. Note that the membrane has two lipid layers (bilayer) in which the fatty acid portions face each other (interiorly) and the polar

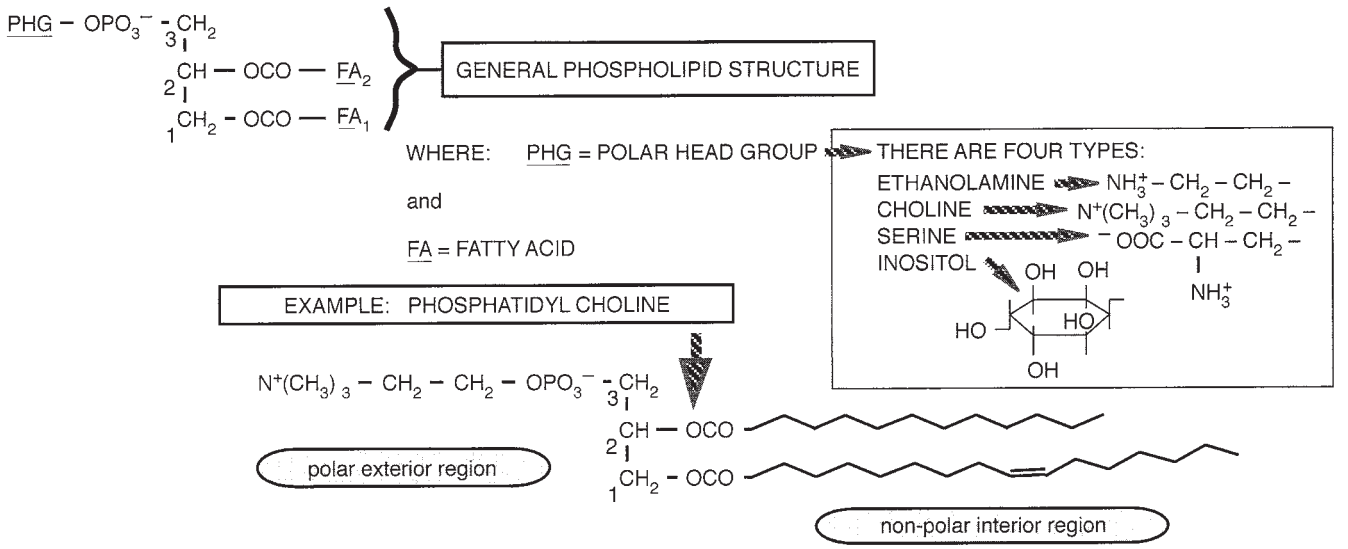
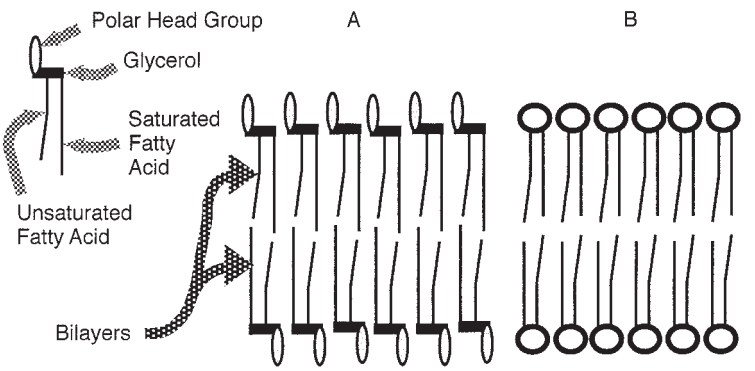


Figure 5–4
Phospholipids consist of glycerol, two fatty acids and one of four possible head groups. ➤ The fatty acids extend into the interior of a membrane while each polar head group is present at the membrane water interface.

head groups are arranged to face the aqueous portions of each side of a cell (plasma membrane) or cell chamber (subcellular organelle membrane). The representation in the figure at B has been used previously in this book (e.g., see Figure 3–18).

The fatty acid composition of phospholipids in membranes is "designed" by the cell according to the cell's functional needs. For example, one may compare (Table 5–2) the fatty acid contents of the phospholipid phosphatidylethanolamine in red blood cell (RBC) plasma membranes with those in photoreceptor rod outer segments. The membrane of the red blood cell must be somewhat rigid to assume its biconcave disc shape. Accordingly, it tends to have a shorter chain, unsaturated fatty acids, and a lower percentage of longer, highly unsaturated fatty acids. Rod outer segment discs, however, require a high degree of membrane fluidity in order to carry out the process of visual transduction, which begins with the bleaching of rhodopsin within the

Figure 5–5
Representations of phospholipids in a membrane. ➤ The B representation is commonly used in membrane diagrams.



Phospholipid components (shown on the left) may be represented in a membrane as at A (above) or, more simply, as at B (above).

TABLE 5–2 ➤ COMPARATIVE PERCENTAGE OF FATTY ACIDS IN PHOSPHATIDYLETHANOLAMINE

Fatty Acid	Red Blood Cell[1] (%)	Rod Outer Segments[2] (%)
16:0	18	10
16:1	1	—
18:0	12	36
18:1	20	6
18:2	7	—
20:3	1	—
20:4	22	4
22:4	8	—
22:5	5	—
22:6	6	34
Unknown	—	10

[1]Red blood cell plasma membranes. (Data from McGilvery and Goldstein, 1983.)
[2]Rod outer segment disc membranes. (Data from Anderson, 1983.)

membrane. Therefore, the percentage of 26:6 (cervonic acid) is almost six times greater than that of RBC plasma membranes.

ISOPRENOIDS

This lipid group represents a family of lipids metabolically built up from five-carbon units known as isoprene (Figure 5–6, insert). Members of this class include: (1) cholesterol and its allied steroids such as the hormone cortisol; (2) lipid soluble vitamins like vitamin A; (3) coenzyme Q as discussed in the previous chapter; and (4) a variety of so-called essential oils that are found in the plant world, exemplified by eucalyptus oil. Here we will focus on cholesterol and, later in the chapter, discuss vitamin A.

Cholesterol is a molecule composed of four fused rings, two methyl groups, a hydrocarbon branch, and a single hydroxy group. As shown in Figure 5–6, there are 27 carbons present. When the molecule is laid on its side, it is relatively flat and rigid. It is a highly apolar lipid, save for the single hydroxy group, and readily fits into membrane structures where it imparts rigidity to the membrane. Cholesterol is an important lipid for a variety of other reasons besides its participation in membrane rigidity. It is a source of cholesteryl esters, which are important components of the precorneal tear film. It is a synthetic precursor of a variety of steroid hormones (see Chapter 7) that affect both ocular function and dysfunction. Medically, there is much interest in dietary cholesterol and the deposition of cholesterol (and its esters) in blood vessels causing *atherosclerosis* and heart disease. Unfortunately, in spite of much research effort, this area remains controversial due to the complexities of cholesterol intake (diet), synthesis, transport, and metabolic relationships to the fatty acids (Matthews, van Holde, 1990; Voet, Voet, 1995). For even more updated information of how atherosclerotic lesions develop, one should consult Zingg, Ricciarelli, and Azzi, (2000).

The participation of cholesterol in membrane structures is more restricted in certain ocular membranes where fluidity is important. In fact, in the rod outer segment disc where fluidity is vital to visual transduction, cholesterol makes up only 8% of the lipids of the disc membranes. At present, cholesterol does not seem to have any specific role in the retina (Gordon, Bazan, 1997). The detection of cholesterol has been

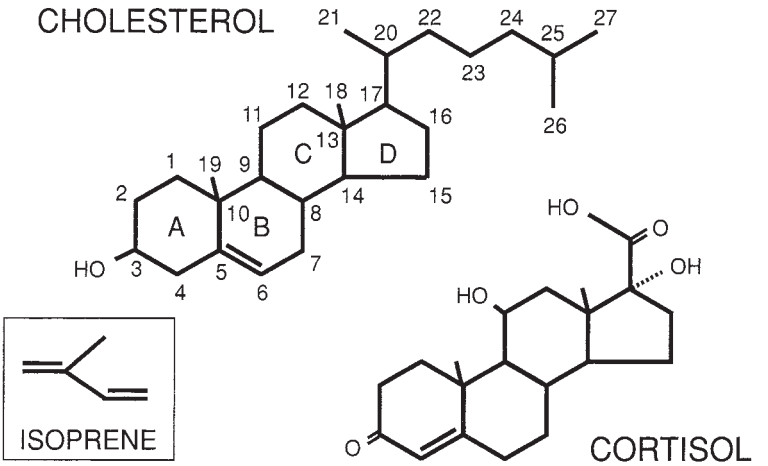


Figure 5–6
Two typical isoprenoids. ➤ The inset is an isoprene unit. The unit consists of five carbons with double bonds shared between four of the carbons. Isoprene units are building blocks of cholesterol and its derivatives. Cholesterol is made up of 27 carbons (as numbered) of which the first seventeen form 4 rings (lettered). Cortisol and other steroids are hormones naturally synthesized from cholesterol.

used to confirm ocular disease origin. In the disease known as *chalazia*, a granulatmatous inflammation of the eyelid margin occurs. The inflammation has been associated with meibomian gland lipids. However, the lipids found in chalazia are rich in cholesterol, a membrane lipid, rather than cholesteryl esters, which are meibomian gland lipids (Nicolaides et al, 1988). Accordingly, the meibomian gland does not cause the disease since the source of this lipid is the membranes of the many neutrophils, lymphocytes, and other white blood cells that bring about the inflammation.

ESTERS

Esters are the bonds formed between a carboxylic acid (which is what a fatty acid is) and either an alcohol (derived from a fatty acid) or a hydroxy group attached to a ring compound (both aromatic and non-aromatic types). Examples of lipid esters have already been seen with bonds formed between glycerol and fatty acids in the formation of triacylglycerols (see Figure 5–3) and phospholipids (see Figure 5–4).

In addition, the hydroxy group of cholesterol will form cholesteryl esters with fatty acids (Figure 5–7). The second example in the figure contains an odd numbered fatty acid peculiar to precorneal tear film cholesteryl esters. A third type of lipid ester is represented by *waxes* (esters of long-chain fatty acids [14 to 36 carbons] and long-chain alcohols [16 to 20 carbons] that are derived from fatty acids). Waxes are usually solid at room temperature and occur in nature as the shiny covering of plant leaves, beeswax, and the oily substances that cover skin, hair, wool, and animal fur. In the eye, waxes are a major component of the lipid layer of the precorneal tear film and exist as liquids at the temperature of the tear film at around 35°C (Figure 5–8).

EICOSANOIDS

Eicosanoids are cyclic lipids derived from eicosanoic acids (20 carbons) such as arachidonic acid (20:4). They include prostaglandins, thromboxanes, and leukotrienes. An example may be found in Figure 5–9. The eicosanoids are short-acting, local hormones and will be considered in Chapter 7.

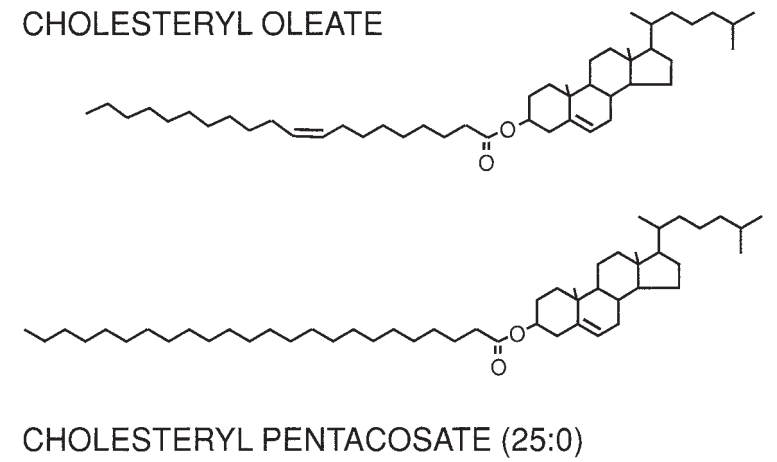


Figure 5–7
Two cholesterol esters formed from cholesterol and a fatty acid. ➤ Cholesteryl pentacosate is a tear film lipid.

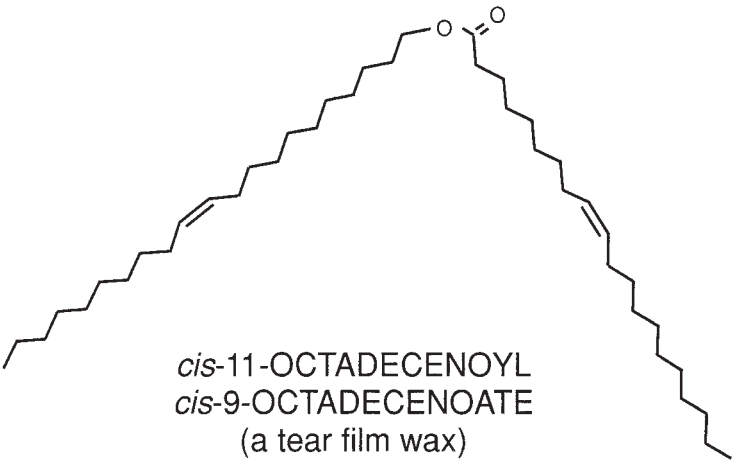


Figure 5–8
Another tear film lipid, a wax. ➤ It is made up of a fatty acid and a fatty acid alcohol. The first substance (*cis*-11 octadecenoyl) is the alcohol.

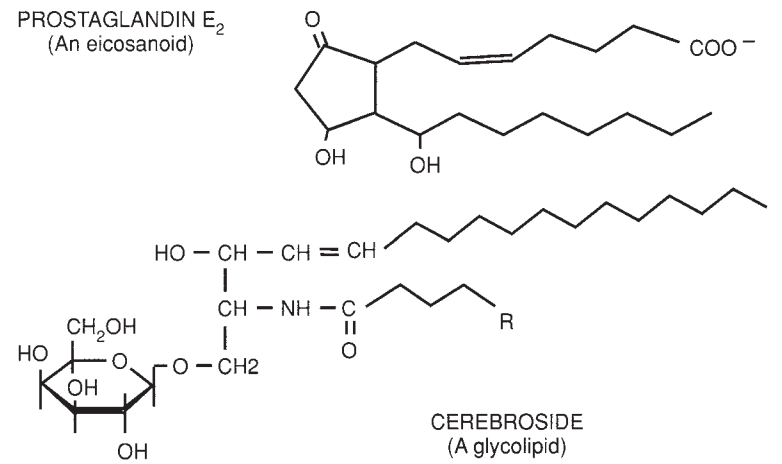
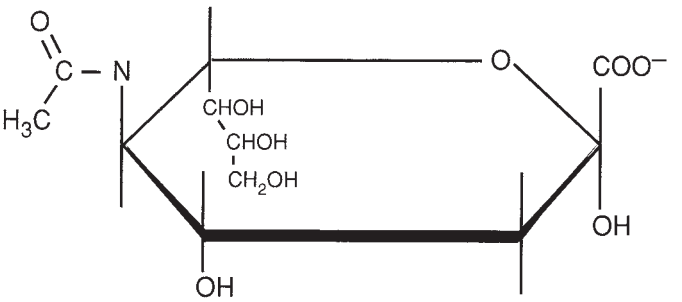


Figure 5–9
An example of an eicosanoid (prostaglandin E_2) and a glycolipid (cerebroside). ➤ See text.

GLYCOLIPIDS

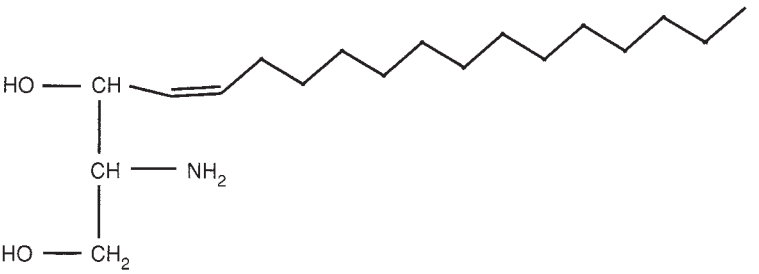
Glycolipids (see Figure 5–9) are important membrane components found in nervous, ocular, and other tissues. Glycolipids, as their name implies, are lipids that contain carbohydrates such as galactose, *N*-acetylgalactose, and *N*-acetylneuraminic acid (*sialic acid*, Figure 5–10). The basic structure that hinges (binds) glycolipids together is not glycerol, but a long-

Figure 5–10
Sialic acid or *N*-acetylneuraminic acid. ➤ This is a complex, modified carbohydrate that is found on glycolipids and glycoproteins. One role of sialic acid is to prevent the oligosaccharide chain from binding to surface receptors of other cells.



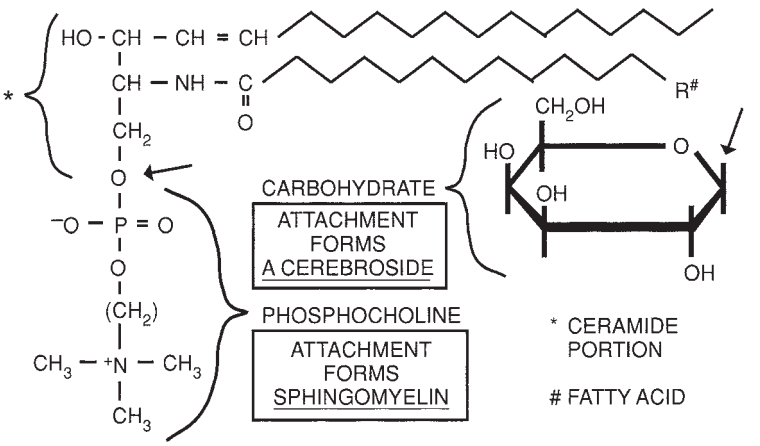
SIALIC ACID

Figure 5–11
Sphingosine is used in place of glycerol to form glycolipids and sphingomyelin. ➤ This molecule has a long chain hydrocarbon (derived from a fatty acid) and an amino group in place of one hydroxy group.



SPHINGOSINE
(The glycolipid "hinge" molecule)

Figure 5–12
A combined example of a cerebroside (a glycolipid having one carbohydrate) and sphingomyelin. ➤ The *arrows* show the point of binding between the galactose and the ceremide moieties.



chain amino alcohol known as *sphingosine* (Figure 5–11). The name is mythological in origin (the Egyptian Sphinx) and refers to something that cannot be understood as was originally the case for this compound. Sphingosine resembles a glycerol ester, but is actually a single fatty acid derivative. When a second fatty acid is bound as an ester to sphingosine the compound is known as a *ceramide* (literally a "wax amide"). If phosphocholine, a phospholipid component, is esterified to a ceramide then the compound becomes *sphingomyelin* (Figure 5–12). If phosphocholine is replaced by one or more carbohydrates, then the compound becomes a glycolipid (or a glycosphingolipid) such as the *cerebroside* and the *ganglioside* shown in Figure 5–12 and Figure 5–13. Note that the

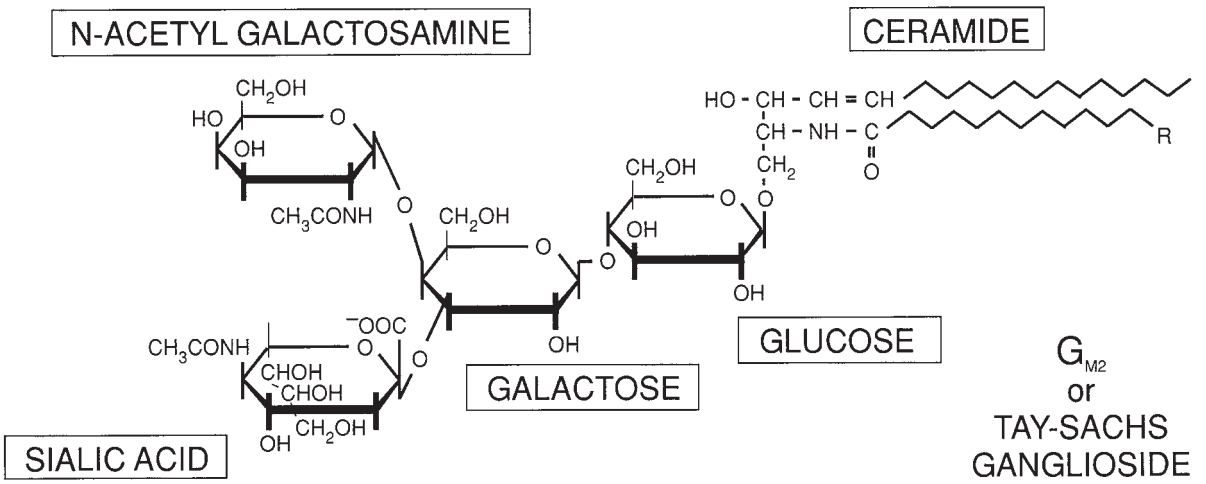


Figure 5–13
The Tay-Sachs ganglioside. ➤ This is a partially degraded ganglioside. The complete, in situ, ganglioside, known as GM_1, has a galactose attached to *N*-acetyl galactosamine which is detached by β-galactosidase in neural and ocular cell lysosomes. In Tay-Sachs disease, *N*-acetyl galactosamine (which would normally be hydrolyzed next) is not removed due to a deficiency of the enzyme hexosaminidase A.

cerebroside and the ganglioside are particular glycolipids having one or more sugars attached to the ceramide portion of the glycolipid. These names are difficult to become accustomed to, so some careful attention is recommended.

Cell Membranes

Cellular membranes are important functional barriers to both cell surfaces and interior compartments of cells (Houslay, Stanley, 1982). All such membranes, ultimately, must be considered as walls built up not only of lipids, but also of proteins and carbohydrates. The simplest membranes contain only lipids.

Membranes are formed from the natural aversion that lipids have to an aqueous environment (*hydrophobicity*). With very simple lipids such as fatty acids, the lipids tend to congregate together such that their hydrophobic bulk will associate together in weak hydrophobic bonds. A sufficient number of these lipids will form spherical *micelles* in an aqueous environment. (Figure 5–14). A more complex hydrophobic association occurs when the lipids introduced into an aqueous environment are phospholipids. In this case, a formation of bilipid layers occurs and such formations are the basis of all cell membranes. Our present knowledge of membrane structure and function is based on the hypothesis of Singer and Nicholson (1972) who developed the original bilipid layer concept of Danielli and Davson (1935) into a working hypothesis of a three-dimensional structure that integrated proteins into the structure.

LIPID COMPONENTS

The lipids found in all cell membranes are phospholipids, cholesterol, and glycolipids. A basic bilipid structure is shown in Figure 5–15, which was included under the discussion of phospholipids. In this structure, the polar groups extend on either side toward an aqueous environment

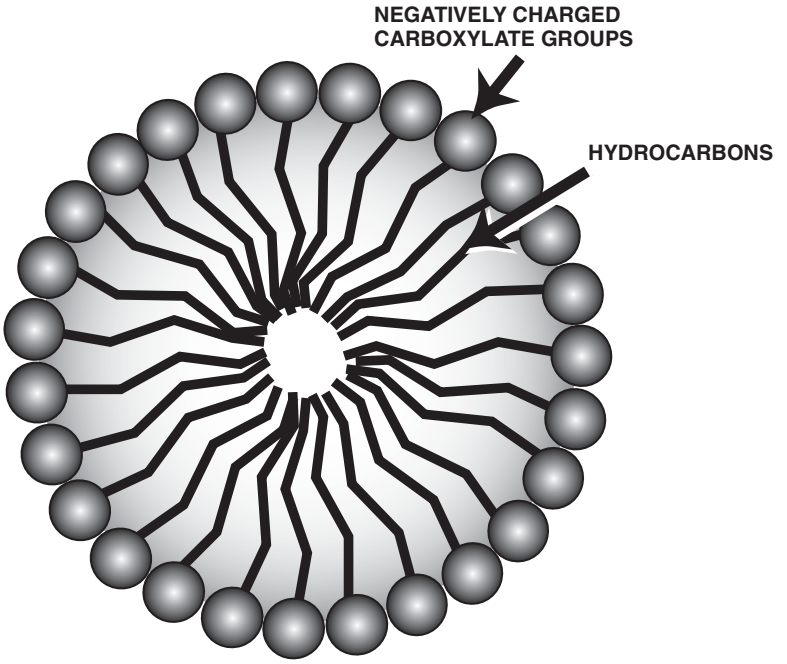


Figure 5–14
An example of a micelle. ➤ This structure forms in the presence of more angled lipids such as fatty acids. That is, there is no bilipid layer.

either inside or outside of the cell. The nonpolar regions, from both sides of the membrane, associate together hydrophobically and consist of variable lengths of fatty acids, which are both saturated and unsaturated. Variation also extends to the different kinds of polar head groups that are present. For example, on plasma membranes there tends to be more phosphatidylcholine molecules on the outer face of the bilipid layer while there is a predominance of phosphatidylethanolamine molecules on the inner face of the bilipid. Essentially, one finds that phospholipids with net positive charges tend to occur *exteriorly*, but the reason for this is uncertain (Lodish et al, 2000). Previously, it was stated that these membranes tend to be more liquid with higher amounts and numbers of unsaturated bonds. Actually, the term *liquid crystal* should be used in as much as these membranes are not so much a liquid as they are more of a structure that tends to be flexible. This means that the fatty acids slide by one another more easily when double bonds are present.

Cholesterol, when incorporated into the membrane structure, counteracts the tendency of a membrane to be flexible at the narrow temperature range of biological membranes just above and below 37°C. Below this narrow temperature range, cholesterol actually promotes some fluidity. The physical chemistry of this behavior, for the curious, is discussed by Yeagle (1991).

Glycolipids serve two functions. They are part of the overall bilipid layer structure, just as phospholipids, and they contribute short-chain carbohydrates into the outer aqueous volume just outside of cells. As such, these complex lipids with their short sugar "arms" form part of the cellular *glycocalyx* (literally "sugar coat"). Glycocalyces are discussed in the section on carbohydrate components.

PROTEIN COMPONENTS

As stated, the structures of cell membranes only began to be understood in the 1970s after much difficult work revealed the conformations of

membrane proteins and their roles in various cell membranes. Lipids form the "walls" of cells, but proteins act as their "doors." To carry the analogy a little further, the doors may swing in one or both directions and a doorkeeper may be present to make sure what goes through those doors, whether what enters changes form (*transduction*), and how fast the traffic flows.

The kinds of proteins found at membranes are divided into two main types: *intrinsic (or integral)* and *extrinsic (or peripheral)*. Intrinsic membrane proteins cross or extend into the bilipid layer of a membrane whereas extrinsic membrane proteins are only associated with either side of the membrane. One can isolate these proteins from membranes on the basis of solubility. Generally, intrinsic proteins require a strong detergent such as methyl octylglycoside for separation while extrinsic proteins may be separated out using aqueous salts. Intrinsic membrane proteins tend to have operational roles: transport (e.g., of glucose), reception (e.g., of insulin), transduction (e.g., of light); and attachment (e.g., to basement membranes). An ocular example of an intrinsic protein is rhodopsin, which transduces (i.e., converts) the presence of light into a chemical signal. Rhodopsin, as may be seen in Figure 2–16, crosses the disc membrane with seven polypeptides. Extrinsic proteins may have more passive roles such as structural (e.g., cytoskeleton maintenance) and anchoring (e.g., for glycocalyx components), but are also involved in transduction, signalling, and cell local movement (e.g., myosin and actin components). An ocular example of an extrinsic protein is *transducin,* which is sequentially included in the light transduction process with rhodopsin. This protein may be seen in Chapter 6 in Figure 6–9. There are hundreds of known membrane proteins, both ocular and nonocular and these will be introduced in later chapters while a few others have already been shown in earlier chapters.

CARBOHYDRATE COMPONENTS

The carbohydrate components of cell membranes were largely ignored by reseach investigators until recently. These components make up the smallest percentage of membrane constituents. They also form the outer sugar covering of a cell that is known as the glycocalyx. On a plasma membrane one will find two kinds of carbohydrates, glycolipids and glycoproteins, both of which have been discussed previously (see previous section and Chapter 2). The roles of carbohydrates on cell plasma membranes is multifunctional. For example, the membranes serve as immunological identifiers for the cell in the animal or human where they occur since they extend out of the membrane as dense carbohydrate branches and interact with the surrounding aqueous environment and its contents. Without their immunological role of "friend or foe" identification, cells would be discarded as foreign substances and the organism would soon cease to exist.

The carbohydrate components also serve as biological bridges and partial bonding agents to any surrounding matrix material. This is how epithelial cells in the cornea may associate with the nearby Bowman's membrane, which is not a true membrane but an extracellular matrix. In Figure 2–38, although not shown, one may insert the sugar portions of glycolipids and glycoproteins between the basal (*bottom*) portions of the epithelial cells and Bowman's membrane. Carbohydrates are principally found on the extracellular surface of plasma membranes. However,

glycolipids and glycoproteins can also occur on the membranes of subcellular organelles.

MEMBRANE COMPONENT SUMMARY

The ratio of proteins to lipids is an important indication of how "busy" the membrane is regarding cell functions that involve transport, metabolism, and signal transduction. Generally, plasma membranes have protein to lipid ratios of approximately 1:1. Mitochondrial inner membranes, which are heavily involved with electron transport and ATP production, have protein to lipid ratios greater than 3:1. On the other hand, myelin membrane, which acts as a biological insulator for nervous transmission, has a small protein to lipid ratio of approximately 1:5. A list of percentage compositions of some membranes is shown in Table 5–3. Figure 5–15 summarizes the assembly of the types of components that are found in cell membranes, but it only gives a suggestion of the membrane complexity that is present.

TRANSPORT: AN ESSENTIAL MEMBRANE FUNCTION

No cell can survive unless it can move substances into and out of its confines. This occurs by a series of processes collectively known as *transport*. Three general types of transport are recognized: *simple diffusion*, *passive facilitated transport,* and *active facilitated transport*. In simple diffusion, very small molecules, which are usually gases such as oxygen and nitrogen, readily cross membranes going from an area of higher to lower concentration. A variety of other manufactured substances, which we know as pharmacological agents or drugs, also enter cells by this process. All of these substances can do this inasmuch as they are, at least partially, lipid soluble, and bilipid membranes do not constitute barriers to them. At one time, it was thought that water entered cells by diffusion. However, it is now known that water is transported by facilitated transport using proteins known as *aquaporins*.

In passive facilitated transport, a protein acts as a gate or channel to assist a substance (such as glucose) in crossing the membrane, going from an area of higher concentration (outside the cell) to lower concentration (inside the cell). An example of this kind of transport occurs with

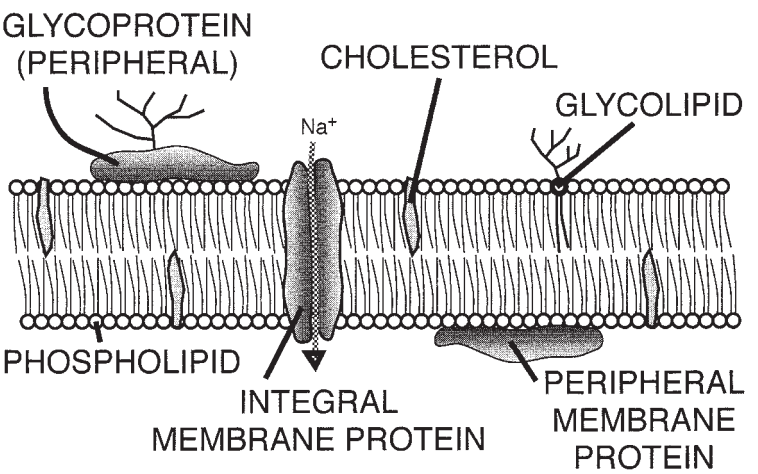


Figure 5–15
The arrangement of the common lipid, protein and carbohydrate components of cell plasma membranes (bilipid layers). ➤ Note that the carbohydrate components (indicated by branching lines) are always bound to either a lipid or a protein and usually are found on the exterior facing membrane.

TABLE 5–3 ➤ PERCENTAGE COMPOSITION (BY WEIGHT) OF MEMBRANE COMPONENTS

Membrane Source	Lipid	Protein	Carbohydrate
Red blood cell	43	49	8
Myelin sheath	79	18	3
Mitochondrion (outer membrane)	47	51	2
Mitochondrion (inner membrane)	23	76	1
Bovine retinal rods	47	49	4

(Adapted from Guidotti G. Membrane proteins. *Ann Rev Biochem* 1972;41:732.)

the movement of glucose inside the cell using the protein GLUT-1 (mentioned in Chapter 4). Graphically, one can distinguish facilitated transport from diffusion by showing that facilitated transport is asymptotically limited by the ability of the protein or enzyme to perform the transport, just as occurs in enzyme kinetics (Figure 5–16). Any form of protein-assisted transport is rate limited (that is, only so many molecules can be transported at a maximal rate). As with enzyme kinetics, such transport is subject to competition or inhibition.

The kinetics of active facilitated transport resemble those of passive facilitated transport. However, there is one important difference. Active transport moves a substance from an area of lower concentration outside the cell to an area of higher concentration inside the cell and, therefore, enzyme catalysis is required for the energy to operate this "pump." Most often, active facilitated transport is linked to the enzyme Na,K-ATPase, which was discussed in Chapter 3. It may be recalled that this enzyme transports three ions of Na^+ outside a cell while transporting two ions of K^+ inwards with the hydrolysis of one molecule of ATP. In other examples, such action may be indirect and actually require two proteins, one of which is often Na,K-ATPase, which supplies Na^+ ions for the process.

Some fine points involving transport are also worth pointing out. When only a single substance is transported, the process is called *uniport* transport as with GLUT-1 and sugar uptake into cells (Chapter 4). When

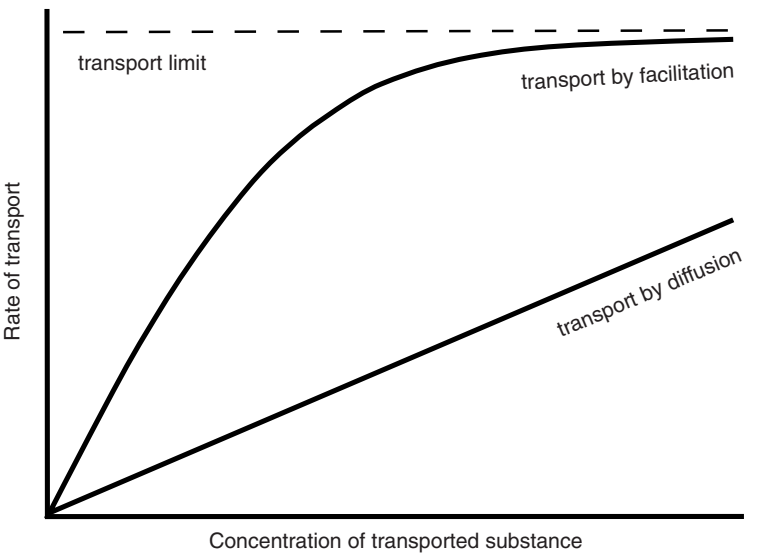


Figure 5–16
Transport kinetics across cell membranes. ➤ When proteins are involved in the transport process (facilitated transport), the rate of transport is always limited by the kinetic ability of the protein/ enzyme involved. In the process of transport by diffusion, no such limitations occur. Compare this figure with Figure 3–4 in Chapter 3.

two substances are being transported simultaneously, the term *cotransport* is used. However, the substances transported may be in the same direction (*symport*) or opposite directions (*antiport*). An example of symport occurs when there is a need to concentrate glucose against a concentration gradient as occurs in kidney tubules. In that case the protein, known as the *two Na^+/one-glucose symporter*, moves Na^+ ions down a concentration gradient while transporting glucose up a concentration gradient with both substances moving in the same direction (Panayotova-Heiermann et al, 1997). Na,K-ATPase serves as an example of antiport transport.

Precorneal Tear Film Lipids

When tears are spread across the cornea after eyelid blinking, the thinned-out liquid that results is called the *precorneal tear film*. This film actually consists of three parts: the most anterior or superficial *lipid layer*, the central *aqueous layer*, and the posterior *mucous layer* (Figure 5–17). Until recently, many investigators (e.g., Holly and Lemp, 1977) considered each layer to have measurable thicknesses of 0.1 μm (lipid layer), 7 μm (aqueous layer) and 0.002 to 0.005 μm (mucous layer) for a total thickness of just over 7 μm. Historically, estimates of total thickness have ranged from 4 μm to 40 μm and one of the latest estimates places it at only 3 μm (King-Smith et al, 2000). This matter is further complicated by recent reports that the mucin layer may be more of a network of epithelial glycocalyx that extends far into the aqueous layer (Chen et al, 1997, Pflugfelder et al, 2000). Accordingly, the current understanding about the distinction of thicknesses and layer boundaries of the tear film is somewhat vague.

The mucous layer or matrix is composed largely of mucoid proteins as discussed in Chapter 2. The aqueous layer contains a variety of dissolved salts and proteins. The lipid layer consists predominately of a large variety of waxes and cholesteryl esters. Tears (and the tear film) represent a protective formulation for the outer surface of the eye. The tears wash away debris from the corneal surface and represent a perfusion fluid for that purpose. The tear film is an optically uniform surface and the aqueous layer contains lysozyme (see Chapter 3) and other proteins that possess antibacterial functions (Milder, 1987). The lipid layer of the film serves the basic purpose of stabilizing the film especially the

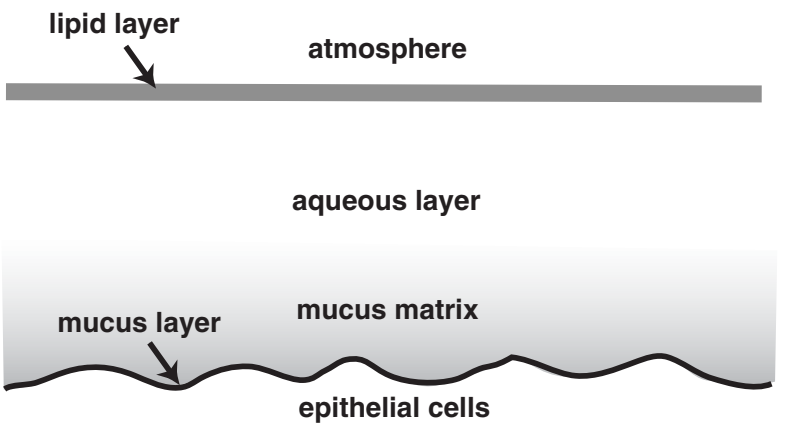


Figure 5–17
Layers of the precorneal tear film. ➤ The thickness of the layers is not known with certainty (see text). Moreover, an extension of the mucus layer, the mucus matrix (grey area), is projected out into the aqueous layer.

TABLE 5–4 ➤ COMPOSITION OF HUMAN MEIBOMIAN GLAND LIPIDS

Lipid Component	Composition (%)
Cholesteryl esters	29.5
Wax esters	35
Triacylglycerols	4
Cholesterol	1.8
Fatty acids	2.2
Unidentified[1]	27.5

[1]These unidentified components can be further subdivided on the basis of their polar/nonpolar properties. Some have been recently identified (see text).
(Nicolaides N: In Holly FJ ed: *The precorneal tear film in health, disease, and contact lens wear.* Lubbock, TX, 1986, Dry Eye Institute, 573.)

time during which it remains intact (15 to 40 sec) before it ruptures and stimulates the next blink (Davson, 1990). That period of time is often referred to as the *tear-film breakup time*.

The composition of lipids in the tears is rather complex. A wide variety of fatty acids, their alcohol derivatives, and cholesterol make up the waxes and cholesteryl esters that are the majority of lipids found there. Other lipid classes are also present as described by Nicolaides (1986) and as shown in Table 5–4. Slightly more than 25% of the lipid types have still not been completely characterized. They include 8.4% double esters or diesters (Figure 5–18) in which hydroxy fatty acids are esterified to two other fatty acid(s) or an alcohol or even a cholesterol molecule. Four percent of the lipids represent uncombined, precursor fatty acids and cholesterol molecules. The varieties of fatty acids are listed in Table 5–5. Nicolaides has estimated that with some 69 different fatty acids, 40 fatty acid alcohols and 11 hydroxy fatty acids in Meibomian gland secretions, about 30,000 ester species are possible! The cooperative physical-chemical properties of these esters provide for the ability of the lipids: (1) to flow from their ducts to the eyelid edges; (2) to form a film over the aqueous layer and maintain contact with it; (3) to adhere to the eyelid skin and act as a barrier to the aqueous layer; and (4) to form a water-tight seal when the lids are closed.

Pathological conditions can alter this exquisite ester mixture and bring about tear film abnormalities. In Meibomian gland dysfunction,

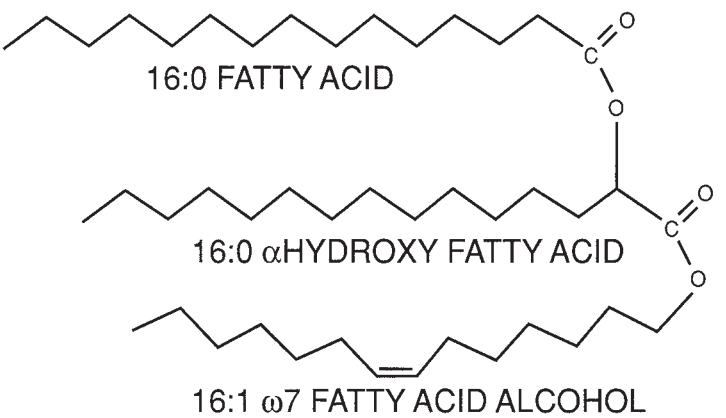


Figure 5–18
A diester component of the lipid layer of the precorneal tear film. ➤ Here the diester is composed of a fatty acid, a hydroxy fatty acid and a long chain alcohol (fatty acid alcohol).

TABLE 5–5 ➤ FATTY ACIDS IN THE PRECOMEAL TEAR FILM

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Please refer to the printed publication.

(Nicolaides N. Recent findings on the chemical composition of the lipids of steer and human meibomian glands. In Holly FJ ed: *The precorneal tear film in health, disease, and contact lens wear.* Lubbock, TX, 1986, Dry Eye Institute, 570–596.)

for example, excessive production of keratin occurs in the ductal epithelium. Keratin is a protein characteristic of skin and hard coverings such as animal horns. Nicolaides et al (1989) have suggested that this process alters the composition of tear film lipids to include the lipid composition of the keratinized epithelial cells themselves. This is reflected in decreased steryl esters, increased cholesterol, and the appearance of ceramides. A further complication of this process is that the epithelial cells may detach from the gland and block the flow of new lipids to the tear film. This results in a more rapid break-up of the tear film. Still another alteration, which may accompany these events, is bacterial infection in the area of the blocked lipids. McCulley and Dougherty (1986) have shown that *Staphylococcus aureus* and other bacteria produce a cholesteryl esterase and a fatty wax esterase capable of hydrolyzing the meibomian lipids.

Lipids of the Retina

In the retina, photoreceptors together with other neural cells operate the complete transduction mechanism for converting light energy into electrical signalling and sending those signals to the brain. Lipids play an important supporting role in that process. The phospholipid/fatty acid composition of the retinal membrane lipids is shown in Table 5–6. Compare the phosphotidyl ethanolamine fatty acid composition in this table with that for photoreceptors in Table 5–2. The compositions, taken from two independent investigations, are virtually the same, showing that the percent of fatty acids in both retina and photoreceptor

TABLE 5–6 ➤ PHOSPHOLIPID AND FATTY ACID COMPOSITION OF MAMMALIAN RETINA

Rights were not granted to include this table in electronic media.
Please refer to the printed publication.

(Broekhuyse RM, and Daemen FJM. The eye. In Snyder F (ed). *Lipid metabolism in mammals.* New York, 1977, Plenum Press, 161.)

membranes resemble each other. That is, they have a high concentration of the highly unsaturated cervonic acid (22:6) that imparts considerable fluidity to cell membranes including photoreceptor discs. It is also worth pointing out that the percentages of phospholipids resemble that of nervous tissue (Broekhuyse, Daemen, 1977). However, the phospholipids in photoreceptors have less sphingomyelin and phosphotidyl inositol than that found in nervous tissue.

The lipid content of rod and cone outer segments is quite high because of the presence of the discs. These discs are separated only by a distance of 300 Å. The lipid composition is 15% of the wet weight of a rod outer segment. By comparison, the lipid content of most cells is only about 1%. The high concentration of cervonic acid in photoreceptors, mentioned above, correlates with the low viscosity of photoreceptor membrane discs. That is, the high fluidity of the discs contributes to the important rotational and lateral movements of rhodopsin needed for phototransduction. There seems to be a preservation mechanism for this fatty acid since the reduction of essential fatty acids from the diets of laboratory animals have no effect in reducing the amount to cervonic acid in their retinas. Polyunsaturated fatty acids such as cervonic acid (22:6) are vulnerable to destruction by oxidative processes in the retina (Broekhuyse, Daemen, 1977). However, Anderson (1983) has suggested that there are sufficient concentrations of vitamin E there to prevent such destruction. Vitamin E (α-tocopherol, Figure 5–19), itself a lipid soluble vitamin, acts by absorbing free radicals (unpaired electrons) that are found in active forms of oxygen and peroxides. If vitamin E were not present, such free radicals would attack the double bonds of membrane fatty acids and break them up into fragmentary aldehydes (Mayes, 1985).

Vitamin A

In Chapter 2, we discussed the linkage of 11-*cis* vitamin A aldehyde (retinal) to opsin to form rhodopsin. Vitamin A is a hydrophobic vitamin that belongs to the isoprenoid class of lipids. It is also called a fat soluble vitamin. The dietary sources of vitamin A are β-carotene and retinyl esters (Figure 5–20). The former occurs in yellow vegetables such as carrots and sweet potatoes (Lehninger, 1982) while the latter comes from animal sources. In the gut both sources are enzymatically converted predominately to vitamin A alcohol (retinol, see Figure 5–20). Some retinoic acid is also formed. The retinol becomes re-esterified and is incorporated into *chylomicra* for transport to the liver. Chylomicra are lipid spheres in which the more hydrophobic lipids are incorporated interiorly and the

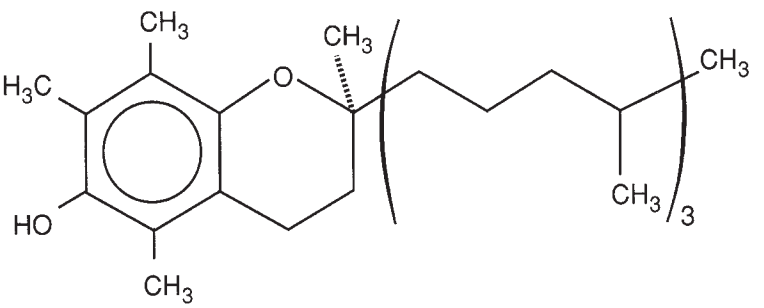


Figure 5–19
α-tocopherol (vitamin E). ➤ This is an isoprenoid lipid with vitamin properties. It is also an antioxidant that acts by absorbing highly reactive, unpaired electrons into the resonance system of the left hand ring.

more hydrophilic lipids coat the surface of the sphere where they are complexed with proteins. Chylomicra are transport complexes that are compatible with the aqueous environment of the bloodstream. Vitamin A transport is conveyed principally to the liver where re-esterification and storage occur. When needed, mobilization of the vitamin A, primarily as retinol, takes place after binding to two proteins: retinol binding protein (RBP) inside the cell and then prealbumin (PA) in the blood stream. In this complex form, retinol is transported through the blood stream to its target cells such as the pigment epithelial cells of the retina and corneal epithelial cells. Upon reaching its target cell, retinol is released and transported, via a receptor protein, into the cell cytoplasm (Chader, 1982). There, as in the liver, it may be stored as an ester after binding to a cellular RBP (CRBP) or converted to a useful form for the cell. These actions, to this point, are shown in Figure 5–21. By now it should be apparent that there are several chemical forms of vitamin A. These forms are summarized on Table 5–7 as *retinyl ester, retinol, retinal,* and *retinoic acid*. They have many roles in addition to that of visual transduction. In addition to acting as a transport form, retinol functions as a hormone to control certain kinds of protein synthesis. Retinoic acid is involved in both the formation of glycoproteins and the maturation of epithelial cells including corneal epithelia. Retinal, as the 11-*cis* form, binds to opsin to form rhodopsin as discussed in Chapter 2. Figure 5–22 shows the sequence of metabolic steps that occur between and within the pigment epithelial cells and the photoreceptors leading to the formation of 11-*cis* retinal and its reformation from all-*trans* retinol (Gordon, Bazan, 1997). Upon entering the pigment epithelial cell either from the circulation or the photoreceptor outer segment, all-*trans* retinol is esterified and isomerized to 11-*cis* retinol enzymatically. Prior to transport to the rod photoreceptor the alcohol is converted to the aldehyde

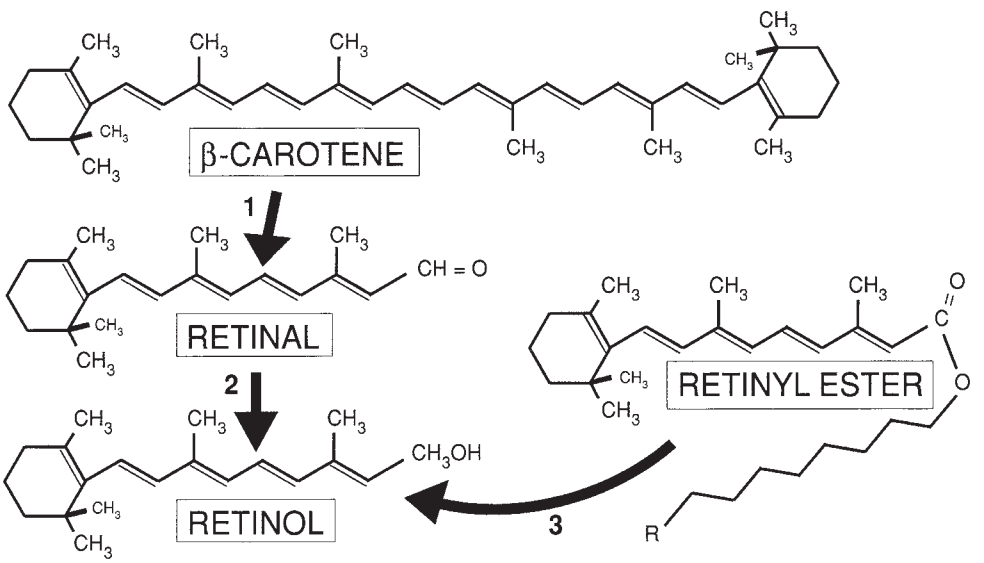


Figure 5–20
Dietary forms of vitamin A. ➤ These are β-carotene (actually a precursor of vitamin A) and retinyl ester. In the intestines, β-carotene is broken into two retinal molecules by β-carotene dioxygenase (reaction 1) with the aid of molecular oxygen and bile salts (cholesterol derivatives). The retinal is reduced to retinol with retinol reductase (reaction 2) using NADPH as a co-enzyme. Retinyl ester is hydrolyzed to retinol with an esterase (reaction 3). The retinol is absorbed into the intestinal cells and, incredibly, reesterified again with long chain saturated fatty acids for incorporation into chylomicra.

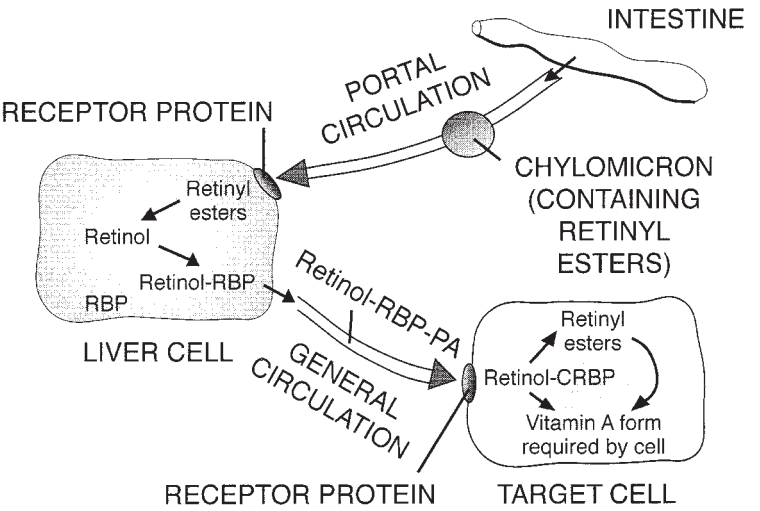


Figure 5–21
The processing and transport of vitamin A to its target cells. ➤ RBP, retinol binding protein; PA, prealbumin; CRBP, cellular retinol binding protein. CRBP is only used to bind retinol inside of cells. See text for description.

form. Some points to note are: (1) all-*trans* retinal in the photoreceoptor is converted to all-*trans* retinol by retinyl dehydrogenase before transport to retinal pigment epithelial cells; (2) RBPs must bind to the vitamin before transcellular transport; and (3) the all-*trans* retinol in *cone* photoreceptors is not transported back to retinal pigment epithelial cells before re-isomerization to the 11-*cis* form.

Most of the scientific knowledge of the roles of vitamin A have come from what occurs to both ocular and nonocular tissues when the vitamin is deficient. In the eye, an early effect is the loss of night vision (*nyctalopia*). This is followed by a hardening of the corneal conjunctiva with the loss of conjunctival secretions (*xerophthalmia* or dry eyes) and, eventually, *keratomalacia* may develop (a degeneration of the corneal epithelium). The latter condition may ultimately result in corneal perforation. Although comparatively rare in the United States; homeless people, the elderly, and others on a poor diet may develop some of these symptoms over time (Skelton, Skelton, 1990). Vitamin A related ocular symptoms are common in nations with poor vitamin A containing diets. Nonocular symptoms generally include adverse effects to any tissues covered by epithelial cells as well as inhibition of the process of bone elongation.

TABLE 5–7 ➤ CHEMICAL FORMS OF VITAMIN A AND THEIR ROLES

Name	Form	Role(s)
Retinyl ester	Vitamin—CH_2—O—C(=O)—fatty acid	Storage
Retinol	Vitamin—CH_2—OH	Transport, hormonal[1]
Retinal	Vitamin—CH=O[2]	Visual transduction
Retinoic acid	Vitamin—C(=O)—OH	Synthesis[3]

[1]Acts at the cell nucleus to influence gene expression.
[2]Exists as the 11-*cis* form (see Figure 1–17). All other forms are the all-*trans* isomers.
[3]Acts as an agent in the synthesis of glycoproteins and in the differentiation of all types of epithelial cells.

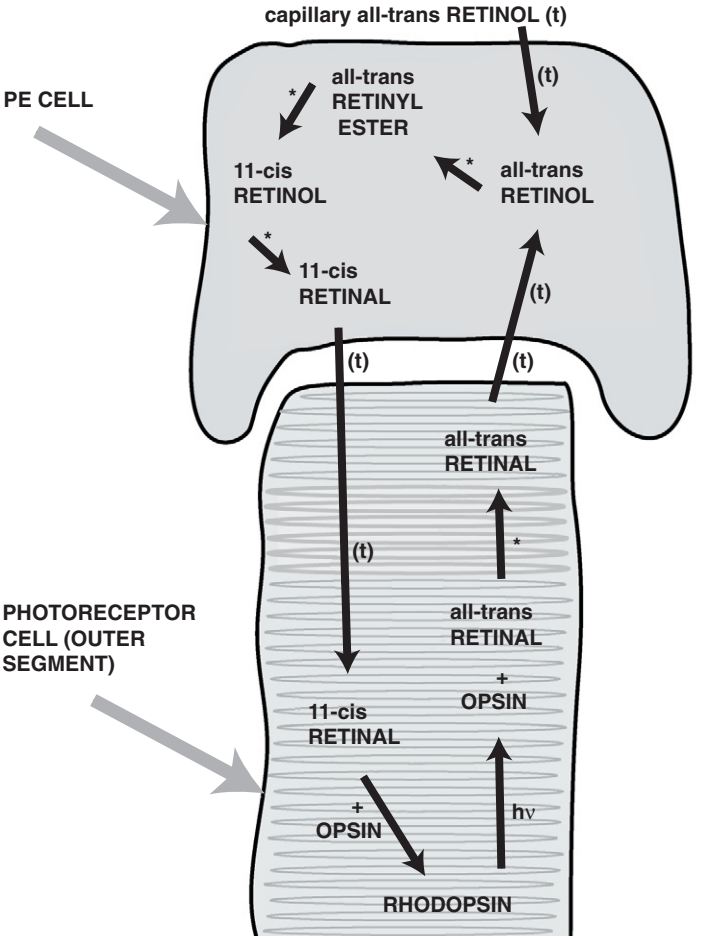


Figure 5–22
The processing and transport of vitamin A at PE (pigment epithelial) cells and photoreceptor outer segments. ➤ At PE cells retinol may be stored as an ester or transported to the outer segment. The transport between and within PE cells and photoreceptor outer segments of 11-*cis* retinal as well as between photoreceptor cells and PE cells of all-*trans* retinol makes use of other transport binding proteins (t), an interstitial retinol binding protein (IRBP) as well as an intracellular retinal binding protein (CRBP) as described by Hollyfield et al (1985). Metabolic transformations of the different vitamin A forms are enzymatically catalyzed (*) in both the PE and photoreceptor cells. See text for further explanations.

Children are, therefore, particularly subject to growth impairments in vitamin A deficiency.

Vitamin A, unfortunately, also has adverse effects when taken excessively. Americans have become so vitamin conscious that they sometimes tend to exceed the recommended amounts, figuring that "more is better." However, Hathcock et al (1990) have shown that when the daily intake of this vitamin exceeds 10,000 IU, adverse symptoms begin to appear. These include abdominal pain, blurred vision, drowsiness, headache, irritability, nausea, and vomiting. Two biochemical aspects of excessive vitamin A intake are increased gluconeogenesis and protein turnover. Other than blurred vision, however, excessive vitamin A intake does not seem to have any other ocular effects. The normal National Research Council (NRC) recommended dietary allowances (RDA) for daily intake are given in Table 5–8.

Glycolipid Storage Diseases and Vision

Gangliosides are glycolipids predominantly located in the membranes of nervous tissue and are characterized by their inclusion of the negatively charged sugar known as sialic acid. The particular ganglioside shown in Figure 5–13 is known as GM_2 or *Tay-Sachs ganglioside*. It is actually a partially degraded ganglioside that accumulates in neural, ocular, and

TABLE 5–8 ➤ NATIONAL RESEARCH COUNCIL RECOMMENDED DIETARY ALLOWANCES (RDA) FOR DAILY INTAKE OF VITAMIN A

Group	Retinol Equivalents (RE)[1]	International Units (IU)
Infants (<1 yr)	375	1200
Children (1–3 yr)	400	1300
Children (4–6 yr)	500	1700
Children (7–10 yr)	700	2300
Males (>10 yr)	1000	3300
Females (>10 yr)	800	2700
Pregnant Females (all)	800	2700
Nursing Females (infant <6 mos)	1300	4300
Nursing Females (infant >6 mos)	1200	4000

[1]Retinal Equivalent (the preferred term) = 3.33 International units. Vitamin pill containers, however, list International Units. 1 IU = 0.3 μg retinol. A revised requirement, known as a Dietary Reference Intake, is under development.

other tissues in the course of Tay-Sachs disease. The disease occurs as a result of a deficiency of the enzyme *hexosaminodase A* that normally catalyzes the breakdown (catabolism) of ganglioside molecules as new molecules are being synthesized. As a result of the accumulation of GM_2 in the retina, the ganglion cells degenerate producing a characteristic cherry-red spot in the macular region (Gravel et al, 1995). Unfortunately, this condition results in blindness at a very early age. It is also accompanied by the failure to develop motor and mental capacities. The patients usually die between 3 to 6 years of age (Brady, 1981). Tay-Sachs disease is one of a number of metabolic storage diseases that involve an enzyme defect (or deficiency) in the catabolism of GAGs or glycolipids. A previous storage disease example was given in Chapter 4 as Hurler syndrome. These diseases generally affect either the cornea (producing cloudiness) and/or the retina (producing degenerative blindness). Each disease is inherited, but is, fortunately, comparatively rare (Grayson, 1979; Townsend, 1998). More information on this subject can be found in Townsend (1998) and Sugar (1998).

SUMMARY ●

Although predominately hydrophobic, lipids have hydrophilic regions that allow them to interact with aqueous media. This amphipathic characteristic of lipids is ideal for their important role in defining cell boundaries as membranes. Lipids also function as hormones, sources of energy, and are an important part of visual transduction. There are seven major lipid classes: fatty acids, triacylglycerols, phospholipids, isoprenoids, esters, eicosanoids, and glycolipids. The lipids that make up cell membranes have a varied composition to suit the requirements of the cell. In photoreceptor discs, for example, a high percentage of cervonic acid (22:6) is used to maintain maximal fluidity of the membrane. The precorneal tear film lipids consist largely of waxes and cholesteryl esters.

This unique and complex mixture assures optimal spreading and stability of the tear film. Vitamin A is important for ocular function in two ways. It combines with opsin, forming rhodopsin, in the form of 11-*cis* retinal. This holoprotein reacts with light to initiate visual transduction. Second, it maintains the proper development of corneal epithelial and conjunctival tissues. A lack of vitamin A in the diet can lead to night blindness and keratinization of the cornea. Glycolipid metabolic enzyme deficiencies can lead to visual impairment or blindness.

PROBLEMS

1. Using the graph in Figure 5–2 as a guide, estimate the melting point temperature of hexadecenoic acid (Table 5–1).

2. Would you predict a high or a low concentration of cholesterol to be present in photoreceptor disc membranes? Why?

3. Rhodopsin can be isolated from the lipid components of disc membranes using octyl β-D-glucoside, a synthetic detergent. Based on the use of this extraction tool, what kind of membrane protein is rhodopsin and what characteristics would you expect it to show in its relationship with a disc membrane?

4. An investigator extracted a lipid from a sample of precorneal tear film. On analysis, the lipid was found to be composed of a fatty acid, a hydroxy fatty acid, and an alcohol. What is the classification of this lipid?

5. Describe the metabolic problem that produces Tay-Sachs disease and the effects of the disease on ocular tissue.

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CHAPTER 6

Hormones

The complexity of multicellular organisms, including humankind, requires the use of biochemical messengers/managers that will maintain control and cooperation among all the member cells of that organism (Lodish et al, 1999). Here then is not a single class of chemicals such as proteins and carbohydrates, but a class of mixed chemical operators that keeps things in order and makes the organism run. These operators, called *hormones*, are in control. Some biochemical control mechanisms have already been seen in the form of enzyme regulation of metabolic pathways (see Chapters 3 and 4). However, such controls are always confined within individual cells. In the case of hormones, one is dealing with the extracellular regulation of multicellular processes such as: glucose uptake into cells, sexual function, physiological drive/mood, self-preservation, blood flow and the like. In the eye, hormonal control does not seem as apparent as in other parts of the body. However, a hormonal mechanism controls visual transduction (essentially, the initial act of seeing) and another set of mechanisms controls a whole series of reactions to ocular injury.

Hormone General Functions: Chemical Micromanagers

Hormonal mechanisms may be conceptualized as occurring in four steps: stimulus action → hormone release → hormone-receptor interaction → initiation of specific cell function. Although oversimplified, this is the basic mechanism of all hormone actions. Initially, hormones were only understood in terms of brain *hypothalamic* function. Essentially, the hypothalamus along with certain organs (endocrine glands) release chemical messengers (hormones) into the blood stream in order to direct specific classes of cells to alter their functions. These hypothalamic messengers were (and are still) called hormones (from the Greek word: *horman* — ‘ορμαν — *to set into motion*). Hormones, as currently understood, now include chemical messengers from: (1) hormones of the hypothalamus and endocrine cells (endocrine hormones), (2) hormones that

function only for cells in a given tissue area (local hormones), and (3) those hormones that function entirely within a cell (intracellular hormones). Only hypothalamic and endocrine hormones travel via the bloodstream (Figure 6–1).

Hormones that are released outside of cells only affect cells that are "targeted." Targeted cells either have receptors (binding proteins) for specific hormones at their plasma membrane surface or are capable of indirectly binding the hormones to specific locations on DNA in the cell nucleus. Cells also have internal hormones called *second messengers* that are activated by those exterior hormones that bind to the cell surface. The second messengers operate within the cell at a variety of intracellular targets, for example, separate locations at cell membranes, Golgi apparatus, or nuclear DNA and bring about one or more physiological response. Those hormones that enter a cell will bind to an intracellular receptor protein and then migrate and bind again to a DNA enhancer (Chapter 7). By this action, the hormone causes an increase or decrease in protein synthesis resulting in one or more physiological responses. The general cellular response mechanisms of these two kinds of extracellular hormone types are shown in Figure 6–2. A given class of cells may also affect its close neighbor cells by secreting local hormones as previously shown in Figure 6–1. These are hormones that affect only a small sur-

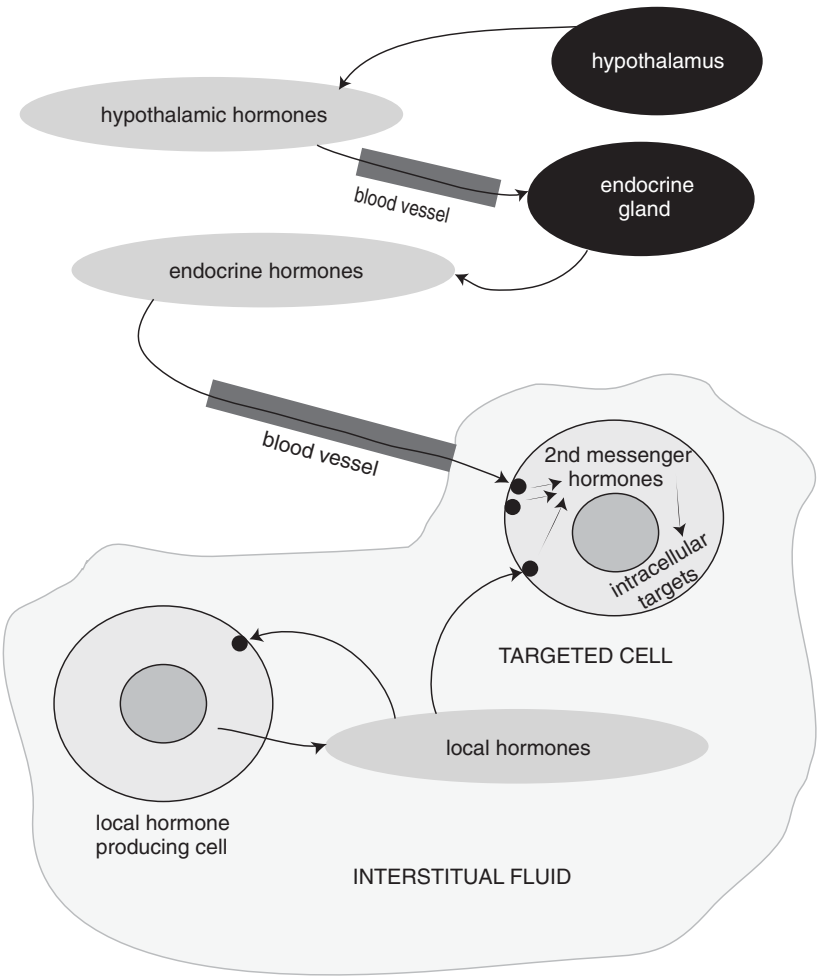


Figure 6–1
Routes taken by hormones to their targets. ➤ Hypothalamic hormones (releasing factors) travel to the first endocrine gland (the hypothalamus). In sequence, endocrine hormones travel either to a secondary endocrine gland or to a targeted cell. All of this transport occurs through blood vessels. At the targeted cell, second messenger hormones travel within the cell cytoplasm to specific intracellular targets after the relative endocrine hormone binds to a receptor protein. In some cases, an endocrine hormone binds to an internal receptor and travels to DNA. Local hormones travel *via* the interstitual fluid to a targeted cell that may be the same cell that produced the local hormone.

rounding area and are said to have a *paracrine* function. In the case in which local hormones affect the same cells that produce them, the term *autocrine* hormone is sometimes used. In general, therefore, it is possible to distinguish four hormone classes: endocrine hormones that bind to a cellular surface, endocrine hormones that enter a cell and bind to its DNA, intracellular hormones (second messengers), and paracrine hormones that travel only locally.

THE ENDOCRINE SYSTEM (NEUROENDOCRINE SYSTEM)

The so-called classical or original understanding of hormones consists of cellular control by means of a hormone "pecking order." In the endocrine (or more properly called neuroendocrine) system there exist primary, secondary, and tertiary targeted cells. As shown in Figure 6–3, the initial hormone release originates from cells of the hypothalamus (hypothalamic nuclei) to targeted cells of the *anterior* pituitary gland, the primary target tissue, using the portal vein. These hypothalamic hormones are often called *releasing factors*. The hypothalamus itself causes hormone release in response to a variety of stimulatory factors that reach the brain: cold, heat, trauma, hunger, satiety, fear and so forth. Certain cells (paraventricular and supraoptic nuclei of the hypothalamus) also make direct synaptic connection to cells of the *posterior* pituitary gland such that a nerve depolarization to these cells *is equivalent to* the primary hormone release to cells of the anterior pituitary. This is an exception to the general endocrine hormone system. Stimulation of pituitary cells, anterior and posterior, by primary hormones or nervous discharge causes the release of other hormones to secondary target tissues; for example, thyroid gland, ovary, and the adrenals. Most of the secondary tissue cells then release their own hormones to tertiary target tissues, such as, muscles, bones, and liver. At this tertiary stage, the hormones elicit those cell actions that can bring about a physiological response due to altered enzyme activity and/or increased or decreased protein production. This "pecking order" has the advantage of both amplifying the physiological effects required as well as producing those

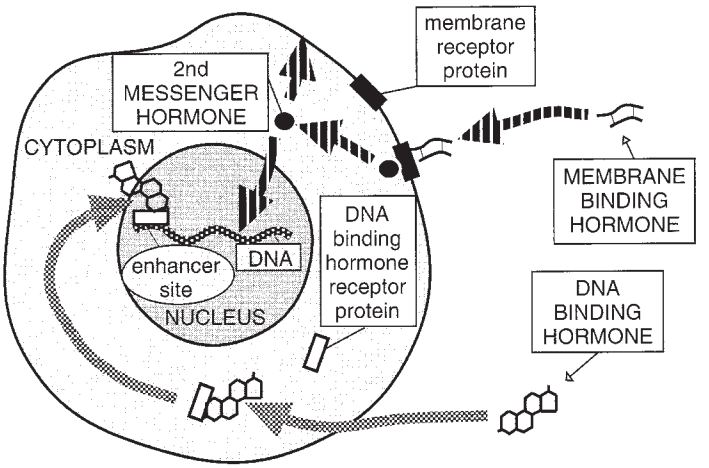


Figure 6–2
General scheme of hormone interaction with a cell. ➤ Membrane binding hormones (from endocrine and paracrine cells) bind to a membrane receptor protein on the cell surface. This activates (or deactivates) the formation of a second messenger within the cell hormone. The second messenger hormone causes amplified biochemical reactions in the cell. DNA binding hormones (from endocrine cells only) penetrate the cell and bind to a cytoplasmic DNA binding, hormone receptor protein. The hormone-protein complex diffuses to the nucleus where it binds to an enhancer site on DNA. Such binding affects the synthesis (or nonsynthesis of specific proteins) in the cell.

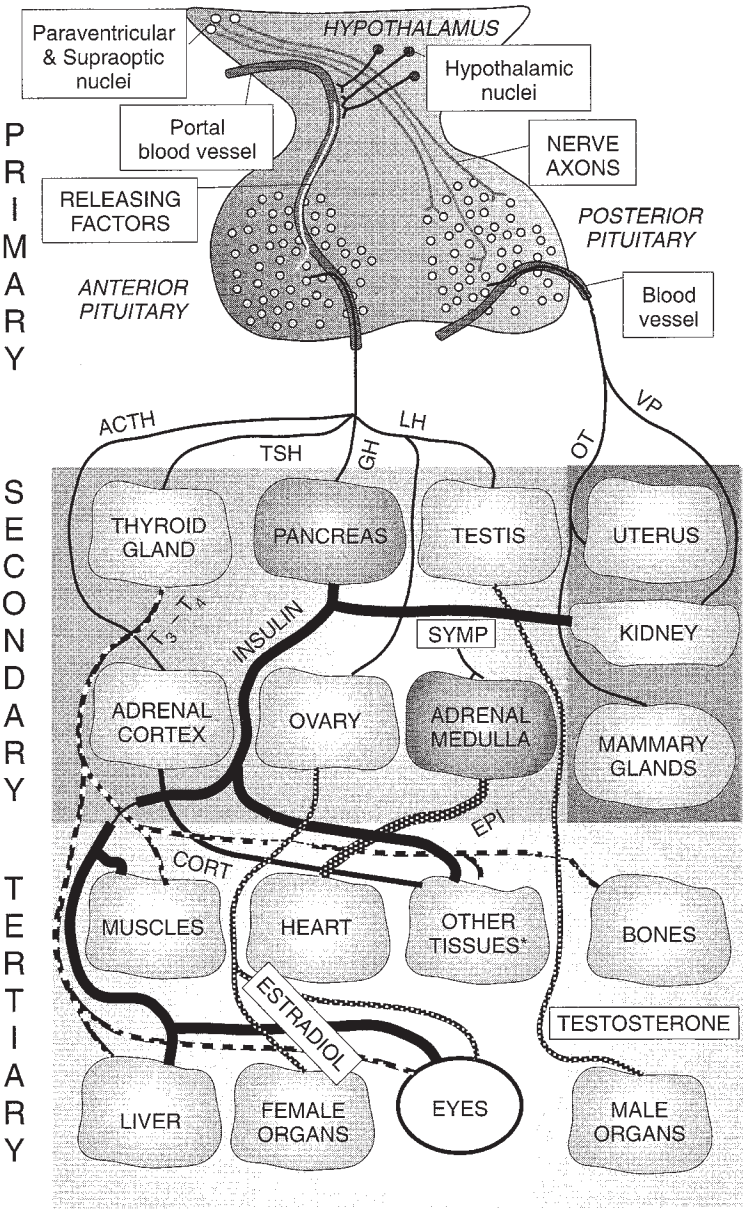


Figure 6–3
General scheme of the endocrine system of hormones. ➤ Initiation of hormone release most often begins in the hypothalamus where there are two systems to activate the release of hormones from the anterior and posterior pituitary. Hypothalamic cells release hormones into the portal blood vessel where they are carried to cells of the anterior pituitary. Paraventricular and supraoptic cells synapse directly to cells of the posterior pituitary. They communicate with them by the release of neurotransmitters (see Chapter 8). Cells of the pituitary are primary target cells of the endocrine system. The primary target cells release a variety of hormones (peptides): including ACTH (thyroid-stimulating hormone), GH (growth hormone), LH (luteinizing hormone), OT (oxytocin), and VP (vasopressin) to secondary cells. The notable exceptions to this system are the cells of the adrenal medulla, which are targeted by neurotransmitters from the sympathetic nervous system (see Chapter 8) and, for the most part, the cells of the pancreas (although growth hormone also influences those cells). The pancreas usually reacts to levels of blood glucose and other nutrients in a somewhat autonomous fashion. Secondary targeted cells receiving input from the anterior pituitary release hormones to a wide variety of tertiary targeted cells (only a limited number are shown here). The hormones (amino acid derivatives, peptides, and steroids) include T_3 and T_4 (triiodothyronine and thyroxine), CORT (cortisone), EPI (epinephrine), insulin, estradiol, and testosterone. The eyes represent a tertiary target tissue also. "Other tissues*" represents every other cell type.

effects in a controlled, coordinated manner. The cells of the eye represent tertiary cells in this system. The physiological effects to all targeted cells (ocular and nonocular) can range from very short periods of time (prostaglandins have effects only for seconds) to extended periods (growth hormone can have effects that last for days).

HORMONE EFFECTS, "SHELF LIFE," AND CONCENTRATIONS

On the average, however, hormone *effects* are usually short lasting—in the minutes to hours range (Kacsoh, 2000). Part of the reason for this is that the hormones themselves have a *short existence* prior to their enzy-

TABLE 6–1 ➤ PEPTIDE, PROTEIN, AND AMINO ACID DERIVED HORMONES[1]

Hormone	Molecular Weight	Source	Target	Mechanism	Effect
Thyrotropin-releasing hormone[2]	363	Hypothalamus	Anterior pituitary	Membrane receptor/ second messenger	Releases thyrotropin
Growth hormone	22,000	Anterior pituitary	Pancreas	Membrane receptor/ second messenger	Releases insulin and growth factors
Corticotropin	4500	Anterior pituitary	Adrenal cortex	Membrane receptor/ second messenger	Releases adrenal steroids
Thyrotropin	26,600	Anterior pituitary	Thyroid gland	Membrane receptor/ second messenger	Releases T_3 and T_4
Oxytocin	1007	Posterior pituitary	Uterus; breasts	Membrane receptor/ second messenger	Contraction; milk release
Vasopressin	1084	Posterior pituitary	Kidney; muscles[3]	Membrane receptor/ second messenger	Resorption of Na/water
Insulin	6000	Pancreas	Liver, other	Membrane receptor/ second messenger	Glucose and amino acid uptake
Glucagon	3485	Pancreas	Liver, other	Membrane receptor/ second messenger	Release of glucose, ketones
Thyroxine (T_4)[4]	777	Thyroid gland	Liver, other	DNA enhancer	Stimulation of metabolism

[1]This list is selective. The reader should consult with other texts for other hormones in this classification.
[2]There are separate releasing/inhibitory hormones for each of the anterior pituitary hormones.
[3]Smooth muscles only.
[4]Four iodine groups.

matic degradation. Endocrine hormones that are water-soluble tend to have a comparatively shorter life in plasma (e.g., insulin has a half life of approximately 4 min) whereas those that are lipophilic tend to have a comparatively longer life (e.g., cortisol has a half life of approximately 80 min). Prostaglandins, which are local hormones, have very short half lives as mentioned above (Mathews, von Holde, 1990). In addition, the hormones released are themselves present in very small quantities (10^{-9} to 10^{-15} M). The reason for this limitation of hormone concentration and existence is to attain reasonable positive and negative controls of physiological responses. Secondarily, the signals generated by hormones are greatly amplified by enzyme activity inside of cells once receptor binding takes place.

HORMONES: CHEMICAL CLASSES

Hormones fall into five biochemical classes: *peptides/proteins*, *amino acid derivatives*, *steroids*, *cyclic nucleotides,* and *eicosanoids*. Representative peptide/proteins, as well as amino acid derivatives, are given in Table 6–1 along with their mechanism of action and biochemical/physiological effects. The peptide/protein hormones that originate from the brain and endocrine organs vary in molecular weight from approximately 350 to 32,000 Daltons. They are targeted to primary, secondary, and tertiary tissues (cells), and accordingly, bring about a large variety of responses. One cannot predict their effects based on molecular size and conformation. Note in Figure 6–3, that while all primary and most secondary tissues release other hormones, the tertiary tissues (cells) cause the ultimate physiological responses. All members of the peptide/protein/amino acid chemical class, except the derived amino acids

thyroxine (T_4) and its cousin triiodothyronine (T_3), function by binding to a membrane receptor and, consequently activating a second messenger in the target cell. The adrenal medulla and the pancreas are notable exceptions. The adrenal medulla responds to sympathetic nervous input (rather than a hormone) to secrete epinephrine as a response to stress. The pancreas secretes insulin and other hormones in response to circulating levels of glucose (see Chapter 4) even though it is also influenced by growth hormone. The chemical structures of four of the hormones named in Table 6–1 are shown in Figure 6–4.

Table 6–2 lists five important steroid hormones. Steroid hormones produce a wide variety of physiological effects by acting at the cell nucleus. All steroid hormones also have some degree of glucose synthesizing properties (*glucocorticoid activity*) in which cortisol is predominant and sodium retaining properties (*mineralocorticoid activity*) in which aldosterone is predominant. Steroids can also produce a long list of undesirable effects such as: potassium depletion, increased weight gain, excessive mental stimulation, osteoporosis (bone resorption), adrenal atrophy, and have the potential for inducing diabetes when they are present in excessive amounts. Some steroids even have the ability to induce cataract formation. These undesirable effects can occur in steroid diseases, steroid intake abuse, or steroid therapy. Cortisol, and its synthetically manufactured cousins, are known for their ability to suppress the inflammatory response (see Chapter 9). This property is sometimes cautiously used to suppress ocular inflammation. The structures of four steroids (which are metabolically derived from cholesterol) are shown in

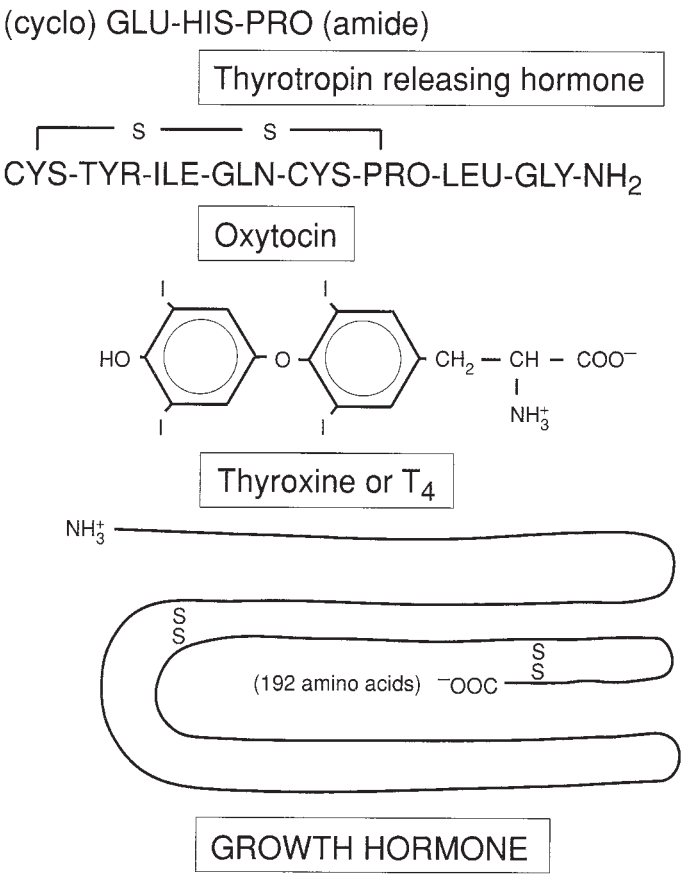


Figure 6–4
Representative examples of peptide hormones and hormones derived from amino acids. ➤ Thyrotropin releasing hormone is made in the hypothalamus. Oxytocin is made in the posterior pituitary gland. Thyroxine is synthesized from tyrosine in the thyroid gland. Growth hormone is made in the anterior pituitary gland.

Figure 6–5. Note that the molecular differences among the steroids are quite small although the effects produced are very different.

In Table 6–3 are found the two *cyclic nucleotide hormones*. The structures are given in Figure 6–5. These are intracellular second messengers. Other second messengers are *Ca^{+2} ions* and *triphosphoinositide (IP_3)* (also found in Table 6.3). Ca^{+2} and IP_3 are components of a messenger system originating from the endoplasmic reticulum and the plasma membrane of a cell. The cyclic nucleotides generally produce opposing effects within a cell and operate by amplifying their signal by a cascade mechanism that will be discussed further in this chapter.

Table 6–4 lists four eicosanoid hormones and their effects. Two of their structures are shown in Figure 6–5. Eicosanoids are local hormones made from long chain fatty acids and produce a variety of physiological responses that also often oppose each other.

Plasma Membrane Hormones and Their Receptors: Mechanisms

Those hormones that bind to plasma membrane receptors (see Figure 6–2) can initiate a variety of intracellular events. In referring to a "targeted" cell for such hormones, it is meant that a particular cell possesses a protein receptor on the cell surface capable of binding to a specific hormone. Not all cells have receptors for every hormone. Those that do may even produce different responses to the same hormone compared to another cell type with the same receptor. That is what causes the specificity and diversity of hormones. An example of a receptor protein for insulin was shown in Figure 4–29. That receptor protein (known as a *tyrosine kinase receptor*) causes intracellular effects by altering the activity of the enzyme tyrosine kinase. The tyrosine kinase is part of the structure of the receptor protein itself. It brings about effects such as increased uptake of glucose, mobilization of lipids into the bloodstream and the active transport of amino acids into cells. Mechanisms for that receptor are described in Chapter 4.

CYCLIC NUCLEOTIDE MECHANISMS

Most receptor proteins, however, cause the activation or deactivation of enzymes that synthesize the secondary messengers: cAMP, cGMP, and inositol trisphosphate/Ca^{+2}. A "G" (guanosine) protein acts as an intermediary in the process. This is indicated in Figure 6–6 for cAMP.

TABLE 6–2 ➤ STEROID HORMONES[1]

Hormone	Molecular Weight	Source	Target	Mechanism	Effect
Cortisol	360	Adrenal cortex	Many tissues	DNA enhancer	Glucose synthesis; anti-inflammatory
Aldosterone	371	Adrenal cortex	Kidneys	DNA enhancer	Sodium retention and potassium excretion
Estradiol	272	Ovary	Female organs	DNA enhancer	Sexual maturation of female
Testosterone	288	Testis	Male organs	DNA enhancer	Sexual maturation of male
Progesterone	315	Ovary	Uterus	DNA enhancer	Preparation, continuance of pregnancy

[1]This list is selective. The reader should consult other texts for other hormones in this classification.

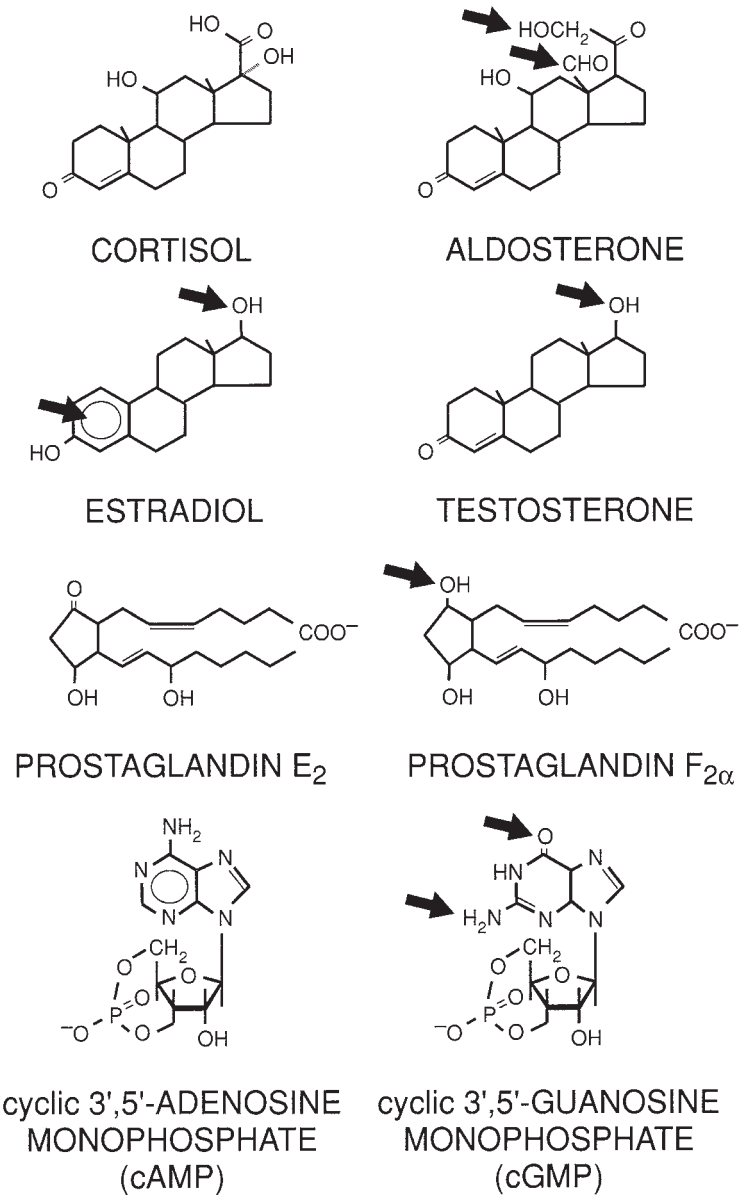


Figure 6–5
Representative steroid hormones (top four), eicosanoid hormones represented by prostaglandins (next two) and second messenger hormones (cyclic nucleotides, the last two on the bottom). ➤ Arrows indicate functional group differences within each class.

When the hormone binds to its receptor protein, known as a *G protein coupled receptor*, it brings about the binding of the nucleotide GTP (guanosine triphosphate) to the α-subunit of its G associated protein. As this occurs, the α-subunit is detached from the other G protein subunits and diffuses to the membrane enzyme, adenylate cyclase (also known as adenyl cyclase and adenylyl cyclase). The α-subunit of the G-protein binds to the enzyme and either stimulates it (if it comes from a G_S protein) or inhibits it (if it comes from a G_I protein). G_s refers to a stimulatory G protein while G_I indicates an inhibitory G protein. Most endocrine hormones operate through G_S proteins in order to simulate adenylate cyclase. In Chapter 8 it will be shown that many neurotransmitters operate through G proteins.

Adenylate cyclase causes the formation of the second messenger, cAMP (cyclic adenosine monophosphate). The corresponding cGMP enzyme is guanylate cyclase. Cyclic AMP and cGMP produce cellular

TABLE 6–3 ➤ SECOND MESSENGER (INTRACELLULAR) HORMONES

Hormone	Molecular Weight	Source	Target	Mechanism	Effect
cAMP[1]	329	Activated PM[5] receptor	Various intracellular locations	Cascade	Often stimulatory
cGMP[2]	345	Activated PM receptor	Various intracellular locations	Cascade	Often inhibitory, operates in visual transduction
Ca^{+2}	40[3]	Activated intracellular vesicles	Protein kinase C (PM) and calmodulin (cytosol)	Cascade	Varied cellular reponses
IP_3[4]	420	Phosphatidyl inositol-4,5-bisphosphate (PM)	Endoplasmic reticulum	Cascade	Ca^{+2} release

[1]Cyclic adenosine monophosphate
[2]Cyclic guanosine monophosphate
[3]Approximate atomic weight
[4]Inositol-1,4,5-trisphosphate
[5]Plasma membrane

TABLE 6–4 ➤ EICOSANOID HORMONES[1]

Hormone	Molecular Weight	Source	Target	Mechanism	Effect
Prostaglandin E_2	352	Ubiquitous cells	Local cells	Membrane receptor, second messenger	Relaxes smooth muscles; causes inflammation
Prostaglandin $F_{2\alpha}$	355	Ubiquitous cells	Local cells	Membrane receptor, second messenger	Contracts smooth muscles
Leukotriene E_4	454	White blood cells, lungs	Local cells	Membrane receptor, second messenger	Exudation of plasma
Thromboxane B_2	370	Platelets, others	Local cells	Membrane receptor, second messenger	Contracts smooth muscle

[1]This list is selective; the reader should consult other texts for other hormones in this class.

effects by means of a cascade mechanism that, essentially, is an amplification of the signal or message brought by the extracellular hormone. This means that the molecule carrying the "message" from the hormone causes the production of many more molecules that magnify the message. This is accomplished by the activation of several enzymes that participate the cascade. Figure 6–7 shows the cascade produced by epinephrine, a hormone produced by the adrenal medulla, to increase the amount of intracellular glucose participating in the Embden-Meyerhof (E-M) pathway (see Chapter 4). In other words, it increases the amount of energy produced by a cell. In the mechanism, cAMP activates a protein kinase (an enzyme causing the addition of phosphate groups). The active protein kinase, in turn, activates a phosphorylase kinase, which activates a third enzyme, phosphorylase. Phosphorylase, as discussed in Chapter 4, causes the formation of glucose 1-phosphate from glycogen stores. Glucose 1-phosphate is a precursor to glucose 6-phos-

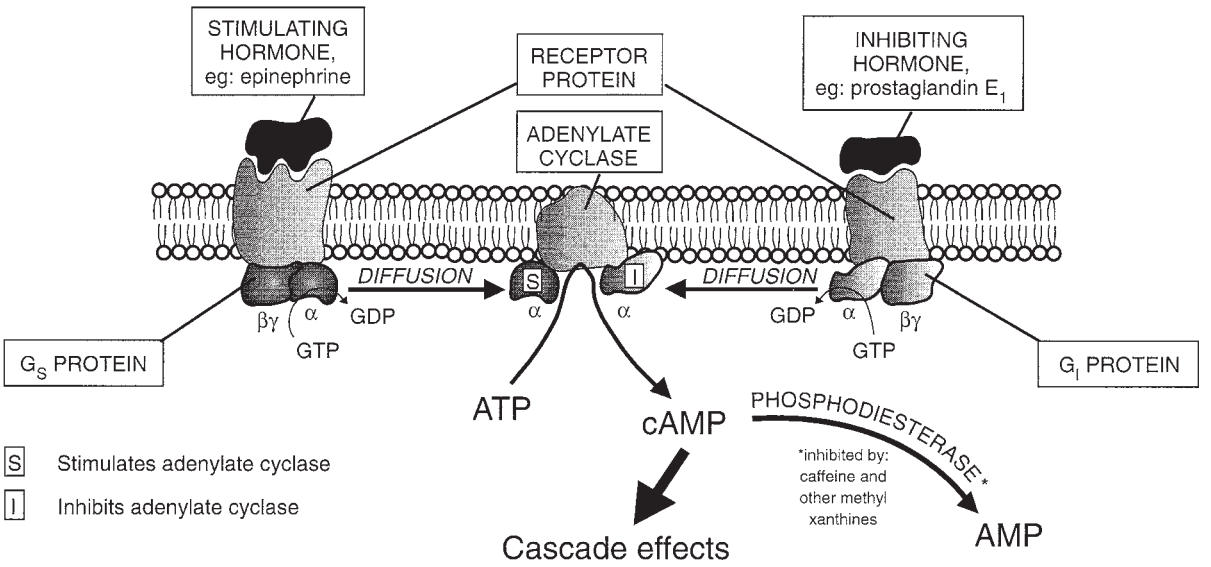


Figure 6–6

Cyclic nucleotide, second messenger reaction mechanisms. ➤ The stimulatory mechanism (shown at the left) is one in which the receptor protein binds to a stimulatory G protein (or G_s). This protein (consisting of α, β, and γ subunits) normally is bound to guanosine diphosphate (GDP, a nucleotide) at its α-subunit. When the hormone receptor protein binds to it, it releases GDP and takes up guanosine triphosphate (GTP). As this occurs, the GTP-α-subunit complex is released from the other two G protein subunits and diffuses to the nearest adenylate cyclase enzyme (bound to the plasma membrane), binds to it and causes its activation. The activated adenylate cyclase catalyzes the formation of cAMP (from ATP) that begins the cascade mechanism of hormone effects. The inhibitory mechanism (shown at the right) occurs less frequently with hormones. It is similar to the stimulatory mechanism except that an inhibitory G protein (or G_i) is involved. The α-subunit of the G_i protein shuts down (or inhibits) the adenylate cyclase. A cytoplasmic phosphodiesterase eventually hydrolyzes cAMP to AMP. Cyclic GMP is activated and broken down in a somewhat similar manner to cAMP.

phate, which participates in glycolysis (i.e., the E-M pathway) (Chapter 4). The number of molecules produced are amplified many times with the activation of each enzyme. McGilvery and Goldstein (1983) estimate that 1 μmole of cAMP (itself amplified from adenylate cyclase by the hormone epinephrine) will cause the synthesis of 25,000 μmoles of glucose 1-phosphate per minute. Similar mechanisms of production and cascade, involving guanylate cyclase, exist for cGMP. Both cAMP and cGMP are inactivated to AMP and GMP respectively by separate phosphodiesterase enzymes (see Figure 6–6). Phosphodiesterases are inhibited by methyl xanthines, notably caffeine and theophylline, substances found in significant concentrations in coffee, certain soft drinks, chocolate, and tea. Accordingly, the increase in the amounts of second messengers, particularly cAMP, account for central nervous system stimulation, cardiac stimulation, and gastric motility when these substances are consumed (Rall, 1985). Although these enzymes are present in the eye, vision is not improved by drinking coffee or eating chocolates!

In the eye, much effort has been spent in trying to show a specific role for a hormonal stimulation of adenylate cyclase and cAMP in the control of intraocular pressure via effects on the ciliary body. However, no conclusive studies have yet shown that any such relationship exists (Abdel-Latif, 1997). In the lens, both cAMP and cGMP may be found with higher concentrations in the epithelial cells (Zagrod, Whikehart, 1981). Harding (1997) suggests that the presence of these second messengers may be associated with the growth of this tissue *via* growth

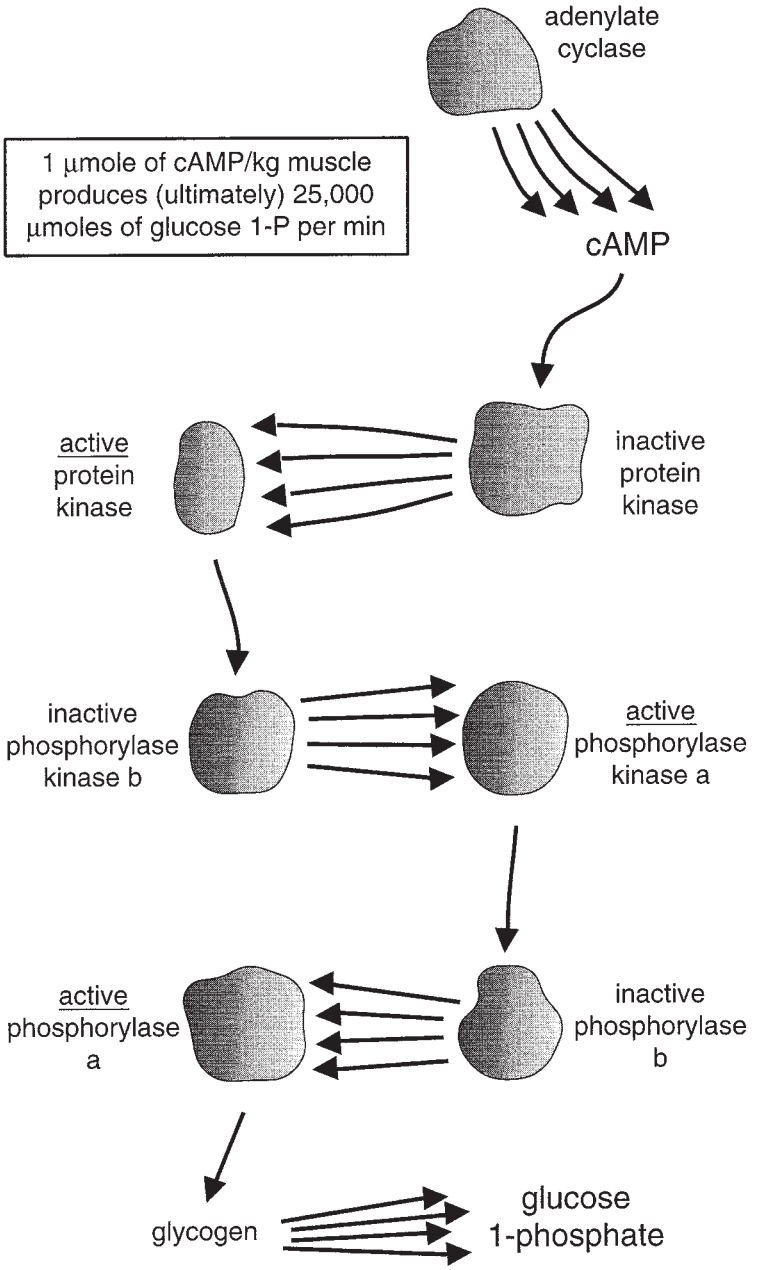


Figure 6–7
A cascade mechanism. ➤ All cascade mechanisms begin with cAMP or cGMP and involve the activation of several intermediate enzymes by adding phosphate (phosphorylation) to inactive enzymes using a kinase enzyme. Cyclic nucleotides activate the first kinase. The particular example shown is for the formation of glucose 1-phosphate from the binding of the hormone adrenaline (epinephrine) to its receptor protein.

hormone. In the retina, the hormone vasoactive intestinal peptide (VIP, released from amacrine cells) can cause increases in cAMP in ganglion cells *via* VIP receptors (Gordon, Bazan, 1997). VIP acts as a local hormone in the retina. The physiological response to this hormone seems to be to fine tune ganglion cell response in a role of neuromodulation, a subject to be taken up in Chapter 8.

INOSITOL TRISPHOSPHATE/Ca^{+2} MECHANISMS

Another hormone second messenger system involves the cooperative association of inositol trisphosphate and Ca^{+2} ions. This mechanism is

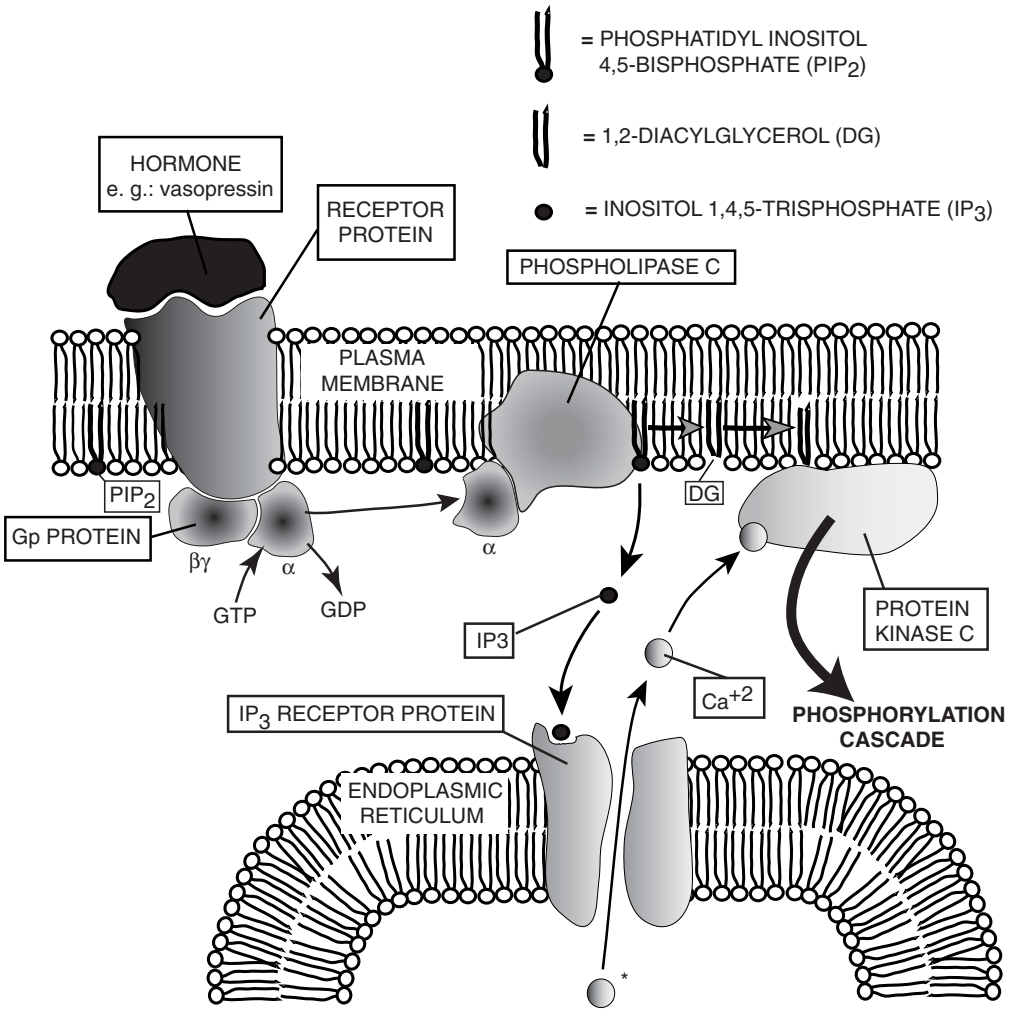
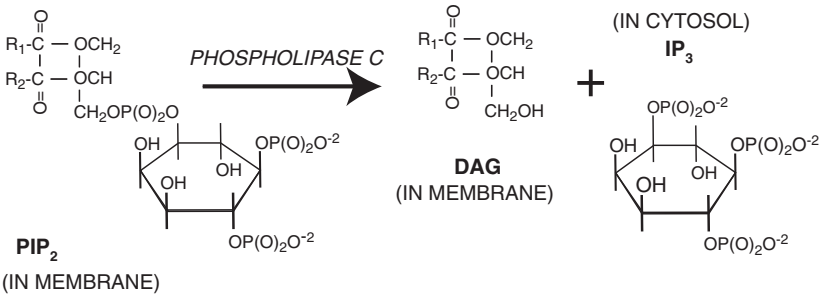


Figure 6–8

Inositol trisphosphate/Ca^{+2} ion, second messenger reaction mechanisms. ➤ In this system, a hormone receptor-G protein complex causes the activation of phospholipase C in a mechanism analogous to that for adenylate cyclase (see Figure 6–6). Phospholipase C hydrolyzes membrane-bound phosphatidyl inositol 4,5-bisphosphate (PIP_2) represented by the dark membrane component in the plasma membrane. The products are inositol 1,4,5-trisphosphate (IP_3) and 1,2 diacylglycerol (DG). IP_3 diffuses to an IP_3 receptor on the membrane of the endoplasmic reticulum of the cell. This receptor is a channel protein that opens to allow Ca^{+2} ions to be released into the cytoplasm. The Ca^2 ions, in turn, stimulate the enzyme protein kinase C that begins a phosphorylation cascade to activate a variety of cellular functions. Some Ca^{+2} ions are bound to the protein calmodulin that independently begin similar phosphorylation cascades. DG, which is still bound to the plasma membrane, also stimulates protein kinase C to produce the same phosphorylation cascades as the Ca^{+2} ions.

illustrated in Figure 6–8. It begins essentially like the cyclic nucleotide mechanism inasmuch as a receptor protein and an associated G protein (here identified as a G_p protein) are similarly activated. However, the α-subunit activates a membrane-bound enzyme, *phospholipase C*. This enzyme hydrolyzes membrane-bound phosphatidyl inositol 4,5-bisphosphate (PIP_2) to 1,2-diacylglycerol (DAG) and inositol 1,4,5-trisphosphate (IP_3) as shown on the next page.



The products of the reaction each contribute to the continuation of the mechanism. IP_3 causes the release of Ca^{+2} ions into the cytosol from *endoplasmic reticula* storage sites. Both DAG and Ca^{+2} ions stimulate another enzyme, *protein kinase C,* which begins another cascade series by initiating its own phosphorylation of a substrate protein. In addition, the Ca^{+2} ions released from the endoplasmic reticula may also bind to a protein known as *calmodulin* (see Figure 2–4). Calmodulin, with bound calcium, activates several protein kinases, which are capable of modifying gene expression (Lodish et al, 1999). Calcium/calmodulin also activates cAMP phosphodiesterase so that it may oppose the effects produced by the cAMP cascade.

In the eye, second messenger systems involving inositol phosphates and calcium/calmodulin have been studied in relation to the contraction of the iris-sphincter muscle. Since these systems are connected to neurotransmitter function, they will be taken up in Chapter 8. The inositol and calcium/calmodulin second messengers are also found in the lens, but exact functions have not been described.

NITRIC OXIDE

A very unorthodox second messenger in a gaseous form that has recently been discovered is nitrous oxide, NO (Furchgott, Jothianandan, 1991). This gas is produced in one cell and passes to a second cell where it stimulates guanylate cyclase to produce cGMP and a consequent cascade. Not many studies have been done in the eye involving NO, but Fleischhauer et al (2000) have shown that the activation of the NO-cGMP pathway takes place in the pig ciliary epithelium and may be involved in the modulation or fine control of aqueous humor production.

Light Transduction: A cGMP Mechanism

In Chapter 2 the molecular properties of rhodopsin were described. The role of rhodopsin in vision is similar to that of a receptor protein for a hormone. Light replaces the hormone, however, and the G protein associated with rhodopsin causes the activation of a phosphodiesterase rather than a cyclase. The G protein, known as transducin (G_t), functions like other G proteins because it incorporates GTP (while removing GDP) when interacting with the activated rhodopsin molecule. The α-subunit of transducin detaches and diffuses to an inactive cGMP phosphodiesterase. The α-subunit of transducin binds to the γ-subunit of this phosphodiesterase causing that subunit to leave the enzyme. This action activates the enzyme, which lowers the concentration of cGMP in the cytoplasm of photoreceptor outer segments. This may be seen in

Figure 6–9. Note that the orientation of rhodopsin (the receptor protein), transducin (the G protein), and phosphodiesterase (the cyclic nucleotide enzyme) seem inverted when compared to Figure 6–6. However, rod discs are internal cell organelles and the molecular orientation must be inverted in order to interact with the photoreceptor cytoplasm. It is emphasized again that the relationship of the cyclic nucleotide enzyme to the G protein in this mechanism involves a phosphodiesterase rather than a cyclase. The role of the GMP cyclase will be described later.

The questions now arise about why cGMP is important for vision and why lowering the concentration of cGMP ultimately translates into a perception of light in the brain? The answers lie in the physiology of the controlled operation of the flow of sodium and calcium ions into the photoreceptor at the outer segment (both rods and cones) and the flow of potassium ions out of the photoreceptor at the inner segment. This flow is described as the *dark current* (Molday, 1998). It is so named because the ion flow is maximal in the dark. This is shown in Figure 6–10, A. The flow of Na^+ and Ca^{+2} ions inward is controlled by gate proteins at the outer segment while the flow of K^+ ions outward is dominated by a voltage gated K^+ channel protein in the inner segment. Maintenance of open channels through the outer segment gate proteins require that cGMP be bound to the gate proteins. When the concentration of cGMP is lowered through the activity of cGMP phosphodiesterase, cGMP dissociates from the gate proteins which close and interrupt the ion flow (see Figure 6–10, B).

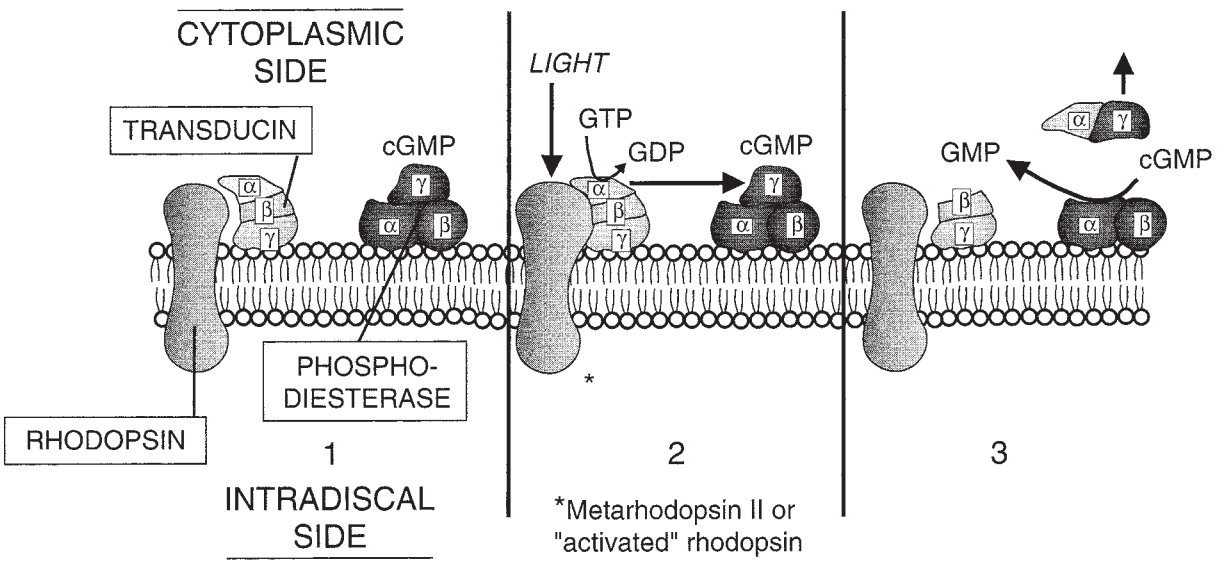


Figure 6–9

Disc membrane, light-receptor protein transduction mechanism. ➤ Similar to the hormone-receptor protein mechanisms described in previous figures. Here light acts as a "hormone" and activates rhodopsin and, ultimately, affects the level of a cyclic nucleotide. On the cytoplasmic side of each disc, rhodopsin (the receptor protein), transducin (the G protein), and guanylate phosphodiesterase (instead of a cyclase) are located in close proximity (shown at 1) on the disc membrane. When light strikes a rhodopsin molecule, the molecular rearrangement of vitamin A (to an unprotonated Schiff base) and the protein conformation of the opsin portion (to metarhodopsin II) causes close contact with transducin. As before (see Figure 6–6), the α-subunit of transducin takes up GTP, breaks away from the other subunits and diffuses to the phosphodiesterase molecule (shown at 2). The α-subunit of transducin binds to the (γ-subunit of the phosphodiesterase causing it to be released from the enzyme. The release activates the enzyme bringing about the catalytic hydrolysis of cGMP to GMP.

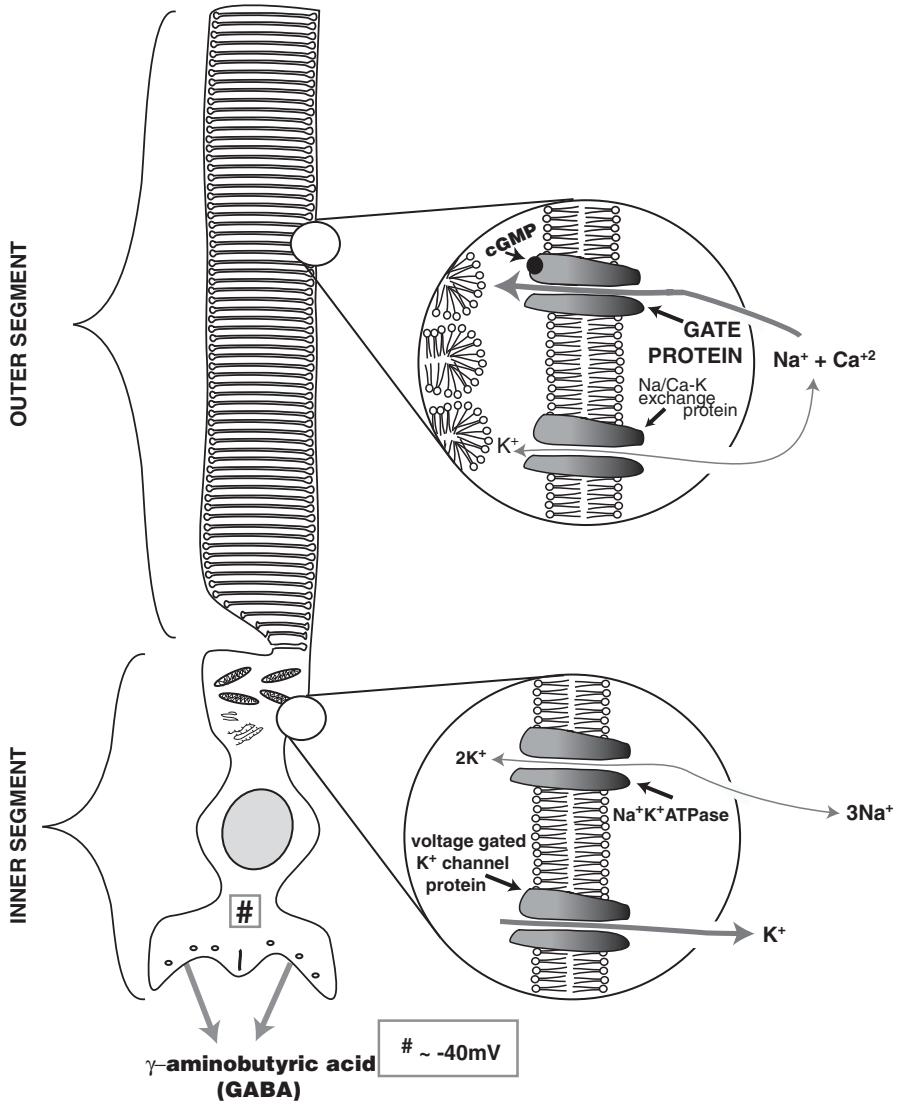


Figure 6–10

(A) The dark current ion flow in photoreceptors. ➤ In the outer segments of photoreceptors, two kinds of proteins maintain a constant flow of Na^+ and Ca^{+2} cations into the outer segment. The gate protein allows the passage of Na^+ and Ca^{+2} cations into the outer segment. The channel is maintained in the open state by the binding of cGMP and even Ca^{+2} itself. The Na/Ca-K exchange protein allows a controlled amount of Na^+ and Ca^{+2} ions to exit the outer segment in exchange for the inward passage of K^+ ions. The combined actions of both kinds of proteins in the dark state rigidly controls the internal cation concentration as well as the internal potential which at the inner segment is approximately 40 mV. On the inner segment, cation flow is controlled by the actions of Na,K-ATPase (see Chapter 3) and a voltage-gated K^+ channel protein. Na,K-ATPase maintains an excess of Na^+ cations on the outside of the cell while the voltage-gated channel protein selectively extrudes K^+ ions in a voltage dependent manner. While the Na,K-ATPase enzyme tends to establish a normal excels of K^+ ions inside the cell, the exact function of the voltage-gated protein is not presently well understood. While dark current conditions exist at approximately 40 mV, photoreceptors normally release glutamate neurotransmitters to bipolar cells.

Illustration continued on page 174.

The activity of phosphodiesterase is controlled, as stated, by light affecting the rhodopsin-transducin-phosphodiesterase cascade. When the sodium/calcium influx is interrupted, potassium outflux continues and the departing, positively charged ions cause the photoreceptors to acquire a net negative charge (see Figure 6–10, B). The cells hyperpolarize. This hyperpolarization is unusual since it stops the flow of current

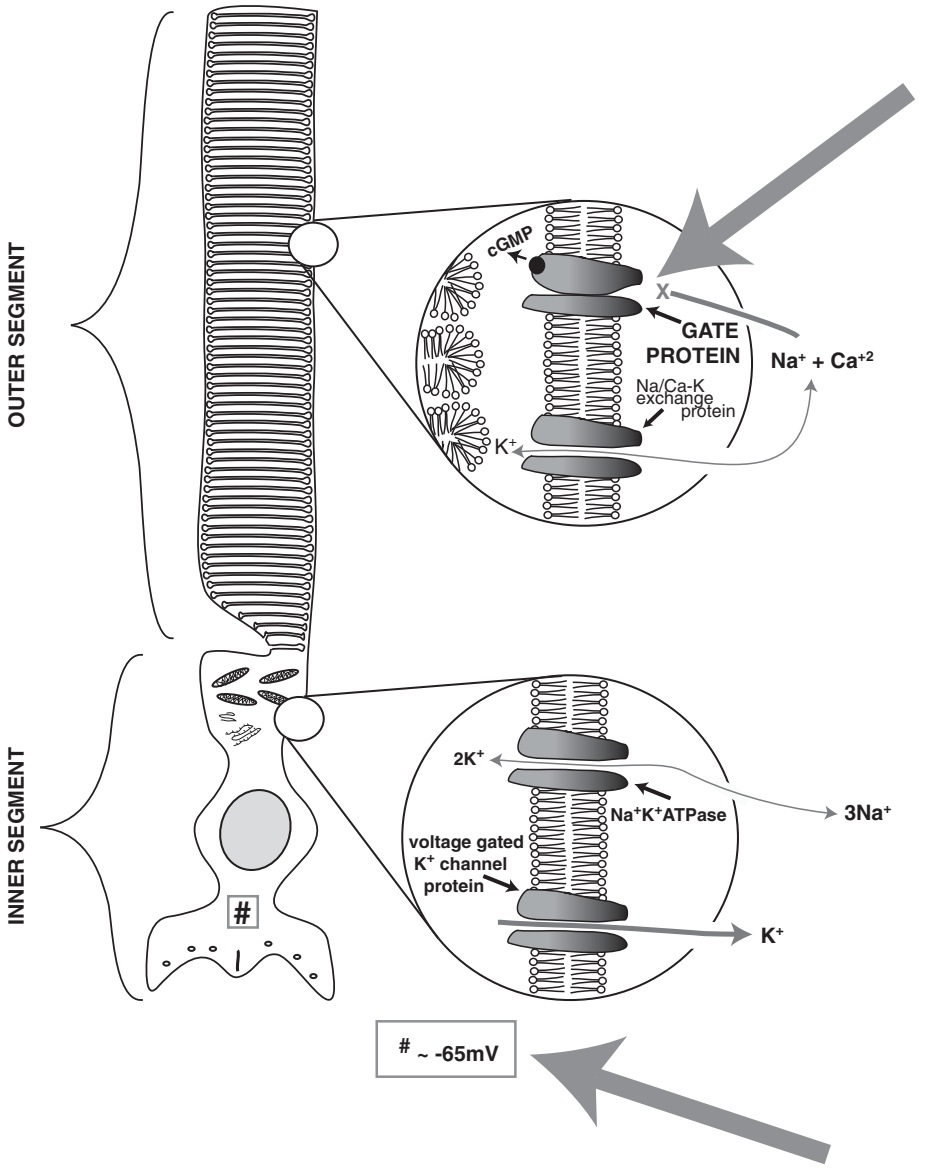


Figure 6–10, cont'd.
(B) Events occurring when the dark current is interrupted. The initial event occurs when the lowering and loss of cGMP to gate protein binding causes the gate protein to close (top arrow). This depletes the outer segment of Ca^{+2} and Na^{+} ions since the exchange protein continues to function and establishes an interruption in the dark current. The increase in internal negative potential spreads to the inner segment where it causes the voltage gated K^{+} channel to open bringing the potential at the synaptic area to approximately –65 mV. This new negative potential inhibits the normal release of glutamate to the bipolar cells (bottom arrow).

(i.e., the discharge of the neurotransmitter *glutamate* is interrupted) to the ganglion cells of the retina causing the ganglion cells to discharge! This discharge is ultimately transmitted to area 17 of the brain where it is perceived as light. The entire visual transduction process is diagrammed in Figure 6–11.

Biochemically and physiologically, there are some additional fine, but important points to be made. First, the ion flow in and out of the photoreceptor plasma membrane involves four different kinds of proteins (as shown in Figure 6–10, A). In the outer segment, in addition to the previously discussed *cGMP gated protein*, there is a *Na/Ca-K*

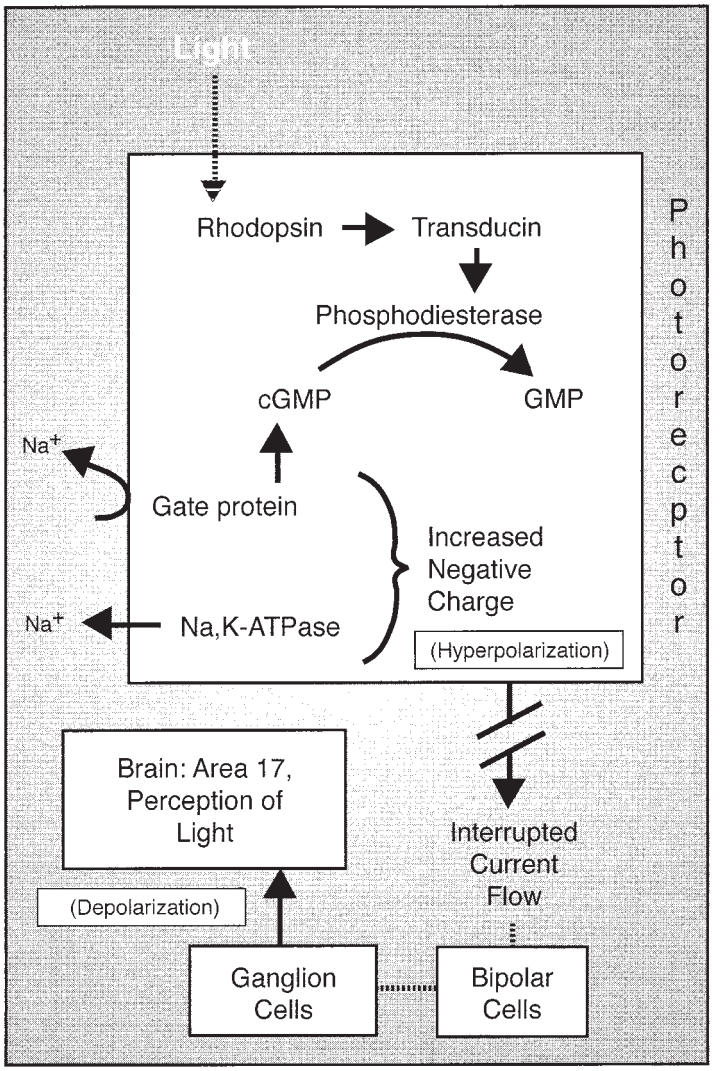


Figure 6–11
Overall diagram of visual transduction.

exchange protein that allows both Na^+ and Ca^{+2} ions to flow back out of the membrane, but at a reduced rate compared to that controlled by the inflow protein (cGMP gated protein). The function of the exchange protein is to maintain internal cytoplasmic Ca^{+2} levels at approximately 400 nM in the dark and at approximately 40 nM in strong light (Fain et al, 2001). The higher concentration is necessary to control other visual transduction functions described below. In the inner segment, the *voltage gated K^+ ion channel protein* normally maintains a steady flow of K^+ ions outward to contribute to the overall dark current flow. That flow is countered at a slower, controlled rate by the enzyme *Na,K-ATPase,* an enzyme whose structure and function were explained in Chapter 3. In this case, Na,K-ATPase acts to restore normal intracellular levels of the two ions. When there is a continual interruption in the dark current, as is currently thought by some investigators (Klumpp, Song, Pinto, 1995; Pinto, Klumpp, 1998), the change in membrane potential eventually will alter the K^+ outflow (this is why the protein is called a voltage gated K^+ channel) to adapt the cell to the continued presence of light. This is a form of *light adaptation.*

However, the influence of changes in the internal Ca^{+2} ion concentrations on these K^+ channels and the process of adaptation (retinal adjustment to light levels) must also be considered. As it is, this process is not yet completely understood. According to Fain et al (2001), more emphasis should be given to the numerous and subtle roles played by Ca^{+2} ions rather than changes in K^+ ions. In fact, this has been the emphasis historically by many investigators.

Calcium ions in photoreceptors (Figure 6–12) have the following functions: (a) self-modulation of Ca^{+2} channel protein sensitivity (Hsu, Molday, 1994); and (b) inhibition of guanylate cyclase in the outer segment (Dizhoor et al, 1995). Self-modulation occurs when Ca^{+2}, bound to *calmodulin*, binds as a complex to the Ca^{+2} channel protein and decreases its ion permeability. This mechanism acts together with the exchange protein to limit the upper concentration of Ca^{+2} ions in the outer segment. Guanylate cyclase is inhibited by Ca^{+2} ions when the ions are bound to *guanylate cyclase binding proteins* (*GCAP*s) (Polans, Baehr, Palczewski, 1996). Accordingly, when the intracellular Ca^{+2} ion concentration drops, guanylate cyclase is activated to produce more cGMP to bind to the channel proteins in order to allow more Ca^{+2} into the cell. This process is limited by the eventual inhibition of guanylate cyclase, channel protein self-modulation, and the continued activity of the exchange protein.

A possible direct and/or indirect influence of Ca^{+2} on voltage-gated K^+ ion channel proteins in the inner segment has also been proposed (Pinto, Klumpp, 1998). A fourth and controversial role is that involving the protein *recoverin* (known as *S-modulin* in amphibians) with bound Ca^{+2}. When recoverin and Ca^{+2} bind to each other in rod outer segments, it has been suggested that they inhibit rhodopsin kinase and, therefore, extend the lifetime of activated rhodopsin. However, conflicting evidence cannot allow a definitive conclusion about this yet (Fain et al, 2001).

The previous diagrams in Figures 6–9, 6–10, A and B, and 6–11 strongly indicate how cGMP is involved in visual transduction. However, the additional information about Ca^{+2} ions tends to challenge comprehension of the entire process. Accordingly, a final figure is presented that shows how Ca^{+2} ions contribute to visual transduction (Figure 6–12). This, together with the previous figures, should put it "all together."

Graves' Disease: Autoimmune Mischief with Thyroid Hormones

In normal thyroid function, the thyroid gland releases two hormones: T_4 (thyroxine or tetraiodothyronine) and T_3 (triiodothyronine). The structure of T_4 is shown in Figure 6–4. These two hormones stimulate general metabolism and growth in virtually all cells of the body. Figure 6–13 illustrates how T_3 and T_4 are produced in the thyroid follicular cells and their adjacent lumen prior to release into the circulation. Essentially, thyroid-stimulating hormone (TSH) binds to its receptor protein and its associated G protein is modified in the usual manner to stimulate adenylate cyclase to synthesize cAMP. In turn, cAMP causes three known effects in the follicular cell: a stimulation in the uptake of iodine; the transport of thyroglobulin back into the follicular cell; and an increase in the volume of the cell (presumably so that the cell can increase its ability to produce more T_3 and T_4). The actual production of T_3 and T_4 take place on the protein *thyroglobulin* (TG). Essentially, TG has iodine

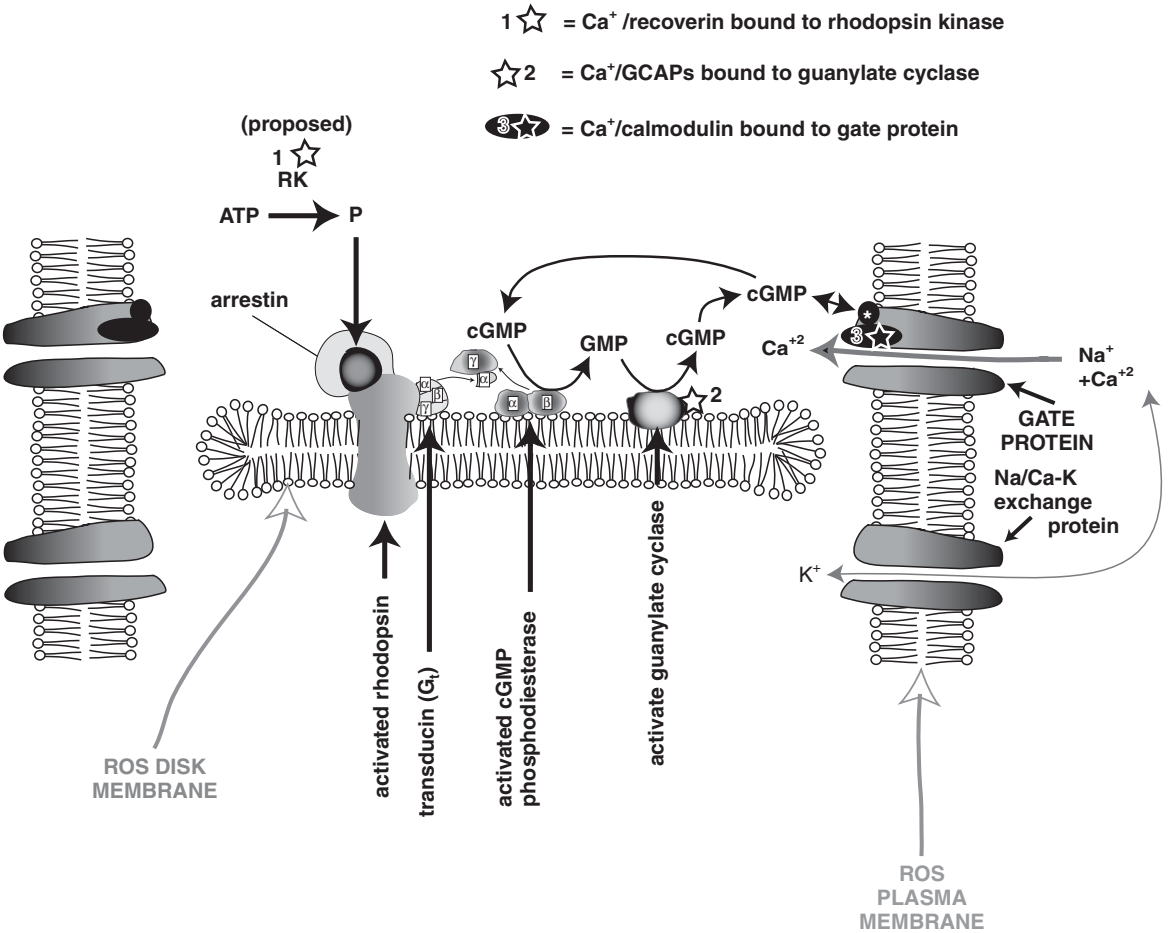


Figure 6–12
Diagram of visual transduction showing roles proposed to be played by Ca^{+2} and cGMP in visual transduction. ➤ Cyclic GMP levels are maintained by guanylate cyclase during the dark current. Cyclic GMP levels are reduced by cGMP phosphodiesterase during visual transduction under the influence of light/activated rhodopsin/G_t. Ca^{+2} ions bind to recoverin to inactivate rhodopsin kinase preventing inactivation of activated rhodopsin by phosphorylation at 1*. Ca^{+2} ions bind to GCAPS proteins to inactivate guanylate cyclase. Ca^{+2} ions bind to calmodulin in order to negatively modulate ion flow through the gate proteins. In essence, Ca^{+2} ions are self-modulating at higher concentrations by at least three mechanisms. See text for further explanation.

incorporated into its Tyr residues as the iodinium (I^{+1}) ion by the enzyme *thyroid peroxidase* while TG is present in the lumen between the follicular cells. Tyrosines have one or two iodine atoms added to them although some inaccessible Tyrs are never iodinated. In a manner that is not well understood, two iodinated Tyrs are coupled together with an oxygen bridge to form precursors to T_3 and T_4 while still bound to TG (Vassart et al, 1996). After returning to the follicular cell, TG molecules (encased in a *lysosome*) release T_3 and T_4 upon hydrolysis of TG by lysosomal enzymes. The released hormones rapidly leave the follicular cell by crossing the cell membrane, which is not a barrier.

Hyperthyroidism (or Graves disease) is a condition in which the thyroid gland releases excessive amounts of T_3 and T_4. The disease was first described in 1786 by Parry, but was later associated with a description of the condition made by Graves in 1835. Nonocular symptoms of Graves' disease include nervousness, irritability, fatigue, weight loss, heat sensitivity, palpitations, and weakness. All of these symptoms result from

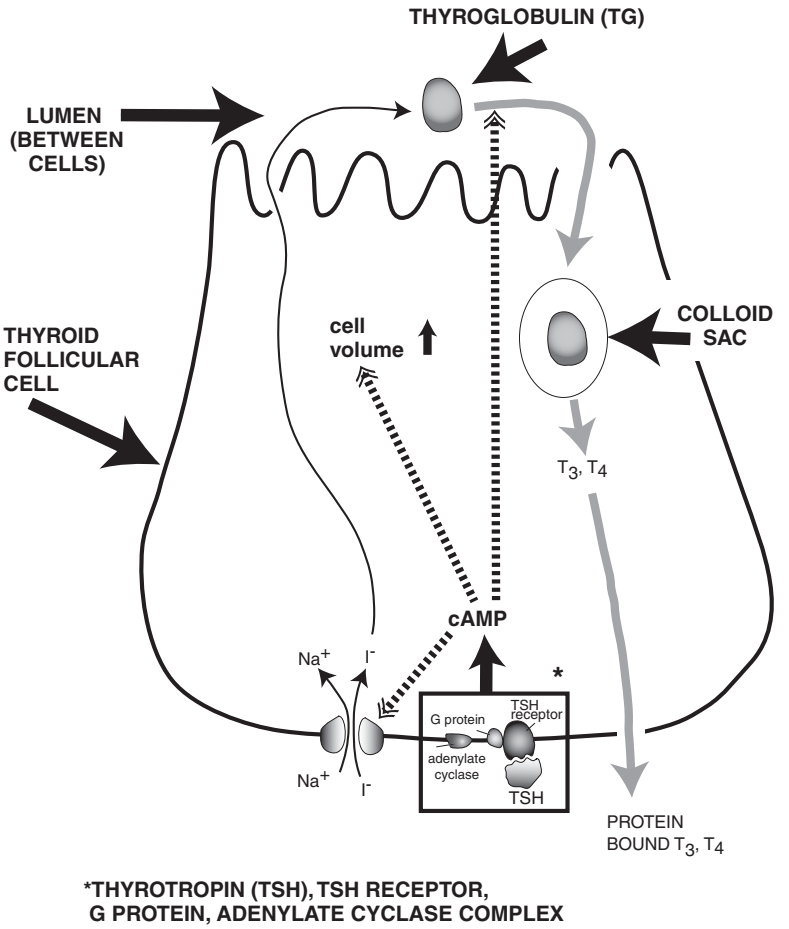


Figure 6–13
Diagram of hormone synthesis mechanisms in the thyroid gland. ➤ Iodide ion is taken up into thyroid follicular cells and transported to thyroglobulin (TG) proteins at the lumen between follicular cells. The iodine is incorporated into this protein's tyrosine residues by the enzyme thyroid peroxidase. Afterwards, iodinated TG is incorporated into colloid sacs and moved back into the follicular cells where enzyme hydrolysis releases T_3 and T_4 hormones, which diffuse from the cell into the blood stream. At least three events support T_3 and T_4 production by the cascades initiated by cAMP: (1) iodide uptake; (2) increase in cellular volume; and (3) cellular re-uptake of iodinated TG. Cyclic AMP itself is produced by adenylate cyclase using the traditional mechanism shown in Figure 6–6 and outlined in the box at the bottom of the figure. In this case, the hormone is thyroid-stimulating hormone (TSH). In Graves' disease, TSH is supplanted by a thyroid stimulating immunoglobulin to produce cAMP.

the increased metabolic rate caused by higher than normal amounts of circulating T_3 and T_4 in the bloodstream. The thyroid gland is usually enlarged. Between 30 and 65% of hyperthyroid patients also have ocular symptoms (Char, 1990). The range depends on the method of evaluation. The eyes are often *proptosed* or partially pushed forward from their sockets, a condition known as *exophthalmos*. This condition causes retraction of the upper eyelids and difficulty in being able to close the eyes. The eyes are pushed forward due to a tissue build-up around the extraocular muscles. Further extremes of this build-up can result in corneal drying (from the inability to close the eyes) and optic nerve damage (possibly due to compression of the optic nerve by the extraocular tissue build-up). Graves' disease results from the existence of one or more proteins that act as if they were the thyroid stimulating hormone (TSH or thyrotropin), a hormone that is released by the anterior pituitary (see Figure 6–3), and that binds to the TSH receptor protein. The impostor proteins are, in fact, antibodies (discussed in Chapter 9) called *thyroid-stimulating immunoglobulins or TSI* (Utiger, 1987). These antibodies regard the thyroid gland to be a foreign tissue or *antigen* and bind to it as a means of tagging the thyroid for immunological rejection (Figure 6–14). However, by binding to the receptor protein for TSH and, by successful competition with TSH, they cause an increase in the circulation of T_3 and T_4 (see Figure 6–13). Graves' disease is, therefore, an autoimmune disease that uses a hormonal mechanism to produce its effects.

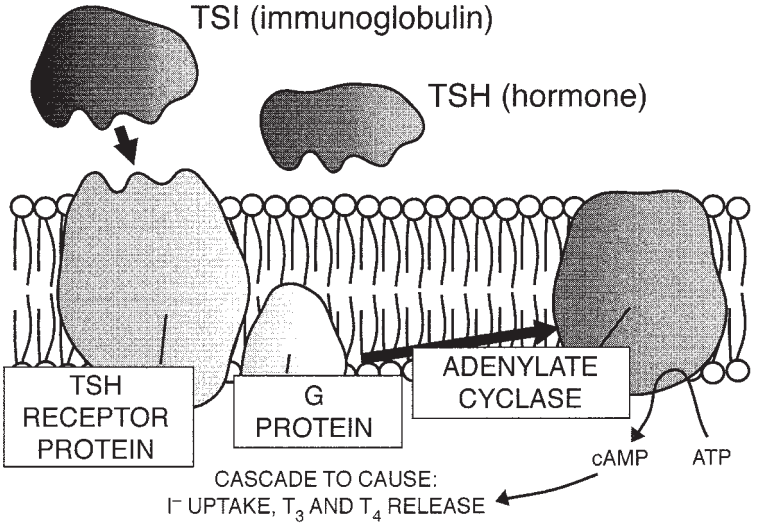


Figure 6–14
Plasma membrane, hormone receptor protein reaction mechanism in the thyroid gland. ➤ Here, in Graves' disease, a thyroid-stimulating immunoglobulin (TSI) mimics the response achieved by the thyroid-stimulating hormone (TSH). The result is an increase in the release and circulation of T_3 and T_4.

The causes for the ocular pathology associated with Graves' disease have never been resolved (Burch et al, 2000). This pathology involves swelling of the orbital fat, connective tissue, and extraocular muscles (Weintraub, 1995). In the eye, the increase in circulating T_3 and T_4 in Graves' disease only directly affects the sympathetic innervation of the lids by inhibiting their closure (Utiger, 1987). Therefore, it would seem that some other factor or factors contribute to exophthalmos. Contributory evidence suggests that thyroid stimulating antibodies (TSI, mentioned previously) also have receptor proteins located in the affected retrobulbar tissues (Ohtsuka, Hashimoto, 2000). The serum levels of TSI are also elevated in patients with marked exophthalmos making the association between the presence of TSI, their receptors and the development of exophthalmos more than coincidental. The subject of immunity will be taken up in Chapter 9.

DNA Binding Hormones: Mechanism

Besides its many specific targets in the cell, second messengers can also affect RNA transcription and ultimately, protein synthesis. However, the influence of steroid hormones and thyroid hormones on RNA transcription is better understood than that of second messengers in this cellular location. Steroid hormones and endocrine hormones from the thyroid gland (T_3 and T_4) bind indirectly to *DNA enhancers*. Such enhancers are regions of DNA that alter the rate of RNA synthesis, and therefore, the rate of protein synthesis. This is accomplished only after the hormone has entered the cell and is initially bound to a protein receptor located in the cell's cytoplasm. In fact, it is the receptor protein itself that actually binds to the DNA enhancer. The general structure of DNA-binding steroid receptors is indicated in Figure 6–15. The action and conformational change induced in the receptor protein when the hormone binds to it, causes the release of a blocking protein from the DNA binding domain of the receptor protein. This action enables the receptor protein-hormone complex to bind to the appropriate enhancer site on DNA after diffusion of the complex into the cell nucleus.

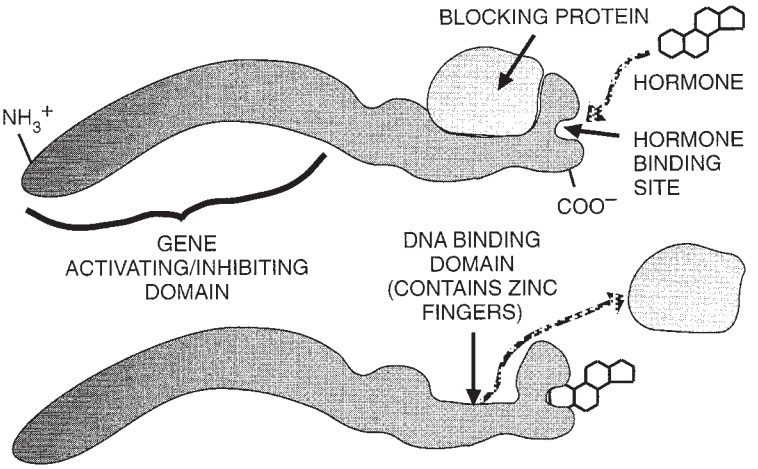


Figure 6–15
Steroid and thyroid hormone receptor proteins. ➤ A blocking protein is normally bound to the DNA binding domain of the protein. When the hormone binds to the protein, it causes the release of the blocking protein.

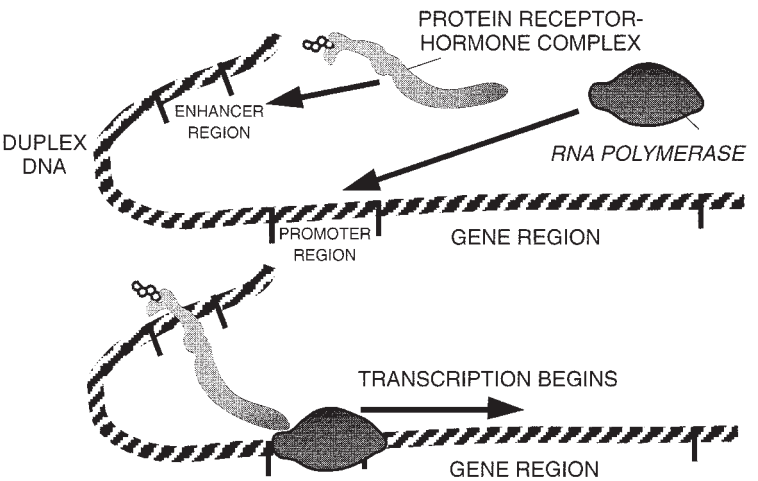


Figure 6–16
Binding of intracellular hormone-binding complex to DNA. ➤ When the complex is formed, it diffuses to the nucleus where it binds to a specific enhancer region on DNA. The gene activating or inhibiting domain extends to the promoter region (lower part of figure) where it either causes transcription to begin, modifies its rate, or prevents it from occurring.

When this takes place, the enhancer region may influence either the initiation or the inhibition of *gene transcription* (and ultimately protein synthesis) by close contact with either the promoter region or RNA polymerase bound to the *promoter region* (Figure 6–16). In the figure, a steroid hormone-receptor protein complex binds to an enhancer while RNA polymerase binds to a nearby promoter. In this case, close contact or binding of the gene-activating region of the receptor protein signals *RNA polymerase* to accelerate, inhibit, or halt transcription. Such altered transcription causes the cell to make varied quantities of specific kinds of proteins of which many are enzymes. An example of this mechanism is what takes place with the steroid hormone aldosterone (see Table 6–2; see Figure 6–5). This steroid causes the retention of sodium ions and the loss of potassium ions in the kidney as a means of fixing specific ion concentrations in the blood (mineralocorticoid activity). An individual would die in a matter of days without such control. Verrey et al (1989) found that aldosterone induces a rapid increase in the rate of Na,K-ATPase gene transcription in cultured kidney cells. As mentioned in Chapter 3, Na,K-ATPase is responsible for transport of Na^+ and K^+ ions and, therefore, an increase in the synthesis of this enzyme supports such a kidney mechanism (Figure 6–17). This is so since raising the concentration of

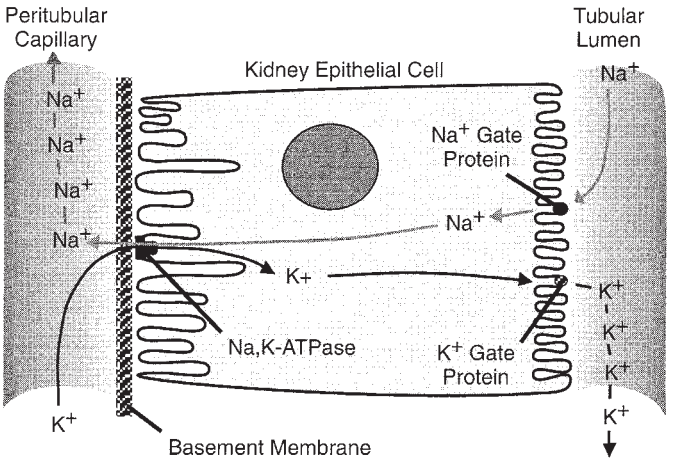


Figure 6–17
Cross section of kidney epithelial cell with capillary (on left) and collecting duct (on right). ➤ The formation of urine in the tubular lumen is partially realized by extracting Na^+ and releasing K^+ into the tubular lumen. In the process, the bloodstream regains Na^+ and loses K^+ by having these ions pass through the epithelial cell. The action is catalyzed by Na,K-ATPase, as well as the facilitation of transport provided by Na^+ and K^+ gate proteins. This action has some similarities to the formation of aqueous from blood in the ciliary body (See Figure 3–20).

this enzyme via enhanced synthesis using aldosterone increases the required transport of ions in the kidney.

In the eye steroid and thyroid hormones produce altered protein synthesis, both positively and negatively, to affect cellular function by similar mechanisms. A practical example is the instance of increased lid retraction that was mentioned in the hyperthyroidism of Graves disease from the high levels of T_3 and T_4. Normally, T_3 and T_4 stimulate optimal metabolism (i.e., via enzyme synthesis) in all ocular cells. Although, clinically, both natural and synthesized steroid hormones are better known for their ability to inhibit inflammatory reactions in the eye (see Chapter 9), they can also produce some undesirable effects in ocular tissues.

In the cornea, an increase in corneal thickness can occur with the use of topically applied steroids just as it can with some steroids that may be present at elevated levels (Soni, 1980). The latter occurs, for example, with pregnant women who have high levels of estriol and pregnanediol (two steroids related in structure to estradiol and aldosterone). More serious, however, is the possible development of posterior, subcapsular cataracts in the lens with the prolonged use of steroids used to treat nonocular diseases such as rheumatoid arthritis. Mayman et al (1979), reported that the enzyme Na,K-ATPase is "inhibited" in lens tissues with the therapeutic use of dexamethasone (an aldosterone-like synthetic steroid). Presumably, (since it has not been adequately demonstrated) these steroids are acting to inhibit the synthesis of Na,K-ATPase by preventing the transcription of the mRNA for Na,K-ATPase. This action is exactly the opposite of the positive enhancement by aldosterone. In effect, some steroids can cause swelling of newly formed lens fiber cells just below the posterior capsule. This swelling represents the manifestation of a cataract due to the decreased ability of the cells to transport Na^+ and K^+ ions. It results in the osmotic inclusion of water. Other explanations for the swelling, such as the binding of steroids to lens proteins have been proposed (Urban, Cottier, 1986), but no satisfactory explanation about the pathological role of such binding has been made. Moreover, Dickerson, Dotzel, and Clark (1997) found that, although some natural steroids may bind to lens proteins, the small amount of steroid that reaches the lens would make the amount bound relatively insignificant in lens tissue. In fact, these investigators imply that a hormone-receptor mechanism is involved. Both Ogueta et al (1999) and

Stokes et al (2000) found evidence for the existence of steroid receptors in the nucleated cells of the lens. That lends credence to a steroidal, hormonal mechanism that could negatively control levels of, for example, Na,K-ATPase in lens tissues and may, therefore, be involved in a cataractogenic mechanism.

If synthetic steroids cause cataracts, why are cataracts not caused by temporary high levels of natural steroid hormones such as the example of pregnancy just given? Three reasons may be presented: the *specific effects of a particular steroid* on an enhancer, the level or *concentration of the steroid* in a tissue and the time or *period that a given steroid is present* in a tissue. Table 6–5 shows a comparison of three steroids with body fluid concentrations, time in those fluids and ocular effects. As can be seen, the length of exposure can be more meaningful than the concentration while the steroid type will determine the ultimate, ocular effect. Although the natural steroid pregnanediol can cause corneal swelling (and difficulties with contact lens wear), the condition is temporary. Extended use of the synthetic steroid prednisone, however, can produce permanent cataracts. The use of synthetic steroids can also bring about an elevation in the intraocular pressure. Although the mechanism for this is presently not completely understood, evidence has suggested that it is an enhancer mechanism (Jaanus, 1989). Recently, in relation to this, Clark et al (2001) have shown that the glaucoma gene *MYOC* may be induced by glucocorticoids in human and monkey trabecular meshwork cells. The induction of this gene also results in the increased synthesis of myocilin, a protein that may increase the outflow resistance in the trabecular meshwork (Polanksy, Fauss, Zimmerman, 2000).

Paracrine Hormones

Paracrine hormones are released by cells in the immediate vicinity of their site of action. This is why they are also termed local hormones. Their effectiveness is limited by the fact that they are very rapidly destroyed after being released. However, this limitation also permits much quicker control of the effects produced. Although *eicosanoids* (see Table 6–4) are the chief members of this class, some small peptides and amino acid derivatives seem to fall within this definition as well. An example of the latter is the substance *histamine* (derived from the amino acid: histidine). Histamine signals the release of white blood cells from blood vessels to an infected tissue. Here, however, we will concentrate on eicosanoids.

TABLE 6–5 ➤ STEROIDS, THEIR BODY FLUID CONCENTRATIONS, DURATION OF PRESENCE, AND OCULAR EFFECTS

Steroid	Present During	Fluid Level (μg/dL)	Duration	Ocular Effect
Cortisol	Lifetime	23[1]	Lifelong	None
Pregnanediol	Pregnancy	206[1]	24 wk[2]	Corneal swelling
Prednisone	Arthritis treatment	35[3]	192 wk	Cataracts
Prednisone	Ocular inflammation treatment	100[4]	<6 wk	None

[1]Levels approach this amount in blood plasma (see Tietz, 1986).
[2]Last 24 weeks of pregnancy.
[3]Levels in blood plasma for treatment of rheumatoid arthritis (see Stubbs, 1975).
[4]Levels in the aqueous humor of rabbits (see Schoenwald and Boltralik, 1979).

Eicosanoids are formed initially at the plasma membrane of the cell as shown in Figure 6–18. Those cells that make eicosanoids do so by cannibalizing arachidonic and other highly unsaturated, long-chain fatty acids from their plasma membranes. This is accomplished by breaking fatty acid ester bonds on phospholipids in the plasma membranes of the cell with the enzyme *phospholipase* A_2. This process may be enhanced by a linked receptor protein-G protein complex that activates the phospholipase A_2 more strongly than would occur without the complex. This means that an outside signaling molecule (not necessarily a hormone) can increase the production of eicosanoids. In the cytoplasm, the released fatty acid is cyclized, oxygenated, and then reduced by a multifunctional enzyme known as *prostaglandin synthase* (also called a *cyclooxygenase* or COX). This enzyme requires both molecular oxygen and a source of electrons to transform the fatty acid to the initial eicosanoid prostaglandin H. Significantly, prostaglandin synthase is inhibited by aspirin. Since some prostaglandins cause inflammatory responses, this explains how aspirin produces an anti-inflammatory effect when taken. It and similar inhibitors are called *nonsteroidal anti-inflammatory drugs* (NSAIDs). Other prostaglandins (and related compounds known as *thromboxanes*) are formed enzymatically from prostaglandin H. Still another enzyme, known as a *lipoxygenase*, forms an additional class of eicosanoids called *leukotrienes* from arachidonate and similarly released fatty acids. The leukotrienes are oxygenated, but not cyclized as the prostaglandins.

When prostaglandins and related eicosanoids are released to neighboring cells, they cause their effects on those cells by binding to a receptor protein and affecting the production of a second messenger. This is accomplished in the same manner as that of an endocrine hormone (see Figure 6–5). Likewise, the effects produced may be either stimulatory or inhibitory. There are at least 13 different prostaglandins and thromboxanes as well as 11 different leukotrienes that can be formed from three major, long-chain, unsaturated fatty acids (Mayes, 1985). A good basic discussion of the tissue biosynthesis of eicosanoids has been made by Piomelli (1996).

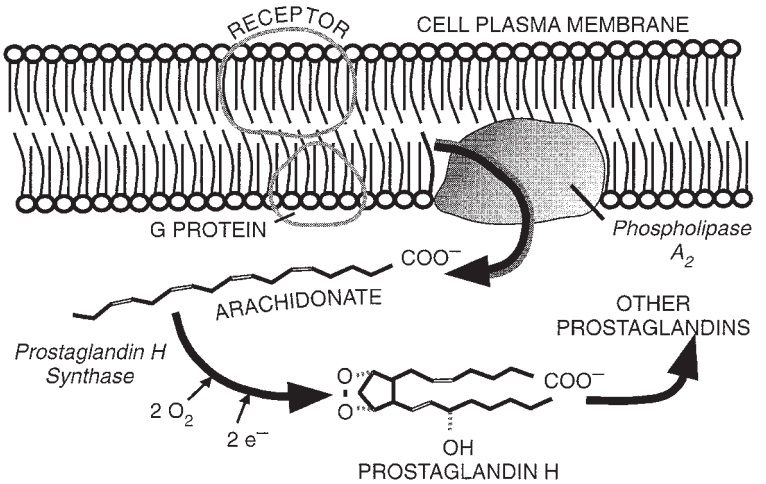


Figure 6–18
The formation of prostaglandins from plasma membrane lipids. ➤ Arachidonate and other highly unsaturated fatty acids are removed from membrane phospholipids by hydrolysis with phospholipase A_2. A receptor protein-G protein complex (outlines) may stimulate the phospholipase A_2. These fatty acids are cyclized, oxygenated and reduced by prostaglandin synthase, also known as prostaglandin endoperoxide synthetase or cyclooxygenase (an enzyme complex with both cyclooxygenase and peroxidase activities) to prostaglandin H. Note the requirement for oxygen and a source of electrons. The H form of the prostaglandin then becomes the substrate for catalysis to other prostaglandins.

The physiological effects produced by these hormones, as with the endocrine hormones, are quite varied. They include control of ionic composition of the blood and blood pressure, modulation of lung functions, influence on cell proliferation, control of the inflammatory reaction (Chapter 9), mediation of gastrointestinal functions, wound healing, and even control of endocrine functions themselves (Watkins et al, 1989). In the eye, eicosanoids are known to affect the function of virtually every ocular tissue (Bito, Stjernschantz, 1989). The principal synthetic pathways important to the eye for the prostaglandins are shown in Figure 6–19. However, the detailed mechanisms of many of the functions of these prostaglandins in the eye have not yet been adequately described. One important observation has been that prostaglandin F2 alpha ($PGF_{2\alpha}$) is able to lower the intraocular pressure of the eye (see, for example, Bito and Baroody, 1989). However, the mechanism for this action has not described although it has been isolated to the *uveoscleral outflow*. Moreover, the use of derivatives of this prostaglandin to treat glaucoma have been frustrated by the presence of side effects attributed

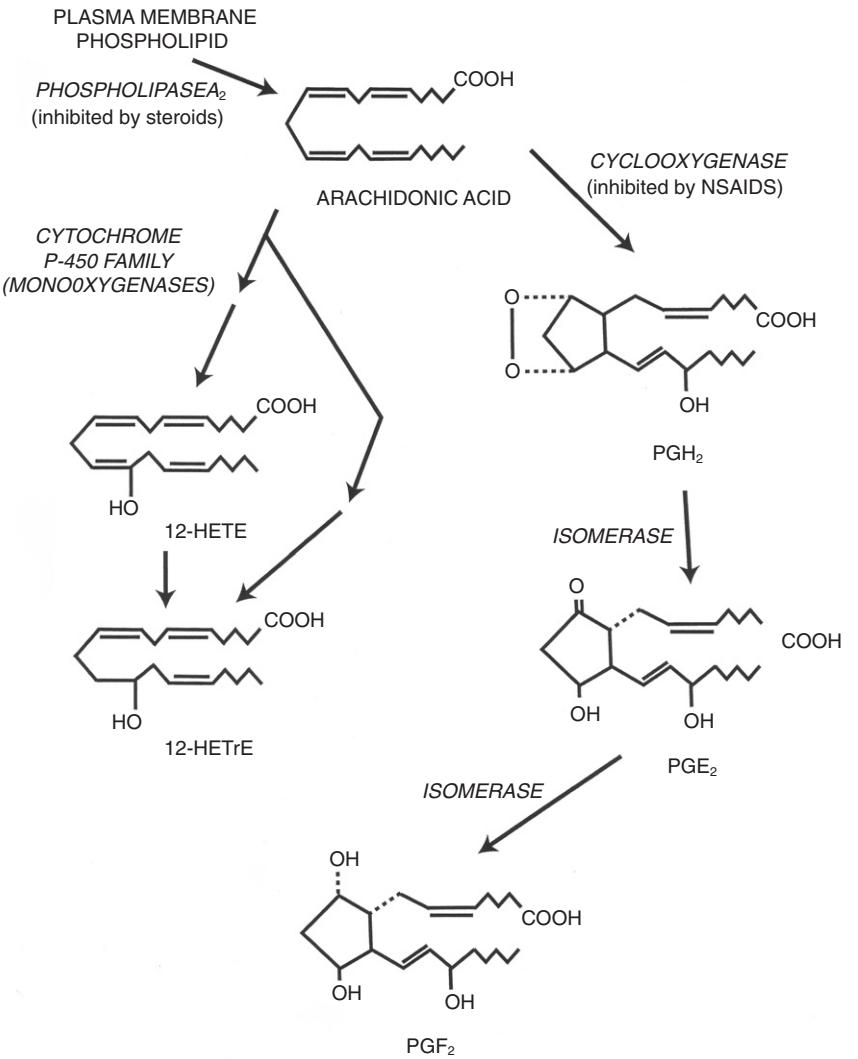


Figure 6–19
Some eicosanoid synthetic pathways. ➤ Numerous pathways exist for the synthesis of a variety of eicosanoids. Shown are relevant pathways for the eicosanoids discussed in the text. Arachidonic acid, freed from its membrane phospholipid component by phospholipase A, is converted to prostaglandin H_2 by the action of cyclooxygenase (prostaglandin synthase) as shown in the right hand pathway. Cyclooxygenase, along with other prostanoid isomerases, is present in the cellular smooth endoplasmic reticulum. Note that cyclooxygenase is inhibited by nonsteroidal anti-inflammatory agents such as aspirin. In the nomenclature for prostaglandins, *PG* stands for prostaglandin while the letters following such as *H, E, and F* refer to the different types of oxygen substitution on the cyclopentane ring on the left of the compound. The number *2* refers to the presence of two double bonds in the compound. In the left-hand pathway, the hydroxytetranenoic and hydroxytrienoic acids (HETE and HETrE) are formed by the action of cytochrome P-450, monooxygenases. They may also be synthesized by lipoxygenases. Both enzymes are also located in the smooth endoplasmic reticula of the cell and none of them are inhibited by anti-inflammatory drugs.

to the existence of prostaglandin receptors in other parts of the eye (Stjernschantz, 2001). A chemical analogue of $PGF_{2\alpha}$ (latanoprost) seems to be effective in 75% of glaucoma patients (Scherer, 2002).

Mittag (1989) has provided some evidence that the iris and ciliary muscles are relaxed by another prostaglandin, PGE_2. Much more recently, Kang et al (2000) demonstrated another effect in rabbit corneal endothelial cells in which PGE_2 can inhibit corneal epithelial cell division by a complex negative feedback pathway affecting *Ras*, a GTP-binding protein that normally causes increased cell division. Therefore, the overall effect of PGE_2 is to control cell division by overriding increased cell division. This initial increase in cell division is triggered by epidermal growth factor, a *cytokine* that also triggers the production of PGE_2. Therefore, epidermal growth factor both increases and, sequentially *via* PGE_2, decreases cell division of rabbit corneal endothelial cells. Whether the same effect occurs in human cells has yet to be shown.

In the retina, Vinores et al (1992) have demonstrated that PGE_2, can cause a breakdown in the blood-retinal barrier by opening the tight junctions between *vascular* endothelial cells. Such a process brings about macular edema. This fills the retinal macula with fluid and can cause visual blurring. Trauma to the retina can be a cause of the release of prostaglandins here. In fact, response to trauma is a common cause of prostaglandin release in ocular and nonocular tissues.

Recently, the activity of a class of eicosanoids known as *hydroxyeicosanoic acids* have been described in the cornea (Edelhauser et al, 1993; and Mieyal et al, 2000). These eicosanoids fall into two main groups: *12-hydroxyeicosatetraenoic acid* or *12-HETE* and *12-hydroxyeicosatrienoic acid* or *12-HETrE*. The metabolism of these compounds from arachidonic acid is shown in Figure 6–15. Note that HETE and HETrE are synthesized from the cytochrome P-450 family rather than the cyclooxygenases that synthesize prostaglandins. Cytochrome P-450 is a family of proteins with enzymatic activity and is present in the cell cytoplasm. The importance of these eicosanoids is their ability to: (1) inhibit Na,K-ATPase in corneal cells leading to corneal swelling (due to HETE); (2) induce chemotaxis as part of the *inflammatory response* (due to HETrE); and (3) initiate capillary proliferation in the usually blood vessel-free cornea (due to HETrE). At least in the rabbit, evidence shows that this is a response to a hypoxic state such as may occur with some types of contact lens wear.

SUMMARY

Hormones are chemical messengers that integrate the cooperative intercellular functions of humans and animals. They are concerned with such functions as growth, metabolism, sexual function, and inflammatory reactions. In the eye, hormones assist in the smooth functioning of the visual process. A hormonelike mechanism is involved in visual transduction. There are five chemical classes of hormones: peptides, derived amino acids, cyclic nucleotides, steroids, and eicosanoids. There are four functional classes of hormones: endocrine hormones that bind to a cellular surface, endocrine hormones that bind to DNA, intracellular hormones (second messengers), and paracrine hormones that bind to a

cellular surface. All hormones have receptor proteins either at the cell surface or within the cell cytoplasm. Receptor proteins at the cell surface react with G proteins and either activate (usually) or inhibit an enzyme that forms (or breaks down) either: (1) cyclic nucleotides; or (2) inositol trisphosphate/Ca^{+2} ions. This process activates or inhibits a cascade mechanism via the second messenger system. Receptor proteins in the cytoplasm bind to the hormone and carry it to the cell nucleus where the complex binds to a DNA enhancer. There are receptors for many types of hormones in the eye. Hormones in the eye cause a variety of responses including lid retraction, cessation of inflammation, uptake of glucose into cells (nourishment), and changes in intraocular pressure. It is also possible that the hormones can bring about corneal swelling, cataract formation, and inflammation. The summary of hormonal influences on the eye is dependent on the type, concentration, and duration of hormones present.

PROBLEMS ●

1. The half-life of a substance is defined as the period of time at which the amount of that substance is decreased by ½ of its original amount. If the half-life of the hormone insulin is approximately 4 min, approximately how much insulin would remain in the blood after 24 min if the initial concentration of insulin is 160 μg?

2. If one ingested a rather generous supply of chocolate candy, explain how this might affect the supply of ATP that is formed from the Embden-Meyerhof glycolytic pathway. [Hint: second messengers are involved.]

3. It is generally believed that phosphorylation reactions, catalyzed by protein kinase C, are involved in contraction mechanisms of the iris sphincter muscle. Vasopressin is a hormone that operates *via* protein kinase C, yet it does not seem to have any effect on the iris. Suggest an explanation for this.

4. A patient is found to have a rare genetic disease in which both arrestin and rhodopsin kinase are deficient. What kind of visual symptoms would you think that the patient might have? Explain your answer.

5. Table 6–5 indicates that prednisone, a synthetic steroid hormone may cause cataracts if given over a period of just over 3.5 years at a level of 35 μg/dL. However, the same steroid given for under 0.11 year (6 weeks) at a level of 100 μg/dL has no effect. How would one establish safe levels and times for prednisone administration to avoid cataract formation.

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CHAPTER 7

Nucleic Acids

Nucleic acids exist for two primary biochemical functions: to maintain the code for the amino acid sequence for all of the proteins contained in a cell and to synthesize those proteins. The study of these functions, known as molecular biology or nucleic acid biochemistry, is one of the most rapidly growing areas of biological research. This is due both to the importance of the roles of nucleic acids and the continuing technological developments of this area. Both multicellular and unicellular organisms rely on the operations of nucleic acids to determine growth, division, specialized functions, development, and hereditary characteristics. In addition, the control and reactivity of nucleic acids is at the center of bacterial and viral infections as is the uncontrolled cellular division which we know by the general term *cancer*. There are also many metabolic diseases that can be traced to enzyme defects caused by some DNA based, hereditary problem. Although there had been a delay in ocular investigations in this area, rapid progress is now being made.

Nucleic Acid Biochemistry

DEOXYRIBONUCLEIC ACID

Two forms of nucleic acids are found in nature: deoxyribonucleic acid (DNA) and ribonucleic acid (RNA). DNA is the nucleic acid form that preserves the code for making all the proteins needed by a cell. RNA translates that code into specific proteins. DNA and RNA have certain chemical attributes in common. They are polymers of nitrogen containing hydrophobic bases (adenine, guanine, cytosine, and thymine [or, in RNA, uracil in place of thymine]) that are joined together by hydrophilic pentose phosphates (Figure 7–1).

When the pentoses, deoxyribose (in DNA) and ribose (in RNA), are each joined to a base, the compound is known as a *nucleoside* and the four bases so joined are called adenosine, guanosine, cytidine, and thymidine (or, in RNA, uridine), respectively. The prefix *deoxy* is used if the sugar is a deoxypentose. In that case, the 2′-hydroxy group is replaced

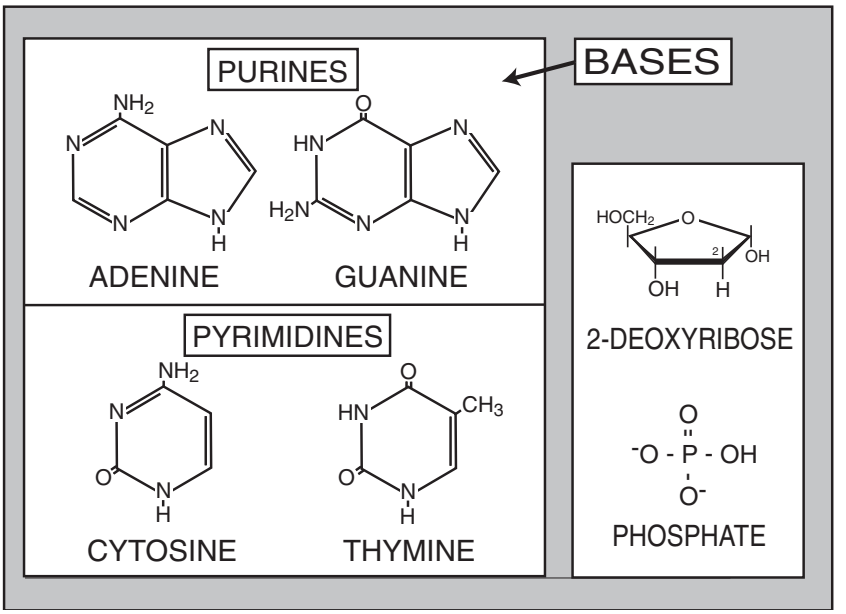


Figure 7–1
The chemical components of nucleic acids. ➤ The bases (heterocyclic rings containing nitrogen) consist of two purines and two pyrimidines. In RNA, thymine is replaced by uracil, deoxyribose by ribose (see Figure 7–10).

by hydrogen. When a phosphopentose, such as deoxyribose phosphate, is joined to a base, the compound is known as a *nucleotide* and the four bases, as just given, add the word phosphate to their name, such as, deoxyadenosine 5′-triphosphate (dATP), adenosine 5′-triphosphate (ATP), guanosine 3′,5′-cyclic monophosphate (cGMP; see Chapter 6) and deoxycytidine 5′-monophosphate (dCMP). These are shown in Figure 7–2. Only the first example would be incorporated into a molecule of DNA. The second example either may act as a source of cellular energy (see Chapter 4) or be incorporated into a molecule of RNA. Cyclic GMP, the third example, acts as an intracellular hormone and deoxycytidine 5′-monophosphate, the fourth example, is a breakdown product of DNA.

The 2′-deoxynucleotides of the four bases (see Figure 7–1) are the only nucleotides incorporated into DNA. In order to be incorporated, they must first exist in the triphosphate form. Biochemically, they become bound to DNA by the catalytic activity of a *polymerase* enzyme. In the process, a diphosphate group is removed from the deoxynucleotide triphosphate. Synthesis proceeds with the addition of the 5′-phosphate end of the added nucleotide to the 3′-end of the growing chain and may be seen in Figure 7–3.

Several features about the DNA chain, as seen in Figure 7–3, should be noted. The DNA, once made, is double-stranded in *eukaryotes*. That is, in cells having a nucleus. The bases are held together by hydrogen bonds: two bonds between adenine (A) and thymine (T); and three bonds between guanine (G) and cytosine (C). The bases themselves are hydrophobic while the deoxypentose phosphates are hydrophilic. Once formed, the double-stranded DNA (or duplex DNA) forms into a helix with the hydrophobic bases held internally and the deoxypentose phosphates located on the exterior of the helix. Helical DNA is a structure that was predicted by Watson and Crick in 1953. This DNA helix, as shown in Figure 7–4, is known as the B-form. An A-form and a Z-form also occur (Wells et al, 1988). These latter two forms are used by cells in special circumstances. Their structures are beyond the scope of this text, but may be seen in Mathews and van Holde (1990).

Typically, on a strand of duplex DNA, one chain contains the code for the synthesis of specific proteins while the other chain contains

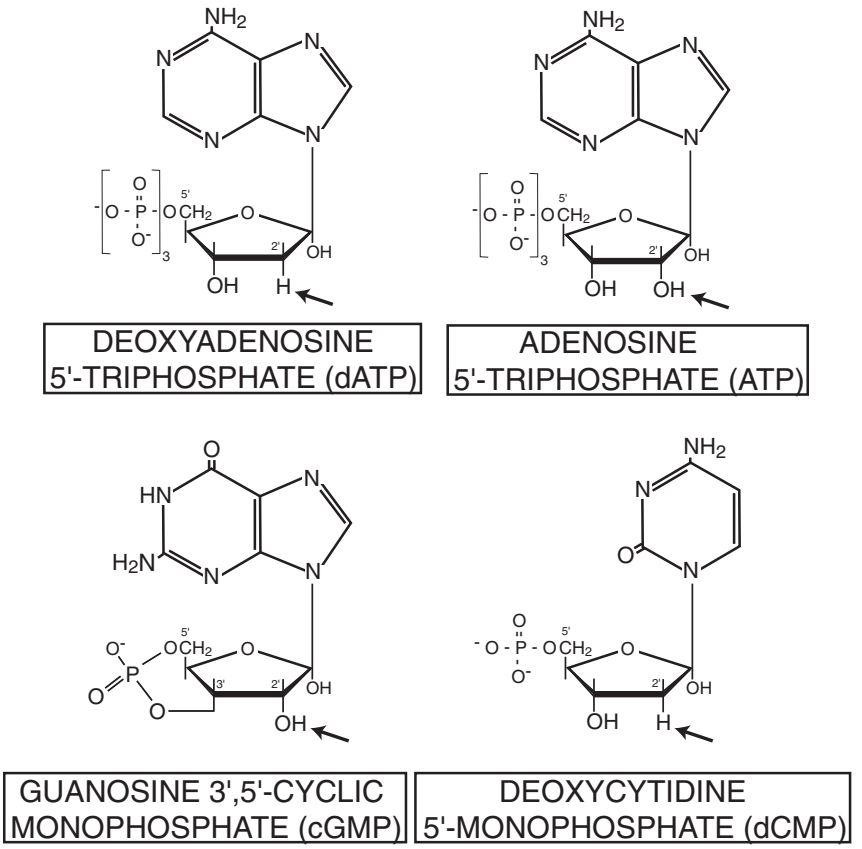


Figure 7–2
Four examples of nucleotides. ➤ The two triphosphates at the top are precursors for incorporation into DNA (*left*) and RNA (*right*). The bottom example (*left*) is not incorporated into nucleic acids but serves as an internal hormone (Chapter 6). The bottom example (*right*) is a breakdown product of DNA. The *arrows* indicate whether each example is hydroxylated or not in the 2′-position of the pentose.

the template or complement of the code. The code itself consists of the sequence of sets of three bases in the chain. Accordingly, three bases in succession code for a single amino acid in a protein. It should be noted that some DNA does not code for any amino acids. Such DNA takes on special roles within the genome of the cell. The genetic code will be taken up in more detail later in this chapter. Human cellular DNA is divided in each cell into 46 *chromosomes*. Each chromosome is actually a super folded complex of duplex DNA and associated proteins. One chromosome contains a single, large duplex DNA molecule when the cell is not dividing. The sum of all the chromosomes in a cell constitutes the *genome* of the cell. Each duplex DNA molecule, as stated, contains a very large number of base pairs. The total number of base pairs in a human cell genome is about 6×10^9 (Alberts et al, 1989a). This is the reason that duplex DNA molecules are highly compacted and folded in the nucleus of the cell. If this were not the case, human DNA from a single cell could be strung out to a length of nearly nine feet (Mathews, van Holde, 1990)! The human chromosomes typically seen in most biology texts are actually two chromosomes joined by a *centromere* at *metaphase*. A centromere is a centrally located, specific DNA sequence required for cell division. At metaphase, a seemingly single chromosome (two joined chromosomes) is called a *chromatid* and is shown in Figure 7–5. The chromatids are composed of super-coiled structures that may be unraveled to show lengths of chromatin fibers. These fibers

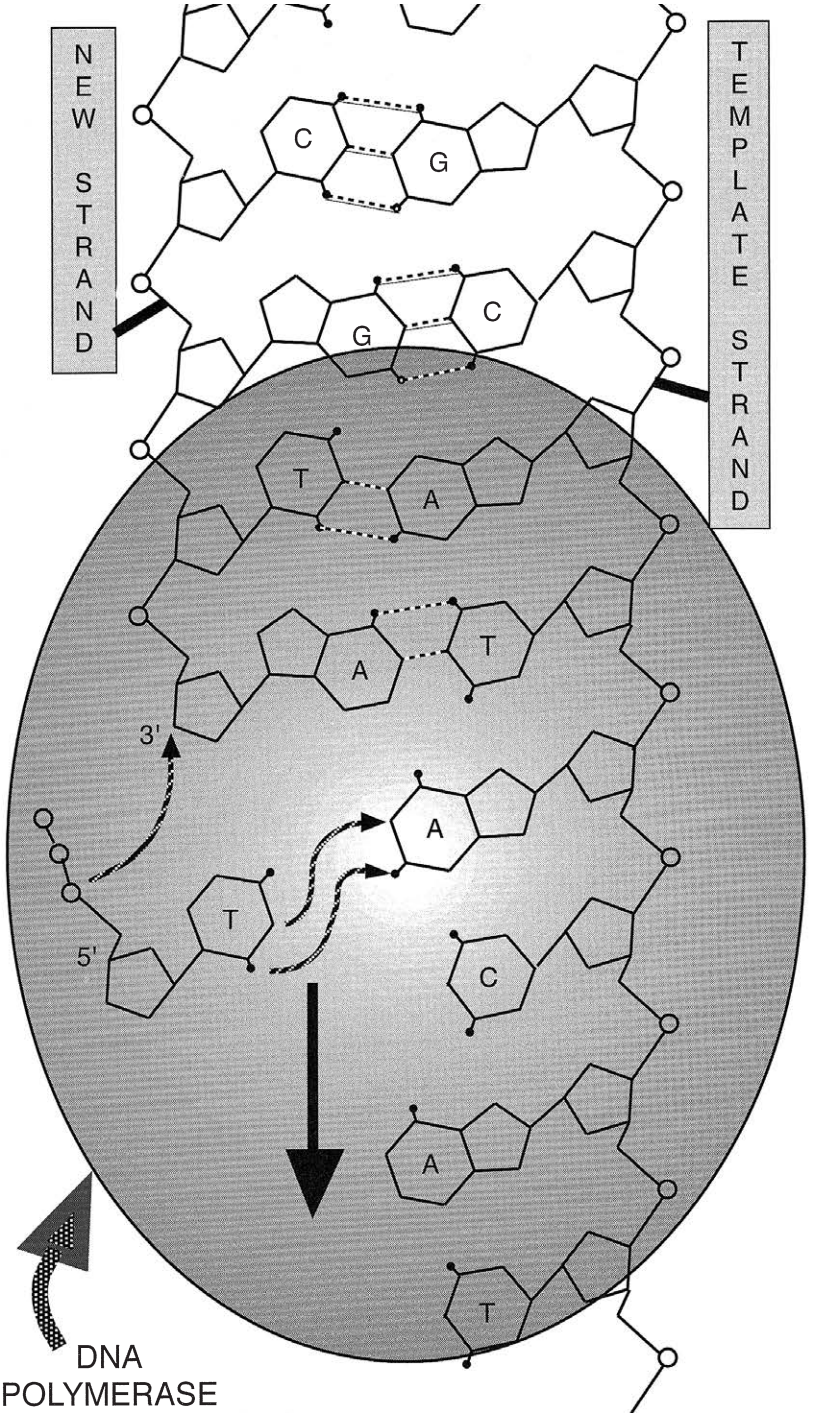


Figure 7–3
DNA replication by DNA-directed DNA polymerase. ➤ The enzyme forms 5′→ 3′ bonds with each incorporated base. The hydrogen bonds A-T and G-C form spontaneously after 5′→ 3′ bonding where the black and white lines are shown. Each triphosphate nucleotide loses a diphosphate group in the catalytic process. The base to be added is determined by the complementary, opposite base on the template strand.

consist of duplex DNA wrapped around the barrel-like structure of histone proteins. Strands of DNA connect each barrel while other nonhistone proteins are bound to the DNA at these strands. Histone proteins assist DNA in compacting itself while nonhistone proteins are involved with the control of DNA and RNA processing (Figure 7–6).

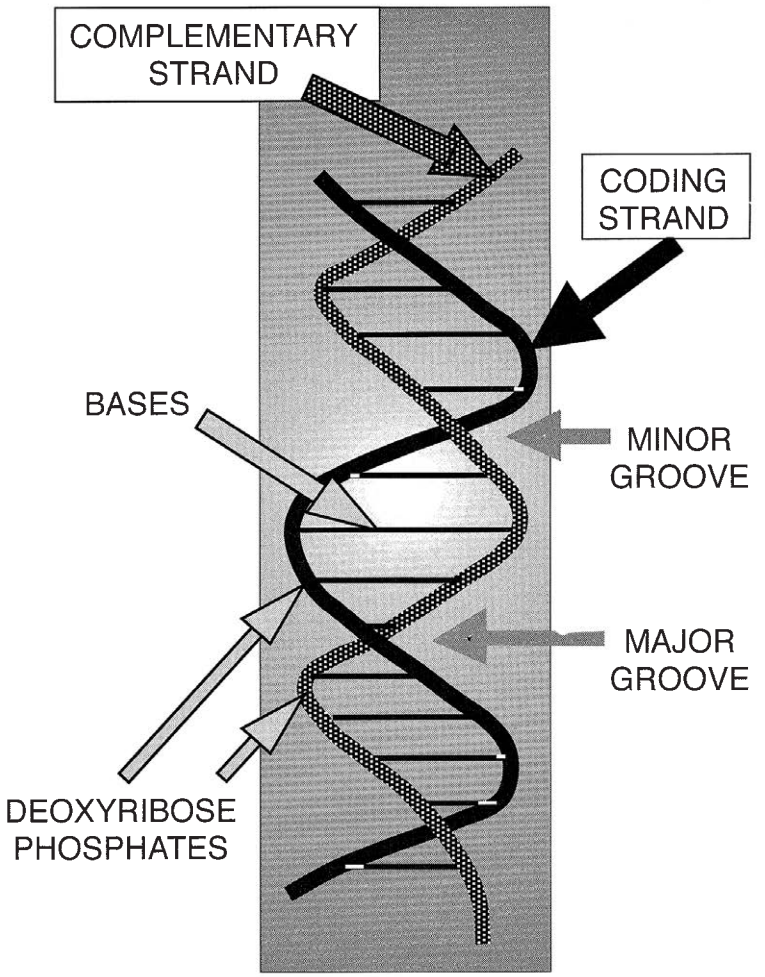


Figure 7–4
A representation of the B-form of the double-helical structure of DNA (duplex DNA). ➤ This is its most common form. Note the presence of both a major and a minor groove in the helix. Functional proteins bind to the DNA at its major groove.

DNA REPLICATION

When a cell divides, it reproduces its DNA by a process known as *replication*. Replication is semiconservative. This means that one original strand of the "parent" duplex DNA becomes incorporated into each of the two new "daughter" duplex DNA molecules. This is to say that one coding strand and one complementary strand of each of the two new strands came from the original DNA before division.

Since the DNA in each chromosome is so long, replication takes place in several locations at one time. It requires many proteins to carry out replication. Some of these proteins, such as DNA polymerase, are enzymes. Each location of active replication is referred to as a *replication fork* in which the parental DNA is the stem and each daughter DNA is a tine of the fork. Several requirements for successful replication make this process complicated. For example, the helix must be unwound before replication and the synthesis of each strand must proceed from the 5′-end of each added nucleotide to the 3′-end of each growing strand. This may not seem complicated intuitively. However, when one realizes that each duplex DNA has its member strands oriented in opposite 5′→3′ directions, a paradox becomes apparent if replication can only proceed in one direction. The problem is solved by the genetic machinery of the cell (Figure 7–7). The figure shows a

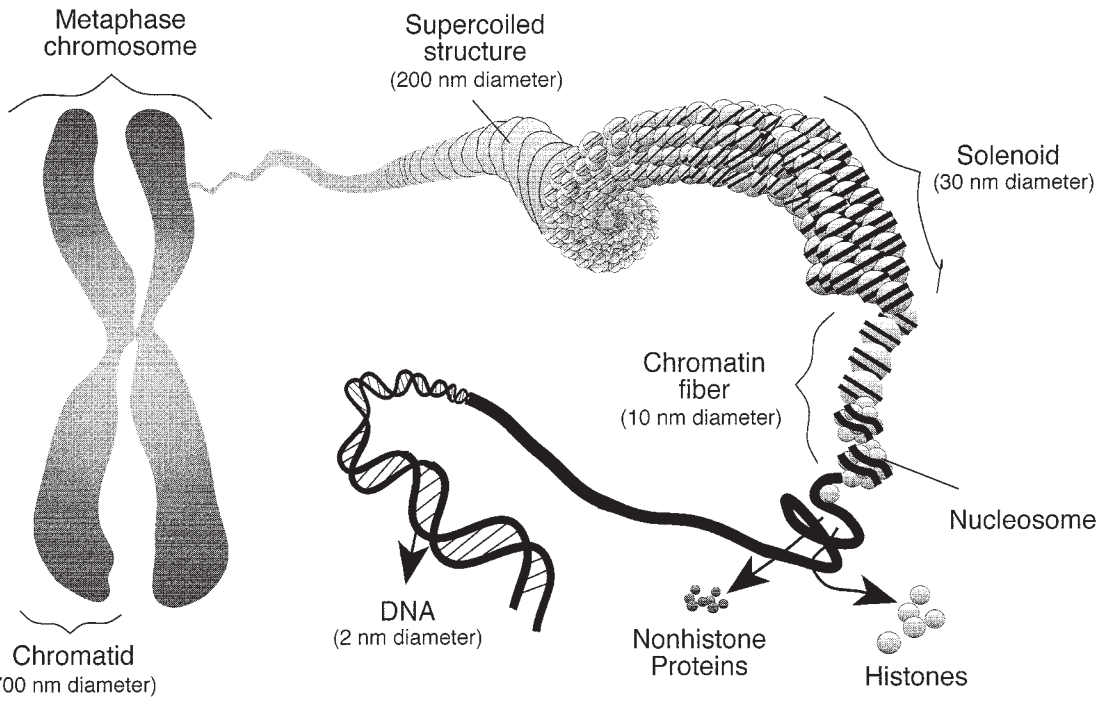


Figure 7–5
Unraveling a metaphase chromosome (two duplex DNA molecules or chromatids). ➤ The supercoiled structures can be untwisted to show chromatin fibers containing nucleosomes (histones and wound DNA) joined by linker DNA. The nonhistone proteins are bound to DNA in between each nucleosome.

Figure 7–6
The appearance of chromatin fibers (Figure 7–5) in greater detail. ➤ H1 histones appear to stabilize the DNA on the nucleosome.

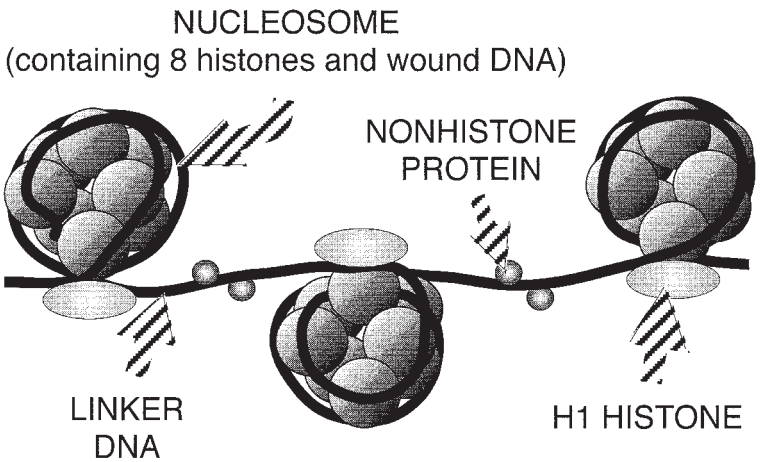


diagram of a replication fork with parental DNA on the left. In the top daughter duplex DNA, the new strand is synthesized in the conventional 5′→3′ direction. This strand is known as the *leading strand*. In the bottom daughter duplex DNA, the new strand is made in discrete, short discontinuous fragments (5′→3′) known as *Okazaki fragments*, named for Reiji Okazaki, the scientist who discovered them (Ogawa, Okazaki, 1980). Each short fragment in the lagging strand (although made 5′→3′) is successively formed backwards in the same direction as that of the opening fork, that is, in the same direction as the growing, leading strand. The fragments are then joined by a *ligase* enzyme and

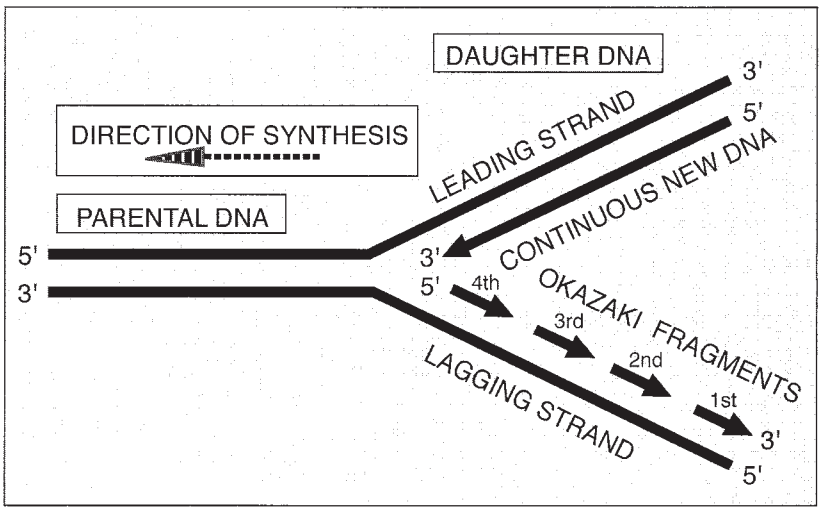


Figure 7–7
Sequence scheme of daughter DNA on the leading and lagging strands. ➤ The numbers on the Okazaki fragments (first through fourth) indicate the order of synthesis of each fragment in time while the arrows indicate their 5′→ 3′ sequence.

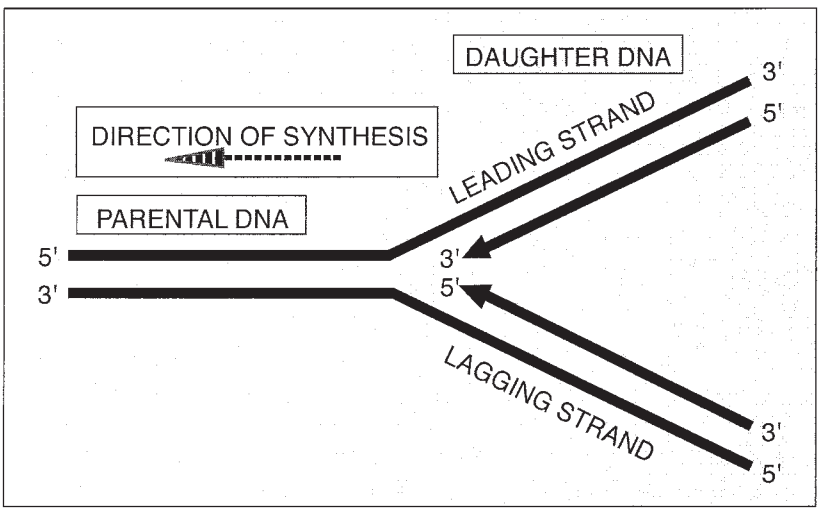


Figure 7–8
The overall effective sequence of synthesis of both the leading and lagging strands.

become a continuous strand. In effect, the synthesis of each strand appears as though it was unidirectional (Figure 7–8). The molecular assembly of a replication fork may be seen in Figure 7–9. As can be seen in the figure, several proteins contribute to the replication process. *DNA helicase*, an enzyme, breaks the hydrogen bonds between the bases to form single strands. Bound to the DNA helicase is a second enzyme: *RNA primase*. This enzyme periodically synthesizes short strands of *primer RNA* that bind to one of the separated parental strands. One of the primer strands becomes the template for the new *lagging strand* and the RNA primer becomes the initiator for that lagging strand. It is important that this parental strand remains a single strand at this stage. For this reason, *double-helix-destabilizing proteins* temporarily bind to it. The parental template for the lagging strand loops around and passes through a *DNA polymerase III* enzyme complex that is actually two polymerase enzymes. The lower polymerize III enzyme synthesizes new DNA bound to the RNA primer to form

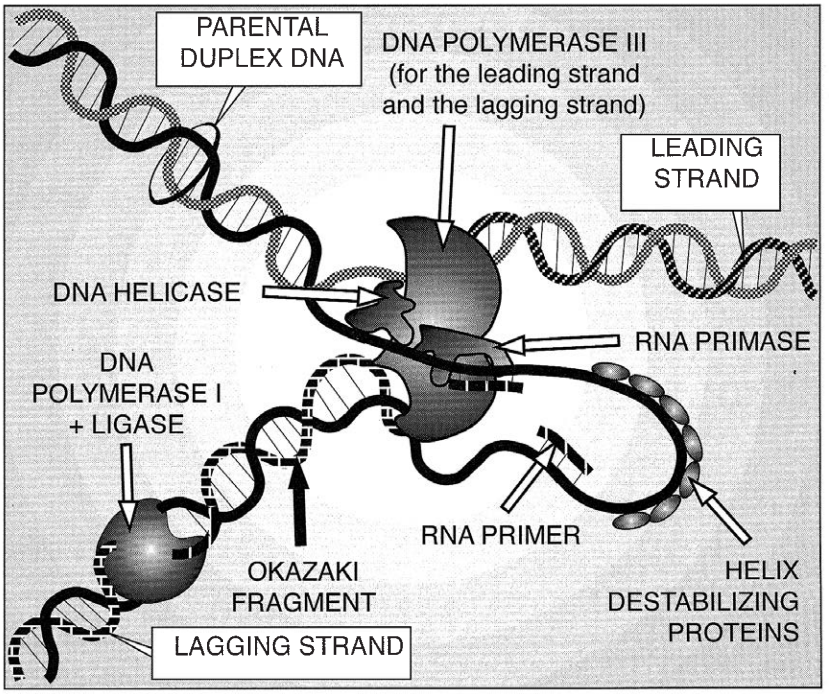


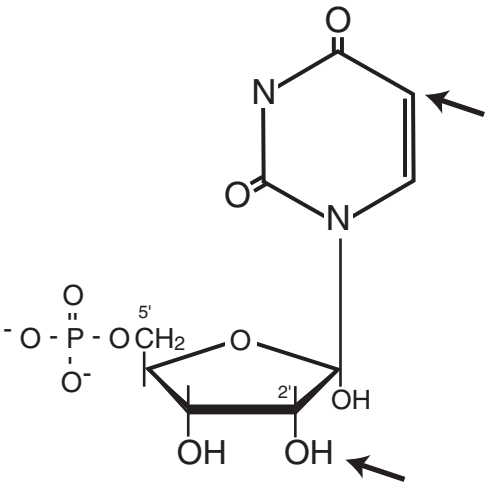
Figure 7–9
The actual appearance of a replication fork. ➤ *White arrows* point out the proteins involved in replication. Although similar to Figures 7–7 and 7–8, it may be seen that the parental DNA forming the lagging strand template must first loop back toward the lower DNA polymerase III molecule before an Okazaki fragment can be formed. For this reason, helix-destabilizing proteins prevent premature helical formation.

an Okazaki fragment on the lagging strand, while the other upper polymerase III forms the new DNA chain for the leading strand. A little further out on the lagging strand a complex of *DNA polymerase I* and *ligase* hydrolyze the RNA portion of the primer and complete the gaps between the DNA Okazaki fragments to form a continuous strand. The ligase enzyme binds the last nucleotide to join the fragment with the new growing strand.

RIBONUCLEIC ACID

Ribonucleic acid, chemically, is similar to DNA except for two important differences: (1) *ribose* replaces deoxyribose as a pentose; and (2) *uracil* (as uridine ribose monophosphate) replaces thymine as a base in most cases. Uridine 5′-monophosphate is shown in Figure 7–10 and its structure should be compared with the bases in Figure 7–1. As a rule, RNA is single-stranded, but there are notable exceptions with regions of both transfer and ribosomal RNA (to be discussed) as well as with some RNA containing viruses.

In eukaryotic cells, the processes of producing proteins require the existence of four different kinds of RNA. These are listed in Table 7–1. *Heterogeneous nuclear RNA (hnRNA)* is the initial coding RNA product made from DNA. *Messenger RNA (mRNA)* is a cellular refinement of hnRNA, made in the nucleus, and represents the form of RNA that carries the exact code for protein sequencing. It is the form used in the process of protein synthesis known as *translation*. It is interesting that this RNA form comprises only 3% of the total RNA present at a given time. Such a low value indicates the strict control necessary for the rate of protein synthesis. *Transfer RNA (tRNA)* is a short chain RNA that attaches to specific amino acids and transports them to *ribosomes* where proteins are synthesized. *Ribosomal RNA (rRNA)* represents 75% of all



URIDINE
5'-MONOPHOSPHATE (UMP)

Figure 7–10
The RNA nucleotide, which is different from its corresponding DNA nucleotide (Deoxythymidine 5′-monophosphate, Figure 7–1). ➤ Uridine lacks a methyl group (*top arrow*), which is present in thymine. The ribose has a 2′-hydroxy group (*bottom arrow*). This is the incorporated form. The precursor form is triphosphorylated.

TABLE 7–1 ➤ TYPES OF RNA USED IN PROTEIN SYNTHESIS[1]

Type	Approximate Size (Base Pairs)	Percentage in Cell %	Function
heterogeneous nuclear (hnRNA)	8000	7	Nuclear precursor of mRNA
messenger (mRNA)	1200	3	Carries the actual code for a protein
transfer (tRNA)	80	15	Attaches amino acids to a protein being synthesized
ribosomal (rRNA)	5000, 2000, 160, 120	75	Possibly catalytic for protein synthesis

[1]Some hnRNA has as many as 20,000 base pairs. The mRNA size given is an average size needed to make a protein of 400 amino acids. The tRNA ranges from 70 to 90 base pairs. The tRNA consists of four types, but their role has not been shown conclusively. (See Alberts et al, 1989, p. 219.)

RNA in a cell and about 66% of the mass of a ribosome where it resides. Presently, the specific roles of rRNA in protein synthesis are not well understood. However, one of these roles is strongly postulated to be catalytic (Alberts et al, 1989a).

TRANSCRIPTION OF RNA

The synthesis of RNA from DNA is called *transcription*. In this process, DNA serves as the template for new RNA formation. The RNA is made by the catalytic activity of three different RNA polymerases that are more properly called *DNA-directed RNA polymerases*. When hnRNA

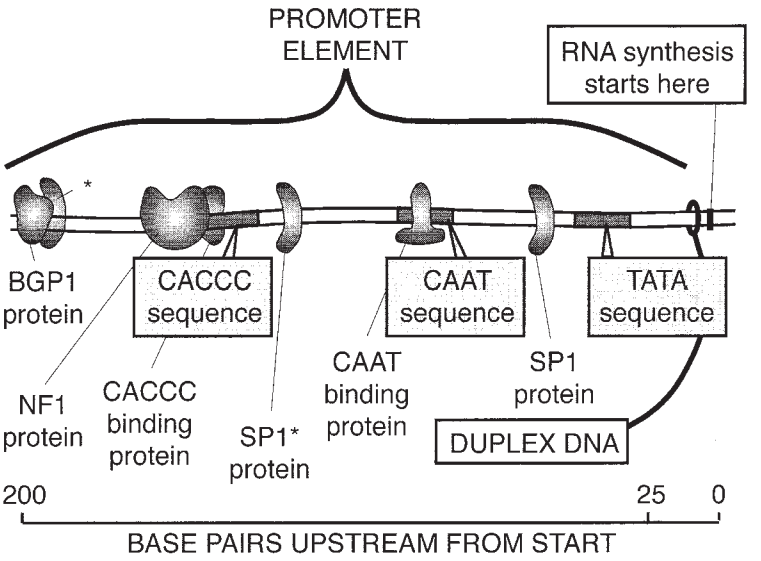


Figure 7–11
An "upstream" promoter element of DNA used to control the synthesis of hnRNA for β-globin in chickens. ➤ Three control sequences, shown in boxes, act together along with the proteins BGP1, NF1, CACC binding protein, CAAT binding protein and SP1 to turn RNA synthesis on or off. These proteins may even control the rate of synthesis.

is formed, it is the first step in protein synthesis. The polymerization is similar to DNA polymerization (replication) using nucleotide triphosphates as building blocks as in Figure 7–3. However, uridine triphosphate replaces deoxythymidine triphosphate. The three RNA polymerases are: RNA polymerase I (for rRNA synthesis); RNA polymerase II (for hn, and ultimately mRNA synthesis); and RNA polymerase III (for tRNA and the smaller species of rRNA).

Transcription can only begin at a *promoter site* on DNA. This site is a device for rigidly controlling protein synthesis by limiting the amount of RNA that is available. A promoter site contains specific short sequences of DNA such as *TATA* and/or *CAAT*. These sequences do not code for any amino acids. Numerous regulatory proteins bind to this region and either support or inhibit the initiation of hnRNA synthesis. The entire region is called an *upstream promoter element*. Figure 7–11 shows such a promoter element of RNA for the eventual synthesis of β-globin proteins in chickens. The proteins in this region bind to the DNA at their major grooves (see Figure 7–4) at linker DNA sites between nucleosomes (see Figure 7–6). When RNA polymerase binds to the promoter element, it will either proceed downstream (5′→3′) on the DNA to begin RNA synthesis or stop depending on the combined influences of the proteins gathered at the TATA, CAAT, or other DNA promoting sequence regions. In addition, there are auxiliary regions of DNA, called *enhancers*, which have further influence on hnRNA synthesis. These enhancers, as well as the appropriate promoter sites, are bound by steroidal and thyroid hormone complexes and were discussed in Chapter 6. A diagram of the operational characteristics for the control of RNA synthesis is outlined in Figure 7–12.

THE GENETIC CODE AND "NONSENSE" SEQUENCES

Previously, it was mentioned that a sequence of three bases will code for a single amino acid in a protein. A number of well-known scientists, beginning with Francis Crick (1958), worked for nearly a decade to determine the code and its mechanism. The code is shown in Table 7–2

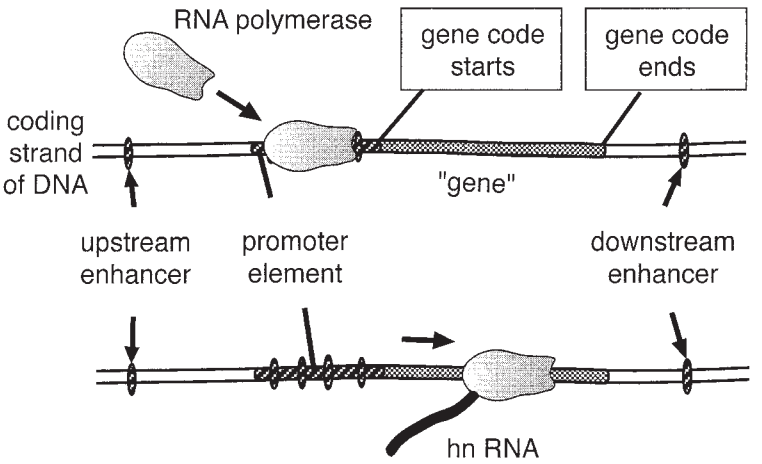


Figure 7–12
RNA polymerase must pass through the promoter element before beginning synthesis of a specific "gene" of hnRNA. ➤ If the right combination of promoter elements are not in place, synthesis cannot begin. The enhancers assist this process (see Chapter 6).

TABLE 7–2 ➤ THE GENETIC CODE FOR PROTEIN SYNTHESIS[1]

UUU Phe	UCU Ser	UAU Tyr	UGU Cys
UUC "	UCC "	UAC "	UGC "
UUA "	UCA "	UAA Stop	UGA Stop
UUG "	UCG "	UAG Stop	UGG Trp
CUU Leu	CCU Pro	CAU His	CGU Arg
CUC "	CCC "	CAC "	CGC "
CUA "	CCA "	CAA Gln	CGA "
CUG "	CCG "	CAG "	CGG "
AUU Ile	ACU Thr	AAU Asn	AGU Ser
AUC "	ACC "	AAC "	AGC "
AUA "	ACA "	AAA Lys	AGA Arg
AUG Met	ACG "	AAG "	AGG "
GUU Val	GCU Ala	GAU Asp	GGU Gly
GUC "	GCC "	GAC "	GGC "
GUA "	GCA "	GAA Glu	GGA "
GUG "	GCG "	GAG "	GGG "

[1]This is the code for RNA (DNA uses T wherever U occurs). AUG acts as the code for both "start" and Met. UUG and GUG occasionally are start signals. UAA, UAG, and UGA code always and only for "stop."

for mRNA. It is obvious that the code is redundant, that is, more than one code of three bases can signal for the same amino acid. Note also that three of these codes signal either synthesis initiation or the incorporation of an amino acid. The three stop codes specify only termination of synthesis.

On both DNA and RNA, there are base sequences that do not code for any amino acids nor for any start or stop signal (Lodish et al, 2000). Surprisingly, a majority of DNA is of this type and is used in a variety of noncoding roles: (1) as spacer molecules to connect coded regions of gene sequences (*introns*); (2) to signal and control transcription (*promoters, enhancers*); (3) to attach to microtubules during mitosis and meiosis (*centromere*, see Figure 7–5) and; (4) to record the number of cell divisions as an aging marker while preserving chromosome integrity (*telomeres*) (see Blackburn, Greider, 1995). Some examples on DNA are

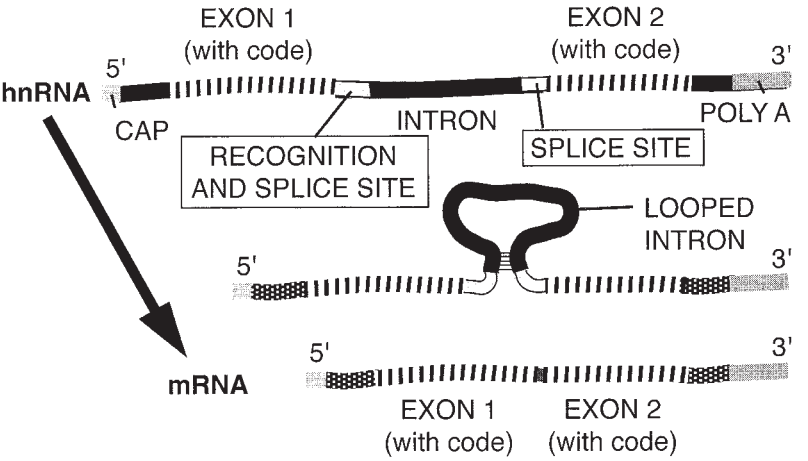


Figure 7–13
The processing of hnRNA in the cell nucleus. ➤ Introns, noncoding spacers, are looped out of the RNA sequence by small ribonuclear protein complexes called spliceosomes. The coded RNA sequence remaining is mRNA.

the recently mentioned sequences TATA and CAAT that are located at promoter elements. Other highly repeated sequences such as $(TTAGGG)_n$ occur in mammalian telomeres. In both DNA and hnRNA, gene sequences that code for either all or part of a protein are known as *exons*. Each exon is separated by a noncoding sequence known as an *intron* (mentioned previously). Introns, acting as spacers, are eventually looped out of a coding sequence prior to protein synthesis.

THE FORMATION OF MESSENGER RNA

As previously mentioned, when hnRNA is synthesized in the nucleus, it usually contains two or more coding *genes* (exons) that are separated by one or more noncoding regions (introns). Heterogeneous nuclear RNA, however, is a collection of pre-RNA, nRNA, and a vast collection of ribonucleoproteins that are involved with pre-RNA being processed to mRNA (Lodish et al, 2000). For practical purposes, we will consider here that hnRNA = pre-RNA. Processing to mRNA begins quickly with hnRNA acquiring a "cap" of guanosine triphosphate at its 5′-end and a "tail" of 100 to 200 bases of adenosine phosphate (polyadenosine) at its 3′-end. The cap has two functions. It protects the RNA from degradation and it is involved with initiation of protein synthesis. The tail may also play a role in preventing degradation. The process of mRNA formation from hnRNA is one of looping out the introns and splicing the exons together. This is illustrated in Figure 7–13. The looping-out of introns is aided by specialized small nRNA as well as numerous small nuclear proteins and involves two transesterification reactions, which break the intron-exon phosphate bonds in order to form a new exon-exon phosphate bond. After mRNA is formed, it is transported out of the nucleus.

PROTEIN SYNTHESIS (TRANSLATION)

The formation of a polypeptide (a protein by definition if the molecular weight exceeds 10,000) is a cooperative process between mRNA, tRNA, and ribosomes as well as specialized proteins. Protein formation from mRNA is known as *translation*. Since mRNA has just been discussed, let us focus on tRNA. This small RNA molecule (see Table 7–1) consists of extensive base-pair binding (double helix formation), looping (cul-

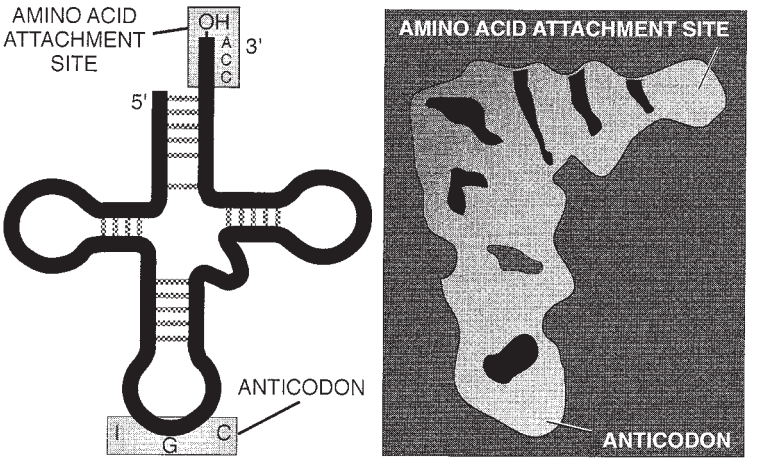


Figure 7–14
Left side: Functional diagram of the tRNA for the amino acid alanine. ➤ The anticodon I (inosine), G (guanine), C (cytosine) will match the mRNA code: GCU, GCC, or GCA (three of the four codes for alanine). Inosine is one of several unusual bases found on tRNA. *Right*: structural diagram of the actual twisted, partially helical shape of tRNA.

de-sacs), an amino acid attachment site, and a site for binding to a three base codon of mRNA. The latter site is referred to as an *anticodon*. This is represented diagrammatically in Figure 7–14 (*left* side). However, the actual shape of the molecule, due to helical folding, resembles a human liver (Figure 7–14, *right* side). As shown in the figure, the important functional regions that contribute to protein synthesis are the anticodon (the three bases that match three bases on mRNA, known as the *codon*) and the 3′-end, which binds to a specific amino acid. The 3′-end is also known as the *acceptor stem*. Transfer RNA also makes use of several unusual bases, such as *N,N′*-dimethyl guanine. These unusual bases are thought to facilitate the twisted conformation of tRNA so that it may carry its amino acid to a codon site on a ribosome (Alberts et al, 1994). During protein translation, tRNA first binds to its amino acid and then enters the ribosome to attach to the codon portion of mRNA with its anticodon site.

The ribosome itself is a two-part assembly with each part composed of both proteins and rRNA. The smaller subunit (with a mass equivalent to 40 *Svedberg units or 40S*) houses the mRNA while the larger subunit (60S) houses the incoming tRNAs and their amino acids. Protein synthesis occurs as one or more ribosomes move along the mRNA chain and simultaneously synthesize a polypeptide. This occurs as each tRNA, with its amino acid, binds to mRNA in succession. As each amino acid comes into the 60S subunit, it forms a peptide bond with the previous amino acid and the peptide chain lengthens (Figure 7–15). The peptide bond is formed by the catalytic activity of the 23S rRNA located on the ribosome and is referred to as *peptidyl transferase activity* even though there is no actual peptidyl transferase enzyme or protein (Lodish et al, 2000). This is an example of a nucleic acid having catalytic activity!

The ribosome continues to move along the mRNA chain as each peptide bond is formed until all of the code is "read." Although rates vary, typically 10 proteins can be formed from one mRNA molecule in one minute (Alberts, 1989a). In eukaryotic cells about 12 separate, associated proteins are required for the initiation of the translation process.

In somewhat more detail, protein synthesis may be described as existing in five stages: *initiation*, *elongation-1*, *elongation-2*, *elongation-3*, and *termination*. The initiation stage is one in which the ribosome is

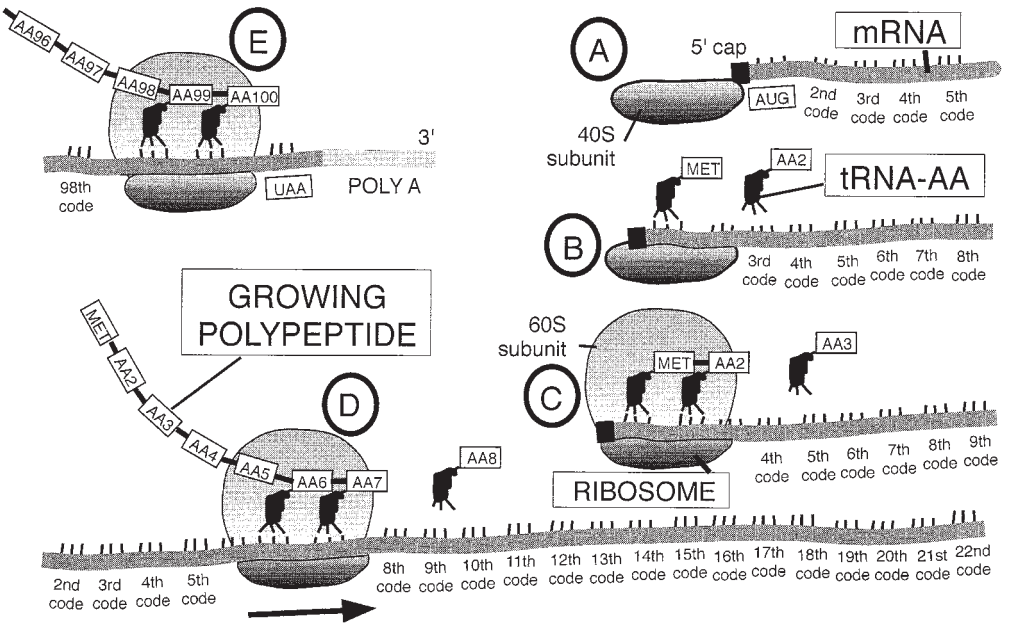


Figure 7–15
The process of translation. ➤ Initially, at A, the 5′cap of mRNA makes contact with the 40S subunit of a ribosome and the first code (AUG) of mRNA signals the beginning of protein synthesis as the tRNA for methionine binds to mRNA at B in the figure. Other tRNAs bearing amino acids (AA) are nearby. At C, the 60S subunit binds forming a complete ribosome. tRNAs with AAs enter the ribosome and bind to the proper codons (codes) on mRNA. As they do, a peptide bond is formed between each AA in sequence. The ribosome shifts to the next codon of mRNA and the process continues as one can see for the seventh AA at D. At E, the ribosome approaches the codon UAA, which will bind to releasing factors (proteins) that cause sequence termination and release of the polypeptide (in this case of 100 AAs, a protein with a molecular weight of approximately 12,500 daltons).

assembled in response to its binding to mRNA and the first tRNA (the one for Met). The t-RNA^{Met} binds to the P-site (P = peptidyl) on the 40S ribosome where the codon for mRNA reads AUG. In response to this activity, the 60S ribosome binds to the 40S ribosome. Several "initiation factors" (proteins) aid this process and GTP (like ATP) acts as an energy source for the process to occur (Figure 7–16). In the first elongation stage, a second tRNA delivers the proper amino acid to the A-site (A = aminoacyl) adjacent to the P-site identifying and binding to the second codon on mRNA (Figure 7–17). Again, GTP is used as an energy source. Peptide bond formation takes place in the second elongation stage. This is accomplished, as mentioned, by the catalytic activity of the 23S rRNA (also known as a *ribozyme*) located in the 60S ribosome. Upon completion of peptide bond formation, the amino acid in the P-site is released from its tRNA on the P-site (Figure 7–18). In the third elongation stage, the initial tRNA is released from its codon as the ribosome moves along the mRNA and the dipeptide is transferred from the A-site to the P-site using GTP to supply energy for the process. Elongation (peptide growth) is repeated as long as there is more code to be "read" from mRNA (Figure 7–19). At the termination stage, a releasing factor (RF) binds to the termination code on mRNA at the ribosomal A-site and the completed polypeptide is released from the ribosome. At this stage, the ribosome components are also released from the mRNA (Figure 7–20).

Some protein synthesizing ribosomes exist independently in the cytoplasm while others attach themselves to the rough endoplasmic

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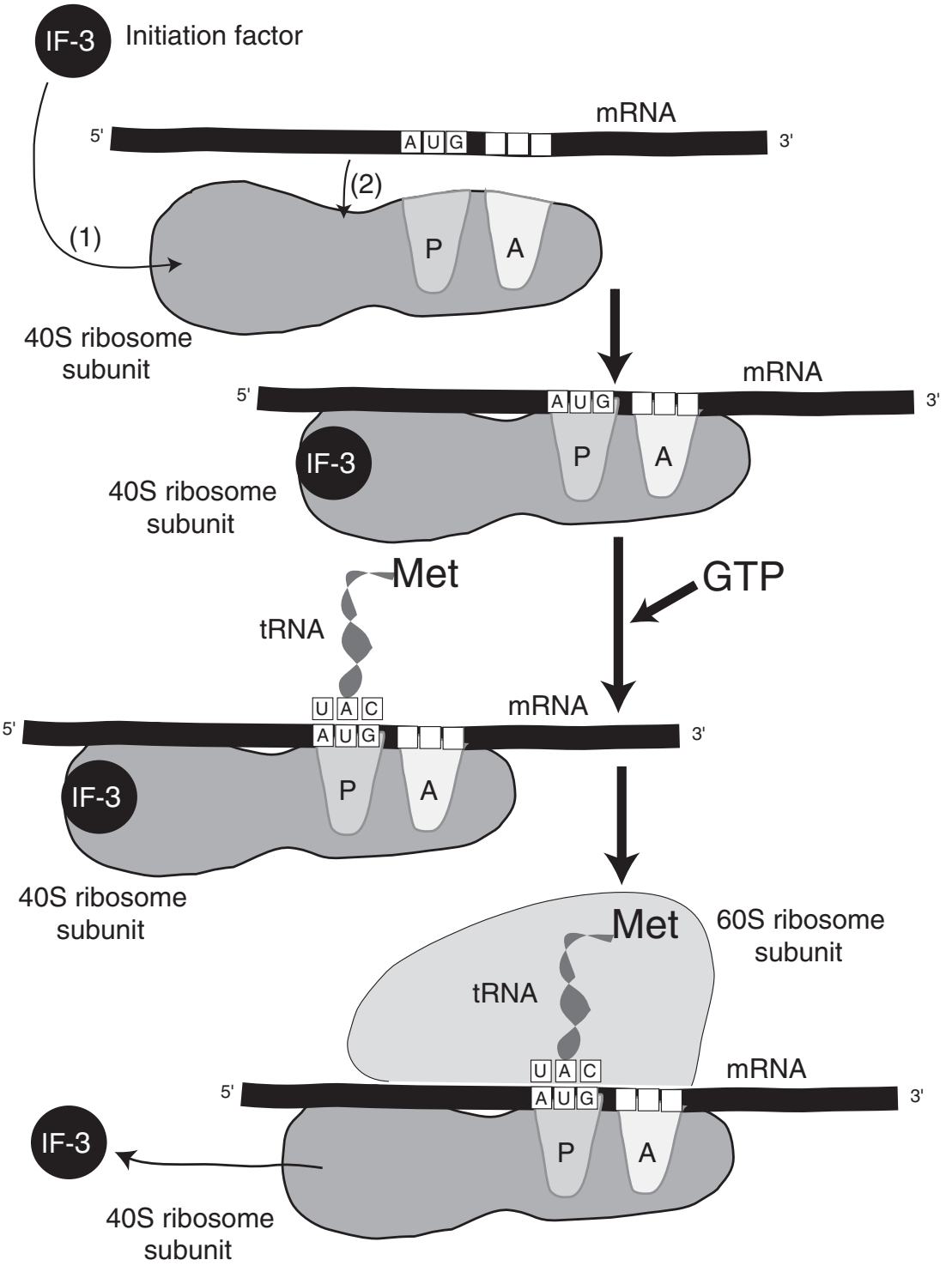


Figure 7–16
The detailed initiation stage of protein synthesis. ➤ The protein initiation factor IF-3 causes mRNA to bind to a 40S ribosome. This, in rapid sequence, affects the binding of tRNA-Met at the AUG sequence at the P-site of the 40S ribosome. When this occurs a 60S ribosome binds to the 40S ribosome.

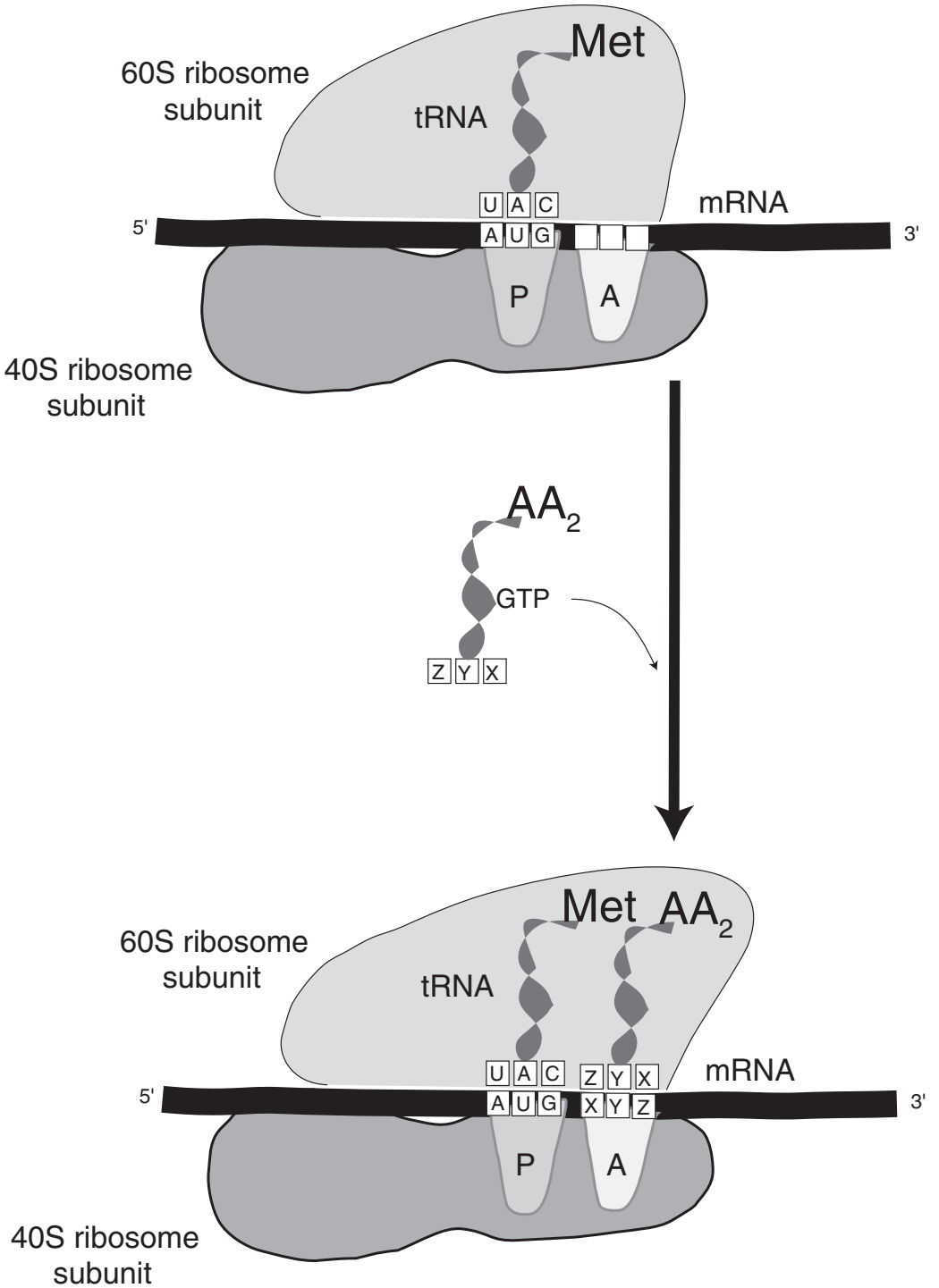


Figure 7–17
The detailed elongation-1 stage of protein synthesis. ➤ This involves the binding of the second amino acid-tRNA complex to the A-site of the assembled ribosome. The binding reaction is enabled by the potential energy in GTP (an ATP equivalent).

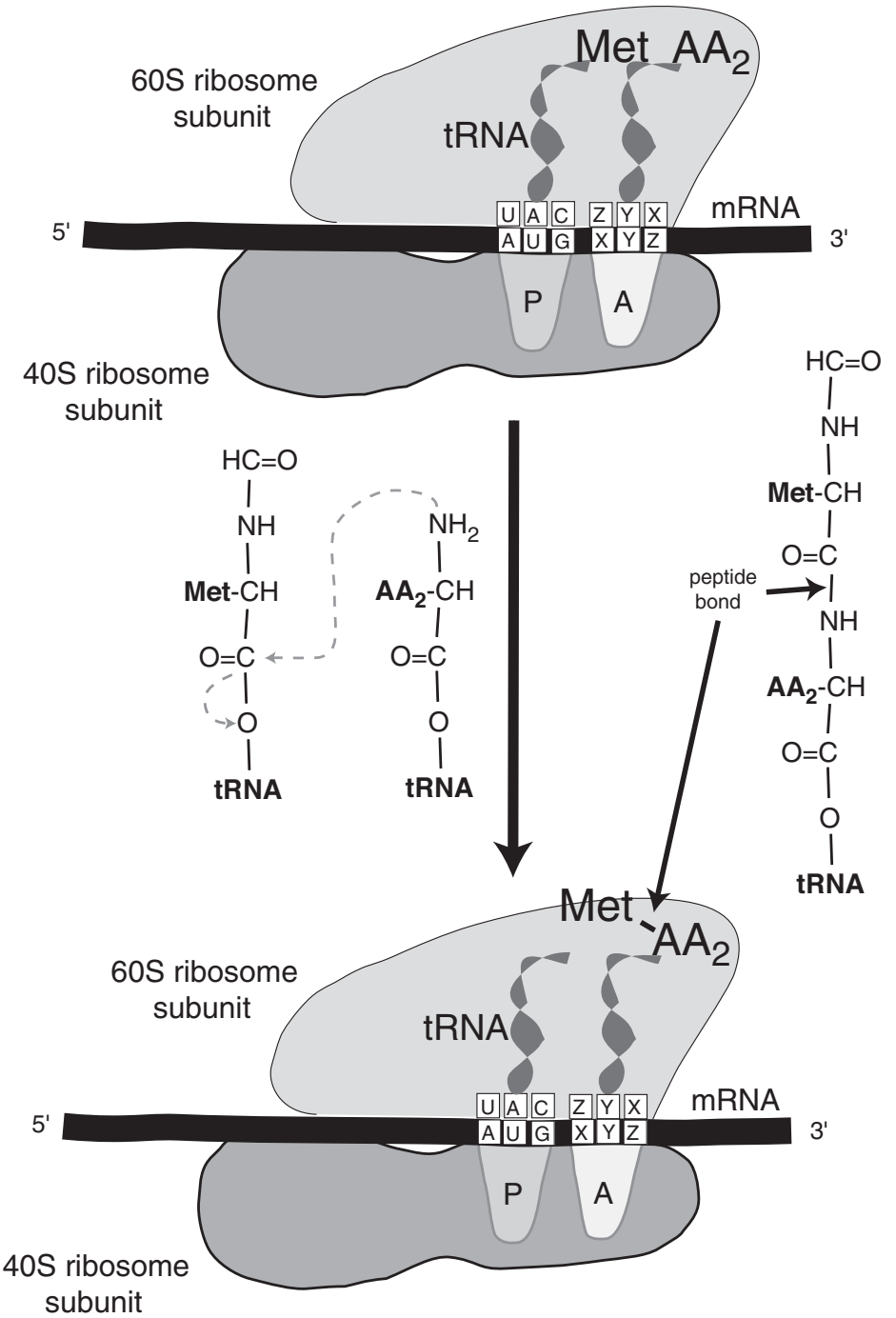


Figure 7–18
The detailed elongation-2 stage of protein synthesis. ➤ In this stage, a peptide bond is formed between the two amino acids by the action of 23S rRNA (not shown) in the 60S ribosome subunit. Although the mechanism of peptide bond formation is known, the detailed participation of the rRNA is not well understood.

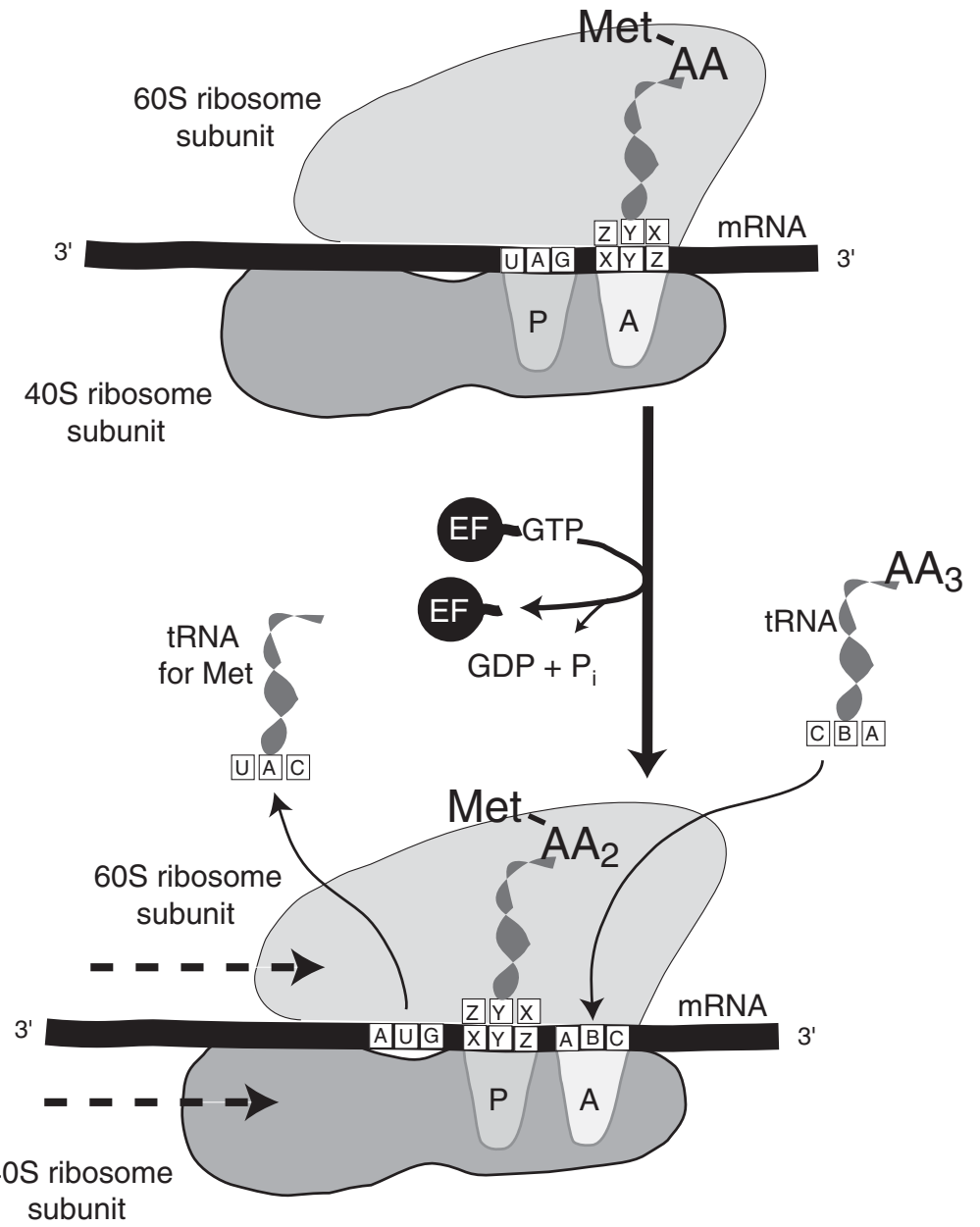


Figure 7–19

The detailed elongation-3 stage of protein synthesis. ➤ Several events occur simultaneously: the ribosomal complex moves one frame (three bases) to the right; tRNA for methionine is ejected from the ribosome; the dipeptide (Met-AA_2) with its tRNA is transferred to the P-site; the empty A-site is then filled with the next tRNA-amino acid (AA_3) as determined by the mRNAs codon; and the entire process is energized by GTP coupled to an elongation factor (EF). In this process, the incoming tRNA-AA_3 is probably bound to the EF-GTP complex.

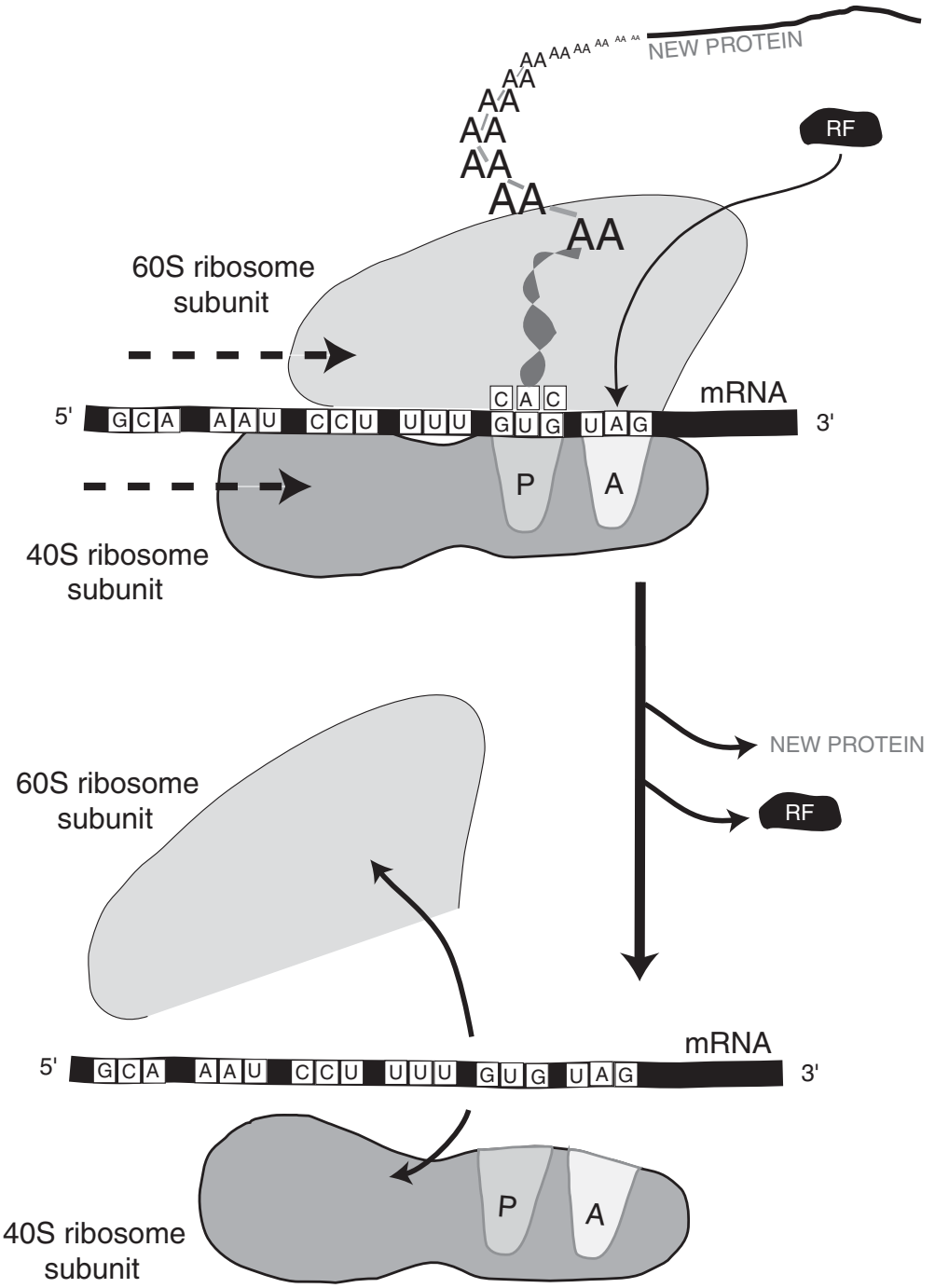


Figure 7–20

The detailed termination stage of protein synthesis. ➤ When a sufficient number of elongation stages have taken place to read the entire code on mRNA, a releasing factor (RF) binds to the A-site as signaled by a termination codon on mRNA. This causes the dissolution of both ribosomal subunits from each other and from the mRNA. The new proteins translation is completed.

reticulum. The latter ribosomes make proteins that will be used for cell membranes, lysosomes (a type of digestion compartment or cellular stomach), and for extracellular functions such as incorporation into connective tissue. The mechanism of protein synthesis just described is identical in both ocular and nonocular cells.

Mutations to DNA and to Nucleic Acid Processing

The functions of nucleic acids may be described as very traditional and very conservative. This is necessitated by the need for the preservation of species and for life itself. When DNA is modified, it has a profound effect upon the organism within which it operates. For example, the substitution of Val for Glu in hemoglobin by a genetic code change in DNA (a mutation in codon 6 of the γ-globin gene) results in *sickle cell anemia* (Lodish et al, 2000). This is a disease in which the red blood cells develop a sickle-like shape resulting in blocked vessels and early destruction of the red blood cells themselves.

Prevention of massive biological chaos and disease development is why DNA copies itself so faithfully and will even enact reparative mechanisms whenever damaged by outside chemical or physical forces. A practical example of how such damage may occur is the alteration of adjacent thymine or thymine-cytosine bases with UV light. This event occurs more commonly than is realized in skin cells when outdoors in bright sunlight. Ultraviolet radiation (in wavelengths near 260 nm) will induce a bond to form between adjacent pyrimidines in DNA (Figure 7–21, *left*). These pyrimidines are located side by side in the same DNA chain rather than as base-pair partners on opposite chains. As such, they are called pyrimidine dimers. When the light induced reaction occurs, the DNA helix is distorted and replication cannot occur beyond the dimer. A cell having a significant amount of such damage may either be doomed to die or to mutate into a cancer cell. This is how skin cancer can develop.

Fortunately, a variety of repair mechanisms usually prevents cells from meeting such fates. One common mechanism functions with the sequential activity of three enzymes: excinuclease, DNA polymerase I, and DNA ligase. The first enzyme breaks 5′,3′-bonds approximately 5 to

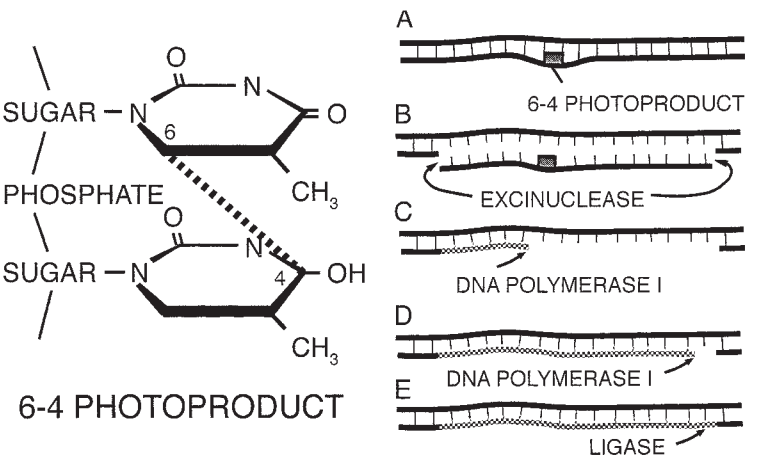


Figure 7–21
Formation of a 6-4 photoproduct on DNA and one type of repair mechanism. ➤ At the left a 6-4 bond between two adjacent pyrimidines on the same DNA strand, caused by UV radiation, is indicated by a dotted line. At the right, at A, the 6-4 photoproduct is seen to cause distortion in the duplex DNA. At B, the photoproduct plus some surrounding nucleotides are removed by breaking phosphate bonds with the enzyme excinuclease. At C and D, DNA polymerase I replaces the missing nucleotides (5′→3′) while, at E, the enzyme ligase adds the final nucleotide to splice the broken ends of the strand.

8 bases on both sides of the pyrimidine dimer. After the damaged sequence is removed, the second enzyme sequences new bases at the gap using the opposite chain as a template. The third enzyme adds the last base to rejoin the two segments of DNA together again (see Figure 7–21, *right*). This cut, patch, and splice mechanism operates continuously to keep DNA intact. Sometimes, however, the repair mechanisms are defective as occurs in the rare skin disease, *xeroderma pigmentosum*. In such a disease, a pre-existing genetic defect makes imperfect excinuclease molecules that are unable to remove pyrimidine dimers efficiently. Patients with this disease develop skin cancer very easily and those that do not at least suffer from atrophied skin, scarred eyelids, and corneal ulcerations.

In the eye, DNA damage by UV radiation is known to initiate reparative processes also (Rapp, Jose, Pitts, 1985). However, such reparative processes are not always successful, depending upon the degree, wavelength, and duration of UV exposure. Pitts et al (1986) reported several kinds of response by the cornea: (1) an inhibition of cellular mitosis as a result of low levels of exposure; (2) swollen nuclei and cell death from moderate to high levels; and (3) complete sloughing of epithelial cells from extreme levels of UV radiation. At higher levels, more is involved than simple DNA damage and the process of damage is known to be assisted by oxygen. At wavelengths below 290 nm, the damage is usually confined to the corneal epithelium. At higher wavelengths, the damaging effects penetrate into deeper layers of the cornea as well as the lens and the retina.

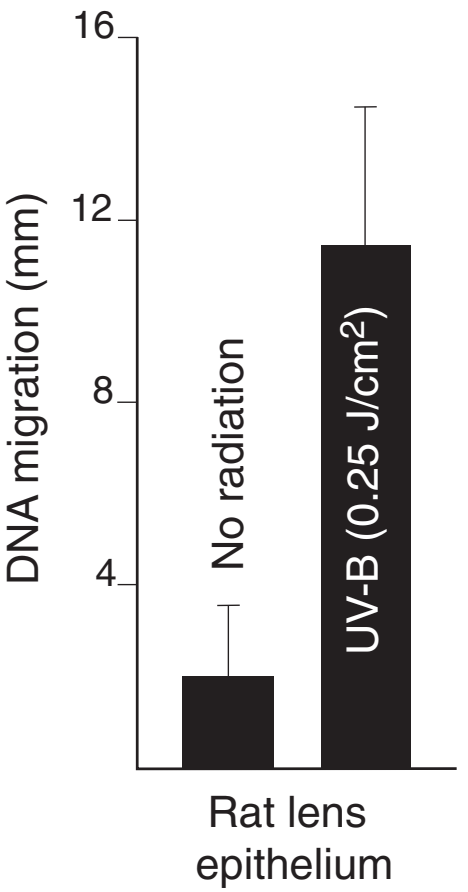


Figure 7–22
DNA damage to rat lens epithelium by UV radiation. ➤ The graph compares DNA damage from UV-B, 290 to 310 nm, applied to *in vivo* rat eyes for 10 min *vs.* untreated eyes. DNA damage was measured in terms of the distance that DNA migrated in an electric field in agarose with imbedded, lysed lens cells. The greater the damage (breakage of DNA), the longer that the smaller DNA fragments migrated (See Singh et al, 1988).

Deeper penetration may also require higher energy sources such as a mercury-xenon lamp to produce DNA damage (Pitts, et al, 1986).

UV radiation is also suspect in producing cataracts (see Chapter 2 under cataract formation). UV-B (280 to 320 nm) induced DNA damage in the lens epithelium has more recently been studied by Reddy et al (1998). In their study, it was shown that DNA strand breaks could be induced in rat eyes after exposure to UV-B (approximately 312 nm). This is shown in Figure 7–22. UV-B has been shown to penetrate the eye to the level of the lens (Balasubramanian, 2000). Since it is a form of the sun's energy that passes through the atmosphere, there is some matter for concern due to its link with the possible formation of cortical and posterior, subcapsular cataracts. In addition to UV radiation (which excites electrons to produce the kind of reactions seen in DNA damage), x-rays and γ-rays are known to produce even greater damage because they possess a higher energy content and displace even those electrons that are closest to the nuclei of atoms (Daniels, Alberty, 1961). This often affects the bases in DNA.

A number of chemical substances can produce DNA damage as well. Among the most well known chemicals are *alkylating agents,* which are chemicals that form covalent alkyl bridges between guanine, adenine, thymine, or cytosine bases on different chains of duplex DNA. Some examples are chlorambucil, carmustine, and busulfan (Figure 7–23). Ironically, these agents also happen to be used to treat cancer. That is, they are used to damage the DNA in cancerous cells so that the cells cannot reproduce (Korolkovas, Burckhalter, 1976). The well-known side effects of chemotherapy for cancer, unfortunately, involve damage to noncancerous cells.

Some chemical agents, on the other hand, are *carcinogenic* to normal cells and may by highly dangerous. 2-Naphthylamine, for example, (see Figure 7–23), is a chemical used in the manufacture of dyes. Unfortunately, this substance was recorded to cause bladder cancer in every exposed worker at one British factory where it was produced after the workers were chronically exposed to the substance (Alberts et al, 1989b). Although the list of such chemicals is continuously growing, the case for the damaging effects of some substances may be

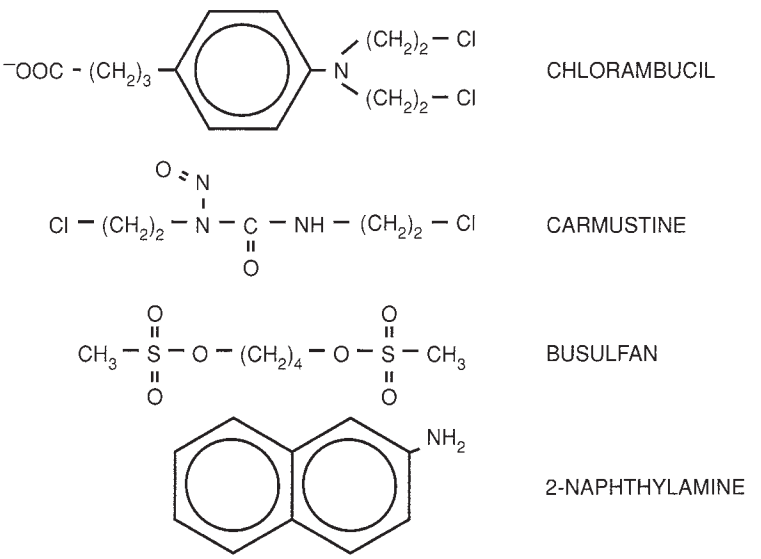


Figure 7–23
Examples of DNA modifying chemicals. ➤ The top three are alkylating agents that add hydrocarbon bridges (principally CH- groups) between bases on opposite sides of DNA strands. These agents are used to modify (i.e., kill) carcinogenic cells. 2-Naphthylamine, on the bottom, modifies the DNA of bladder cells so that they lose control of cell division (become carcinogenic).

overstated due to the presence of the previously mentioned repair mechanisms. It is important to understand that exposure to such agents, even those that are considered dangerous, must be *chronic* (continuous over a period of time) in order to induce a tumor growth. A single mutation of DNA is insufficient to cause cancer and those that develop from external agents often do so only after many years of delay (Alberts et al, 1989b).

Mutations that eventually lead to cancer are disastrous since they cause cells to lose control over their ability to divide. One or more genes within such cells, modified by base alterations, may make excessive amounts of proteins that signal the cells to divide continuously. Alternatively, genes whose proteins suppress division may become inactivated. All of these proteins function by binding to DNA promoter regions.

Consider, for example, the rare childhood ocular disease, *retinoblastoma*. In its hereditary form, retinoblastoma consists in uncontrolled growth that is initiated by neural precursor cells in the immature retina. There may be multiple tumors (masses of abnormal cells) present in both eyes before the end of the third year of life (Vaughan, Asbury, 1980). In this disease, the Rb1 gene on chromosome 13 may be absent or nonfunctional. This gene, which makes a protein that suppresses cell division (Figure 7–24), is one of a pair of such genes. Its absence is sufficient to allow occasional, uncontrolled division to take place whenever occasional failure of the other Rb1 gene occurs. This disease is shown in Figure 7–25.

Molecular Biology of the Crystallins

NORMAL CRYSTALLINS

Crystallins are the major soluble proteins found in lens cells. As discussed in Chapter 2, much interest and study has been invested in this protein class because of its role in supporting lens development, lens clarity, and its association with the development of cataracts. For each major crystallin type in vertebrates it is known that there are 2 *genes* with 3 exons per gene for α-crystallins, 6 genes with 6 exons per gene for β-crystallins, and 7 genes with 3 exons per gene for γ-crystallins (Piatigorsky, 1989). This amounts to 63 separate DNA and RNA sequences that may be used to make three major protein types in the human lens. The genes that determine the synthesis of α-crystallins are located on separate chromosomes (11 and 21). That is to say that they are unlinked. This is also true for the genes of the β-crystallins, but not true for the γ-crystallin genes except γs-crystallin. One of the most amazing facts about crystallins is their diversity of types. Although three types (and many subtypes) occur in humans, there are currently some 13 types known in the animal kingdom (Piatigorsky, Wistow, 1991).

On an evolutionary basis, all crystallins appear to be related to proteins that were originally made for other, nonocular roles before their adaptive use in the ocular lens. It is known that the crystallins, for example, are related to heat-shock proteins. Heat-shock proteins are made in response to an unusual temperature rise in an organism. They protect the organism by solubilizing and refolding any heat-denatured proteins (Alberts et al, 1989c). The chaperone function discussed in Chapter 2 is similarly useful in preserving existing lens protein shape and function. It may be recalled that these proteins are not renewed in lens fiber cells as they have lost their ability to synthesize new proteins.

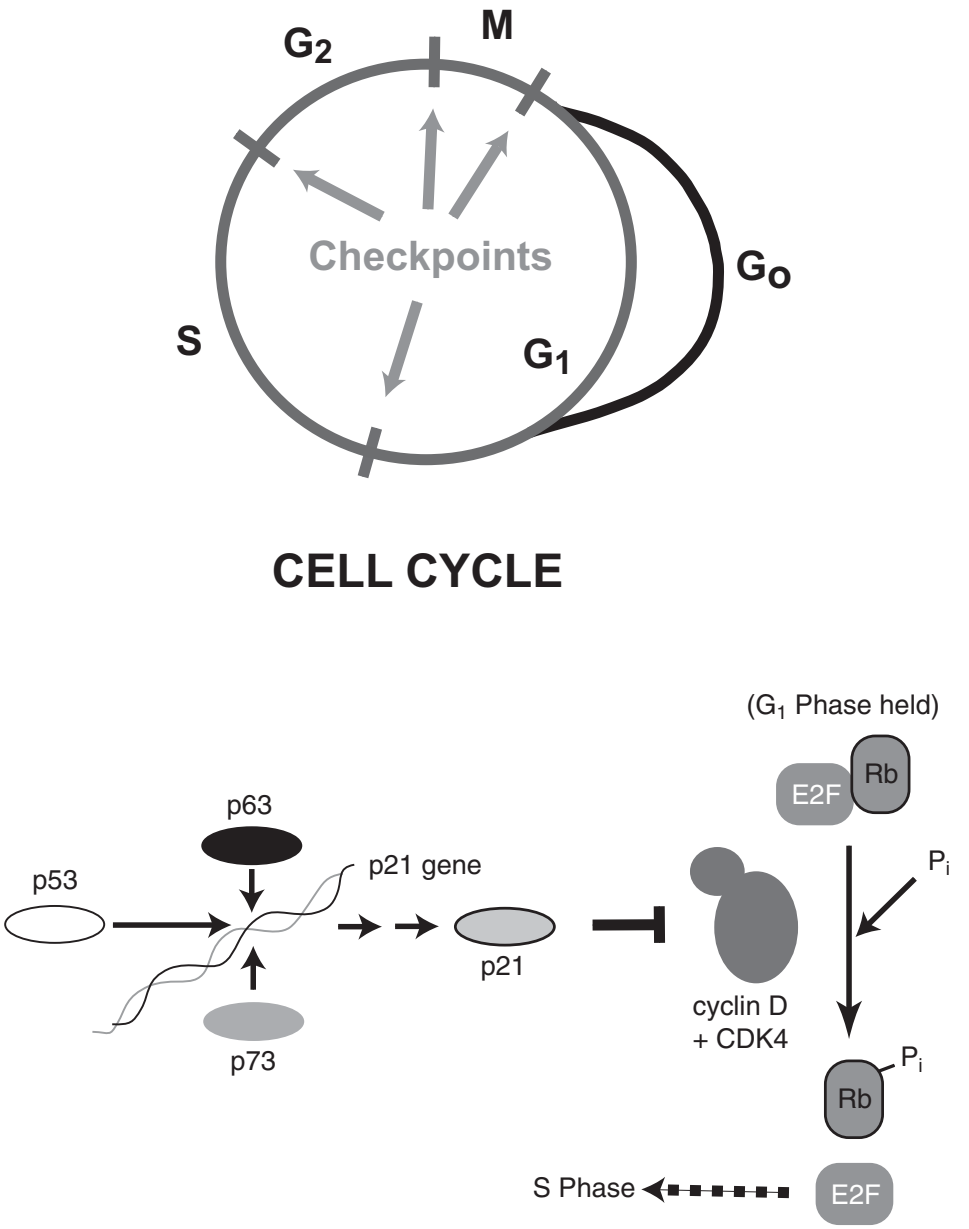


Figure 7–24

The cell cycle and some of the many biochemical pathways that influence it. ➤ A diagram of the cell cycle is shown in the upper part of the figure. In the resting stage, G_1 or gap one, no synthetic processes leading to cell division occur. A subdivision of the G_1 phase, known as G_o, also occurs in which the cell cannot enter into a synthetic process leading to cell division. Cells may be in G_o, for example, when there is insufficient nourishment to begin DNA synthesis. In the S or synthesis phase, synthesis of new DNA (replication) occurs prior to cell division. The G_2 phase (gap two), is an interphase prior to mitosis or cell division. In this phase there are four sets of chromosomes present. In mitosis (the M phase), the complex process of cell division occurs (see, for example, Lodish et al, 2000). The movement or commitment of a cell into its next phase occurs by passage through so called "checkpoints" or "restriction points," which are determined by a variety of biochemical mechanisms. The lower part of the figure illustrates one phase commitment mechanism for passage though the checkpoint from the G_1 to the S phase. Such a mechanism involves the proteins *E2F* and *Rb* (*right*). When E2F and Rb are bound together, the cell is held at the G_1 phase. When the Rb protein is phosphorylated by cyclin dependent kinase 4 (when cyclin D is bound to the kinase) the Rb and E2F proteins are released from each other and both promote checkpoint passage by the stimulation of mRNAs to make proteins necessary for the S phase of the cell cycle. Obviously any influence on the activity of the cyclin D/CDK4 complex will influence the ability of the cell to divide (i.e., to enter the S phase). Three proteins (of many such as KIP [*k*inase *i*nhibitor *p*roteins]) that are known to influence this complex are p53, p63, and p73. These proteins cause the synthesis of mRNA for p21 a protein that inhibits the cyclin D/CDK4 enzyme complex. Any defect in p53, for example, may allow the cyclinD/CDK4 enzyme complex to carry out unchecked catalysis to separate E2F and Rb at a high rate and allow uncontrolled cell division. This, in fact, occurs in some cancer cells in which p53 occurs in mutated and useless forms.

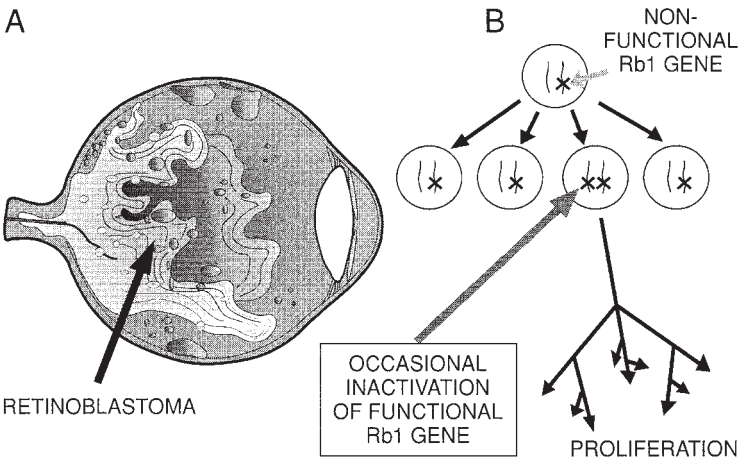


Figure 7–25
Diagram of retinoblastoma and its cause. ➤ "A" shows the presence of numerous retinoblastoma tumors in an eye. In "B," there are two Rb1 genes that limit cell proliferation. In retinoblastoma initially, one Rb1 gene is nonfunctional or missing. When the other gene occasionally fails to function, cell proliferation or uncontrolled growth, takes place due to the absence of Rb protein. See Figure 7–24.

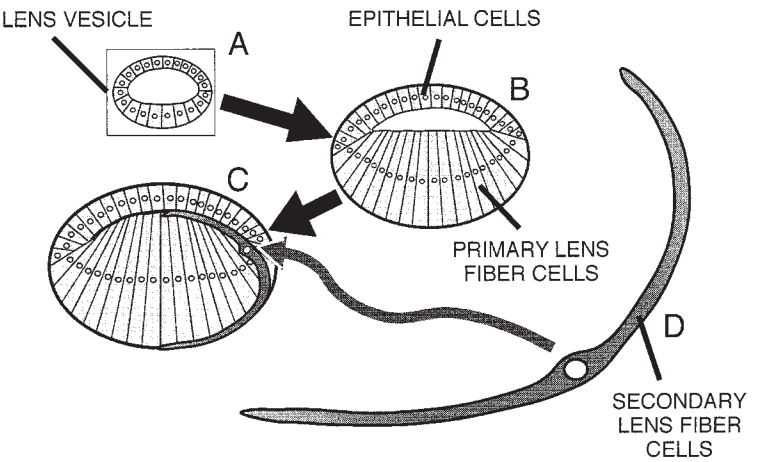


Figure 7–26
The developing fetal lens, which begins as an epithelial vesicle at A, is stimulated by both intralenticular and extralenticular factors to increase in size posteriorly at first. ➤ This is seen by the lengthening of primary lens fiber cells at A, B, and C. Progressively, the lens continues to grow by the development of secondary lens fiber cells (D). The accelerated production of crystallin proteins in these cells contributes to the volume increase (lengthening) of these cells.

The human crystallins have developed, apparently, by duplication of genes that originally made proteins in other species for different roles. In fact, a variety of proteins could probably fulfill the requirements of a structurally clear protein to inhabit the interior of lens fiber cells. However, the chaperone activity of the α-crystallins seems to be vital in the preservation of the structures of all the crystallins in the maintenance of lens clarity.

An important requirement of lens proteins is that they be synthesized in large quantities. When one considers that the genetic machinery of lens fiber cells shuts down as the cells mature, it becomes a crucial matter for the young cell to produce very many proteins. Interest in this area dates back to early 20th century when biologists attempted to determine why a lens would develop from a detached epithelial tissue mass (or lens vesicle) adjacent to the fetal eyecup. One hypothesis has been that increasing the volume of the developing primary fiber cells is a way of causing lens formation (Figure 7–26). This is to say that the relatively rapid synthesis of crystallins during the differentiation of lens fiber cells promotes the elongation of that cell type by filling it with proteins. The factors that turn on the crystallin producing genes are now partly understood. They involve the operation of *PAX-6* genes, which have been linked to proper sequential eye development (Francis et al, 1999).

The chromosomal locations of the human crystallin genes are known. αA crystallin genes occur on chromosome 21 (Quax-Jenken et al, 1985) while αB crystallin genes are found on chromosome 11 (Ngo et al, 1989). Two of the eight β-crystallin genes are found on chromosomes 17 and 22 while six of the γ-crystallin genes occur on chromosome 2. Refer to Figure 7–27. The length and sequence of crystallin genes and their promoter sequences have been heavily investigated. For

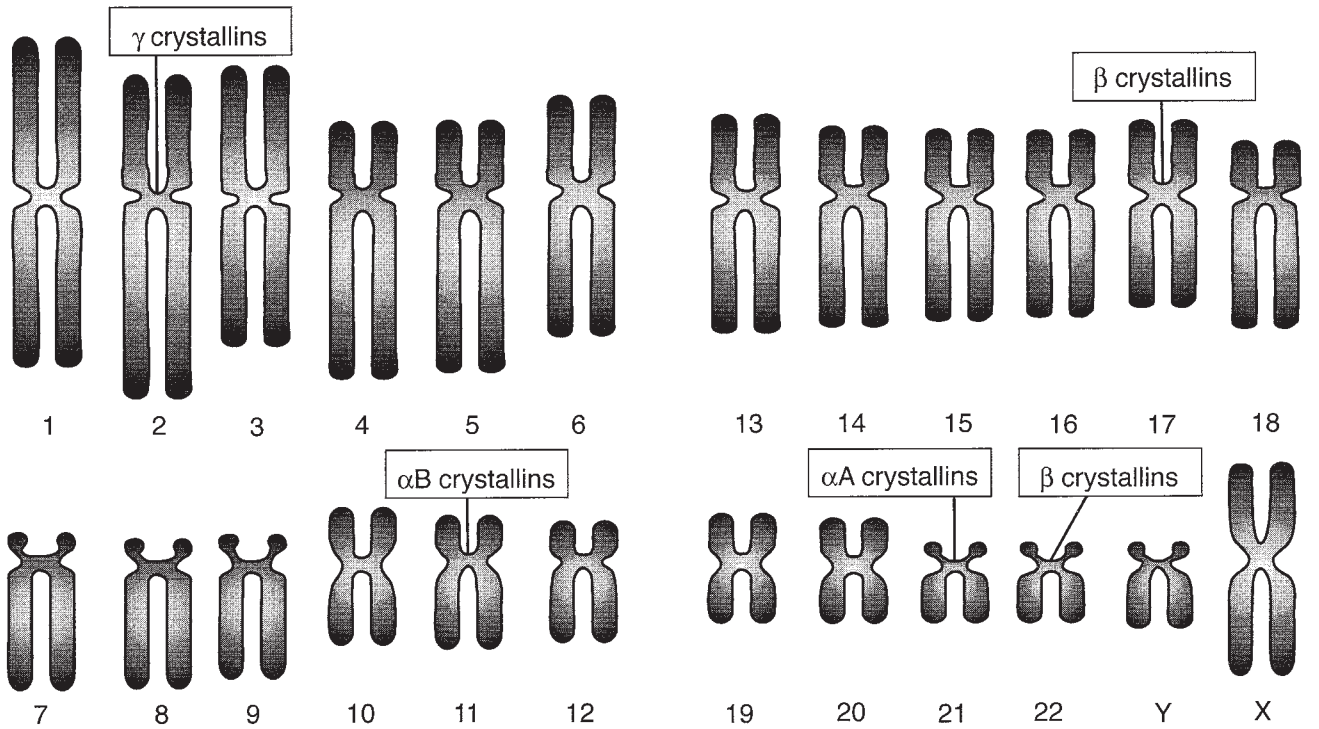


Figure 7–27
The 46 human chromosomes at metaphase (double sets of 1 to 22 as well as x and y). ➤ The synthesis of lens crystallins comes from chromosomes 2, 3, 11, 17, 21, and 22.

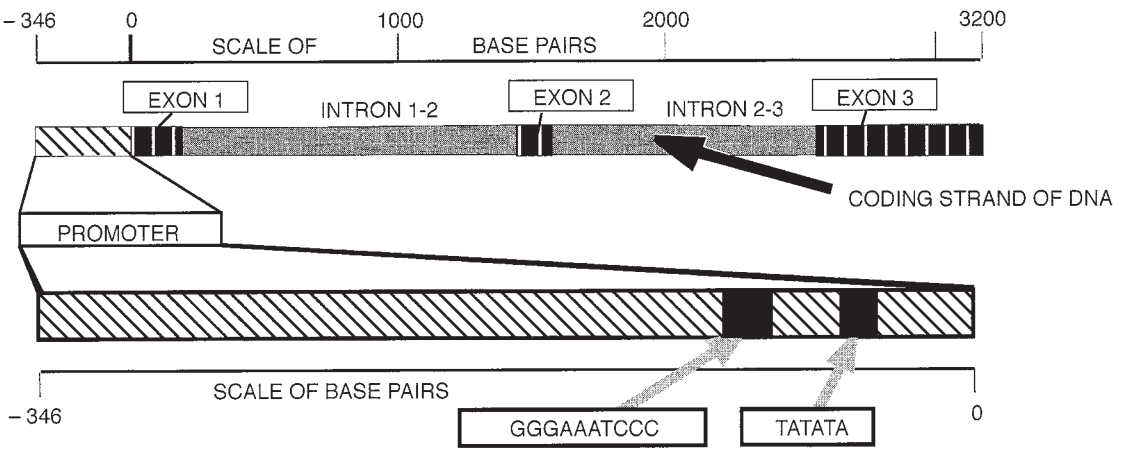


Figure 7–28
The region of DNA that codes for mouse αA crystallin with its promoter on mouse chromosome 17. ➤ It has three exons separated by two introns. The promoter region (magnified) has a sequence: GGGAAATCCC that binds to a protein called αA-CRYBP1, which is necessary for transcription.

example, the mouse αA crystallin gene and its promoter are shown in Figure 7–28. In the promoter element of that gene, the TATA sequence has been found. This is a very common sequence found in the promoter regions of genes that assembles the preinitiation protein complex needed for transcription to occur (Voet, Voet, 1995). There are no CACC or CAAT sequences. However, a nuclear protein termed αA-CRYBP1 has been discovered, which binds to the promoter at sequences −66 to −57 (Nakamura et al, 1990). This protein contains so-called zinc fingers that help the protein to bind to the aforementioned DNA sequences at their major grooves (see Figure 7–29). Slight conformational differences in promoter DNA sequences and the shape of the protein zinc fingers determine where the protein will bind to the DNA. In the case of protein αA-CRYBP1, Nakamura et al (1990), suggest that its binding is necessary for the normal initiation of αA crystallin synthesis. That is, it is necessary for the synthesis (transcription) of hnRNA that codes for this crystallin.

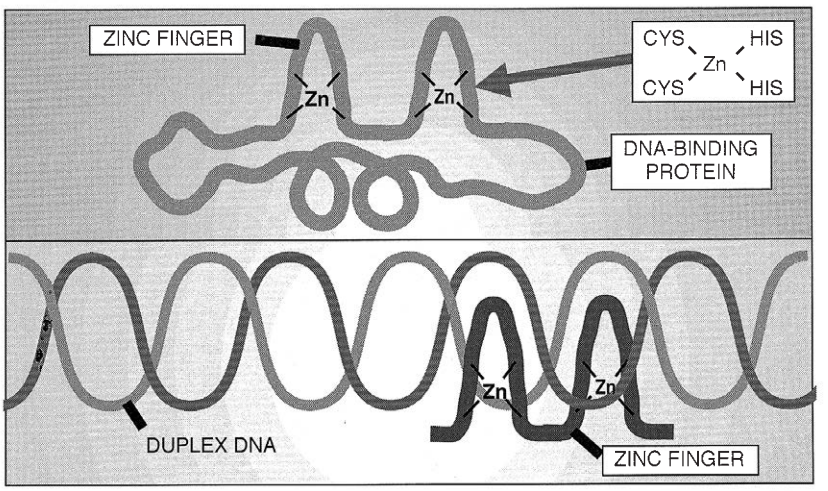


Figure 7–29
Protein zinc finger attachment to DNA. ➤ Proteins binding to DNA have "zinc fingers" that are peptide regions that will slip into the major grooves of DNA. Zinc binds to four amino acids (2 Cys and 2 His) on the peptide chain to form the finger shape.

TABLE 7–3 ➤ TABLE OF KNOWN GENES FOR HUMAN CRYSTALLINS[1]

Gene	Chromosomal Location	Protein Product	Body Locations	Functions
Cryaa	21	αA-crystallin	*lens* (spleen[2])	structural, chaperone
Cryab	11q12-q23[3]	αB-crystallin	*lens*, heart, brain, muscle, kidney	structural
Cryba1	17	βA1/A3-crystallin	lens	structural
Cryba2	2	βA2-crystallin	lens	structural
Cryba4	22q11.2-13.1[3]	βA4-crystallin	lens	structural
Crybb1	22q11.2-12.1[3]	βB1-crystallin	lens	structural
Crybb2	22q11.2-12[4]	βB2-crystallin	lens	structural
Crybψb2	22	None-pseudogene[4]		
Crygs	3	γs-crystallin	lens	structural
Cryga→f	2q33-36[3]	γA→γF-crystallins	lens	structural

[1] Adapted from Graw J: The crystallins: genes, proteins, and diseases. *Biol Chem* 378:1331–1348, 1997.
[2] Present in trace amounts
[3] Present on the q arm of the chromosome given in *centimorgans* from the *centromere*.
[4] A nonfunctional gene.

The crystallin genes that exist in the human lens cells are shown in Table 7–3.

The expression of the αA-crystallin by the Cryaa gene should be considered as lens specific as only trace amounts have been found elsewhere in the body (i.e., spleen). The opposite is true for αB-crystallin expression. Note that, in the lens, "α-crystallin" consists of both types of polypeptides. These crystallin subtypes are synthesized in both lens epithelial and newly formed lens fiber cells. The expression of the members of the β- and γ-crystallins only occur in newly formed lens fiber cells (Harding, 1997).

CRYSTALLIN AND OTHER PROTEIN GENETIC DEFECTS

It should be apparent, from the previous section, that any chemical or physical process that produces a defective gene and causes it to be transmitted, or causes any normal gene to be over/under transcribed, will result in some pathological process or disease. This is so inasmuch as the genetic machinery will produce either abnormal proteins or normal proteins in abnormal amounts. This is just as true for the eye as any other part of the body. In the lens, this often results in the formation of congenital cataracts. Defective genes that may be involved in congenital cataract formation have been studied in animal models that have defects similar to humans. This is important, as, although human congenital cataracts are relatively common, they are more difficult to document.

Patients with such diseases often have a large variety of cataract types and a limited line of traceable descent. Zigler (1990) has described six animal models with traceable pedigrees and congenital cataract types that have been useful. In one of these, the Philly mouse, it was found that the cataract, which began to form 15 days after birth, was due to a deficiency of a β-crystallin (Carper et al, 1982). Subsequently, it was shown that the β-crystallin, a βB2 subtype, is not made due to the mutation of its gene. The gene, instead, makes a defective substitute protein (Nakamura et al, 1988). According to Zigler, cataract formation in the Frazer mouse and 13/N guinea pigs can also be traced to abnormal genes that make defective crystallins. Studies of animal models have more recently been described by Graw (1999) in which the objective was to demonstrate gene type, location, and *phenotype* pathology as a comparison with human types whose *pedigrees* often lacked a sufficiently obtainable history. There now exist at least 150 lines of such cataract types. For example, Klopp et al (1998) have described three mutations of mouse *Cryg genes* that synthesize γ-crystallins. In one of these mutations, known as Cryga^{1Neu}, there is an amino acid exchange of Gly for Asp at position 77 of γA-crystallin. This substitution affects the connecting peptide between Greek-key motifs 2 and 3 (Figure 7–30) and is predicted to alter protein folding to result in cataract formation. Other research on the Cryg gene cluster on mouse chromosome 1 (and human chromosome 2) has been in progress to determine genotype-phenotype relationships to specific types of cataract formation (Graw et al, 2002).

The study of congenital cataract formation in humans has also made remarkable advances in recent years. Therefore, gene mapping of such cataract formation has been generated on eight chromosomes including the crystallin producing regions of chromosomes 2, 17, 21, and 22 (He, Li, 2000). For example, Berry et al (1996) found anterior polar cataracts may be caused by a mutation at 17p12–13. Still another

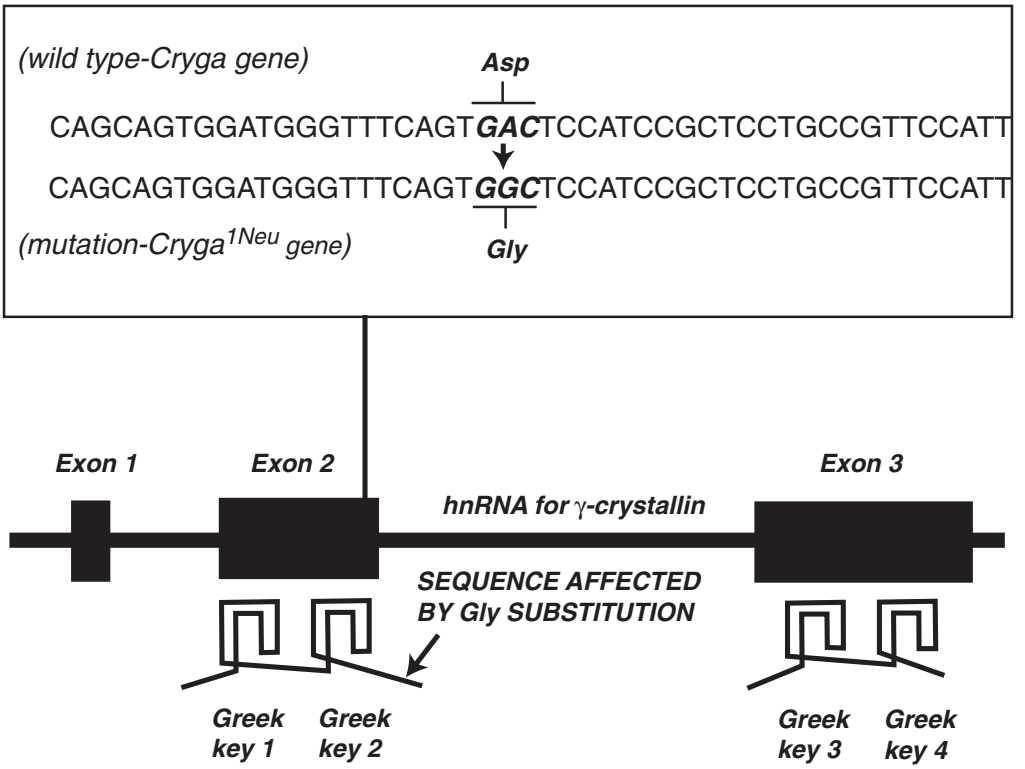


Figure 7–30
Example of a mutation of the mouse Cryga gene for γ-crystallin showing the effects of a point mutation in the gene. ➤ In the upper diagram, the normal gene (wild type-Cryga gene) is replaced by the mutated gene (mutation-Cryga^{1Neu} gene) in which the base A is replace by the base G. This translates into the substitution of Gly for Asp in the protein. In the mutated protein (lower diagram) the random sequence between Greek key 2 (β-pleated sheet 2) and Greek key 3 (β-pleated sheet 3) is sufficiently altered to enact cataract formation.

example, was a zonular *pulverulent* cataract that was inherent in seven-generations of a 30 member family (Ren et al, 2000). This genetic defect involving γ-crystallin was localized to chromosome 2q33–35. It involved an abnormal 5-base insertion.

Of particular interest in recent years has been the role of the PAX-6 gene (and its protein product) in congenital cataract formation. PAX-6 has been described as a centrally important gene in eye development (Halder, Callaerts, Gehring, 1995). Although other protein factors, such as fibroblast growth factors and the protein products of *SIX3, SIX5,* and *PITX3*, also contribute important roles to eye development, PAX-6 protein abnormalities seem to have been more related to congenital cataractogenesis. PAX-6 is a transcription factor, a protein that helps to control the rate of hnRNA formation. In the eye, mutations of the PAX-6 gene have been associated with *aniridia* (absence of an iris accompanied by cataract) and *microphthalmia* (abnormally small eyeballs often accompanied by cataract). Duncan et al (2000) have mentioned that there are more than 70 different mutations of PAX-6 that affect the eye. They showed that truncated forms of the PAX-6 protein can induce cataract formation in mice made transgenic by the injection of a defective PAX-6 gene. That is, the defective (shortened) PAX-6 proteins exhibited repressed formation of mRNA for αA crystallins.

TABLE 7–4 ➤ CHARACTERISTICS OF SOME VIRUSES

Virus	Nucleic Acid/Form/Length[1]	Capsid[2]	Activity Causes
Poliovirus	RNA/single-stranded/7440 b	Simple	Paralytic poliomyelitis
Reoviris	RNA/double-stranded/1196-3896 bp	Simple	Colorado tick fever
Parvovirus	DNA/single-stranded/4680-5176 b	Simple	Animal infections
Bacteriophage[3]	DNA/single-stranded, circular/5700 b	Simple	Infections in bacteria
Herpesvirus	DNA/double-stranded/150,000 bp	Projections	Epithelial and ocular infections
Polyoma	DNA/double-stranded, circular/5000 bp	Simple	Demyelinating disease
Adenovirus	DNA/duplex, linked protein/35,937 bp	Projections	Respiratory and ocular disease
Poxvirus	DNA/duplex, sealed ends/to 300,000 bp	Covered	Smallpox and ocular infections

[1]Key: b, bases; bp, base pairs; duplex, double-stranded.
[2]Capsids are geometrical proteins. Some are uncovered (simple); some have membrane covers (covered) and/or protein projections (projections).
[3]$\phi\chi$174

Viral Intervention in the Cornea

Viruses are relatively simple organisms compared to eukaryotes. They depend on higher organisms to carry out their reproduction. This is accomplished by using the genetic machinery of viral host cells. Although it is simplistic to say that a virus is itself a bag of genes, such a description is close to reality. Viruses exist in a variety of shapes, sizes, and complexities. The more simple ones have only an outside coat or bag known as a *capsid* composed of several types of proteins. The capsid, in more complex viruses, may be covered by a lipid membrane obtained from its host bilipid membranes. The lipid membrane also contains proteins. Either DNA or RNA is found within the capsid. Viral nucleic acids may be either single or double-stranded with some variations in the shape of the strands. Table 7–4 lists some of the commonly known viruses.

In the cornea, one of the more serious viral infections is initiated by *herpes viruses*, especially herpes simplex (types I and II). Type I is often found in the mouth and upper esophagus while type II typically occurs in the genital area. Herpes simplex contains double-stranded DNA (see Table 7–4) and its capsid is covered by both a membrane envelope as well as a protein capsid as seen in Figure 7–31. An area called the *tegument* has also been described as existing between the membrane envelope and the capsid. However, its contents seem to be unknown (Roizman, 1996). Herpes simplex is a *pervasive virus*. In the United States, 90% of the population becomes infected with type I by the time an individual reaches 15 years of age (Burns, 1963) albeit only a small, but significant fraction of this percentage develop corneal infections. The herpes simplex virus is capable of existing in two states. In one state, it actively infects cells and reproduces itself. In the other state, it becomes quiescent in its host for prolonged periods of time (*latency stage*). The mechanisms for attachment/invasion, reproduction, and egress from the infected cell are shown in Figure 7–32. The virus particle or virion attaches itself to the cell membrane of corneal epithelial cells by binding to a proteoglycan (Roizman, Sears, 1996) associated with the membrane surface (part of the "glycocalix" mentioned in Chapter 5 under cell membranes). The viral envelope fuses to the cell membrane and the capsid, with its duplex DNA, is taken rapidly into the cell. The capsid is transported to the nuclear pores of the cell where viral DNA is released into the nucleus.

There viral transcription occurs to produce viral mRNA which is diffused to the cell's cytoplasm where three different classes of over thirty

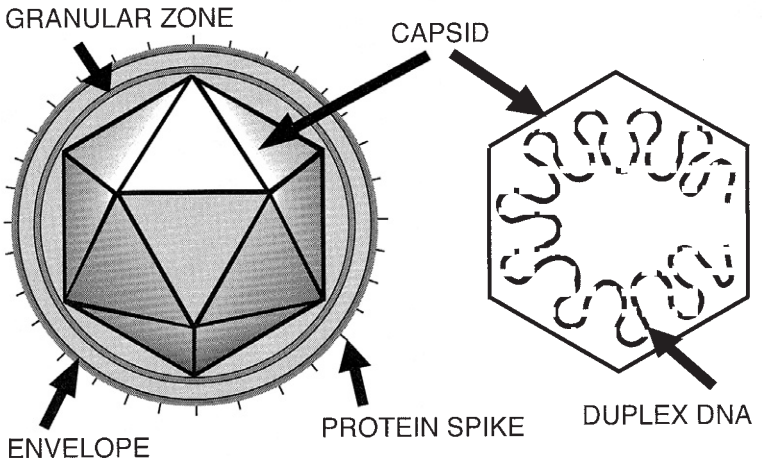


Figure 7–31
Diagram of a herpes virus unit or virion. ➤ At *left*, the capsid is surrounded by a granular zone or tegument of protein, which is covered by an envelope of membrane. Projecting from the membrane are protein spikes. Within the capsid (an icosahedron [Greek: twenty sides]). On the right, a single length of duplex-DNA is packed in the form of a toroid or donut shaped, twisted coils.

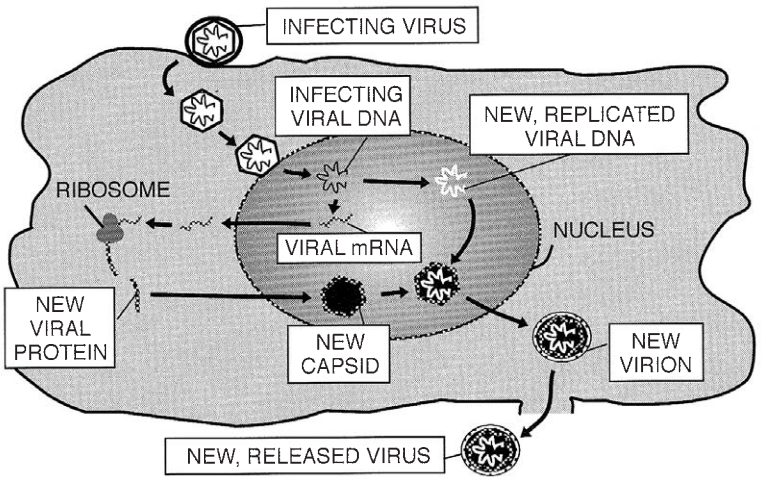


Figure 7–32
Diagram of the mechanism of a herpes virus cell infection. ➤ After making contact and binding to the host-cell, the capsid is internalized and binds to the nuclear membrane. Viral DNA is released into the membrane where it replicates and transcribes itself. New viral proteins are made from the mRNA on host ribosomes. At the third stage of translation, capsid proteins form new capsids in the nucleus. DNA enters the capsids and the virion acquires its membrane from the nucleus. The virion leaves the cell by reversed phagocytosis.

viral proteins are made in sequence: *regulatory* or *α-proteins*, *viral DNA replication* or *β-proteins* and *envelope/capsid, structural* or *γ-proteins* (Roizman, Sears, 1996). The virus requires a high concentration of β-proteins, including its own viral DNA polymerase, to replicate its own viral DNA, which is the next step. The capsids are assembled from viral γ-proteins in the nucleus by a process that has not yet been described.

After capsid assembly, newly replicated viral DNA is packaged in the capsids as shown in Figure 7–32. While this process is taking place, viral specific glycoproteins are assembled and transported to the plasma membranes of the infected cells (see Figure 7–32). These glycoproteins will later have the role of allowing the virus to infect other cells (*infectivity*) and serve unwittingly as immunological markers for Herpesvirus infected cells. The completed virion passes through the nuclear membrane where it becomes coated or enveloped with nuclear membrane and eventually is ejected from the cell by a mechanism akin to reversed phagocytosis (or, as some believe, by a Golgi transport mechanism). As the virus passes out of the cell's plasma membrane it apparently picks up more membrane and protein from the cell surface, but this process has not been well described (Roizman, Sears, 1996). The virion is ready, at this point, to infect another cell. What is destructive or pathological about this entire process is that those cells that are used by the virus are

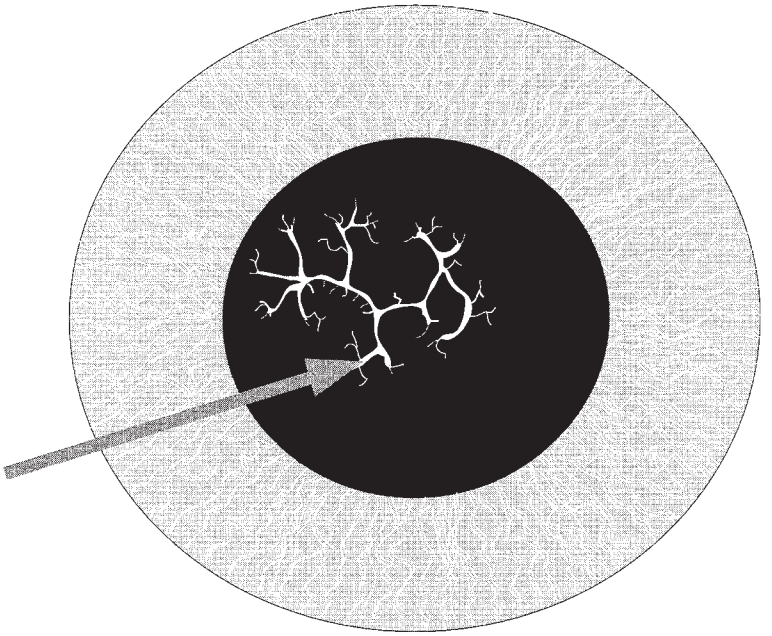


Figure 7–33
A cornea, with its posterior surrounding iris, infected by herpes virus (*grey arrow*). ➤ The diagram shows the typical dendritic lesions of cells killed by this virus.

eventually killed. This occurs for three reasons: destruction of the host nucleolus (where ribosomes are made), terminal alteration of host membranes (especially at the nucleus), and shutdown of host protein synthesis. In the cornea, such a destructive process can be visualized as dendritic (branch-like) ulcers where the virus has killed epithelial cells (Figure 7–33).

A final, but significant property of herpes viral infections is the phenomenon of *latency*. After a primary infection, some virus particles (virions) are transported by sensory nerves to autonomic ganglia. In the case of a corneal infection, the virus particles become stored in the cells of the gasserian ganglion (on the larger root of the fifth cranial nerve) and remain there inactive until reactivated by some stressful stimuli such as emotional trauma, fever, sunburn, or menstruation. Then the viral particles travel back to the cornea and re-infect it. The mechanisms of latency establishment and viral reactivation remain poorly understood. It is known that in latency the viral DNA become circular and produces one mRNA known as *LAT (latency associated transcript)* RNA (Roizman, Sears, 1996). Thompson and Sawtell (2001) have recently found evidence that the LAT gene is important to establish viral latency and that the mechanism actually protects sensory neurons from destruction by shutting down the virus. This was shown by comparing normal viruses with LAT null mutant viruses, that is, viruses that did not possess the LAT gene in mouse hosts. It was also shown that migration of the virus back to the cornea is reduced in herpesvirus null mutants in rabbits by Zheng et al (1999).

Clinically, herpes virus infections can be treated and controlled by several chemicals (i.e., drugs), which act as nucleoside analogues. For example, acycloguanosine resembles deoxyguanosine, but has no pentose (Figure 7–34). It is fortunate that acycloguanosine is catalyzed by *viral* thymidine kinase in infected cells to acycloguanosine triphosphate (AGT). *Cellular* thymidine kinase will not phosphorylate it. Viral DNA polymerase incorporates AGT into newly formed viral DNA, as

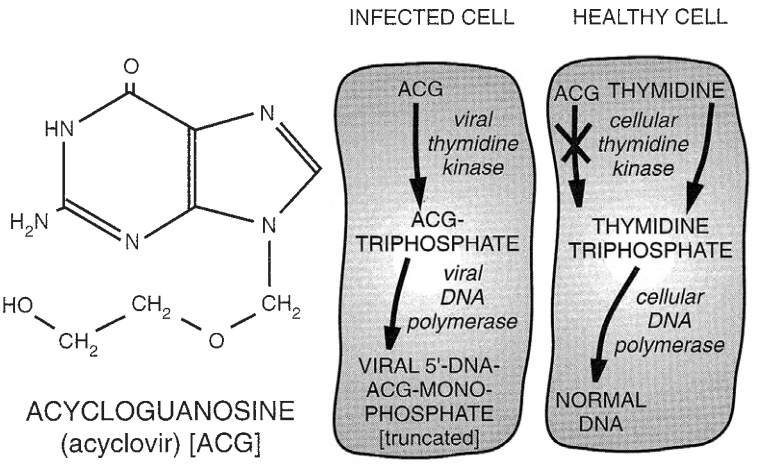


Figure 7–34
Acycloguanosine and its effects on viral infected cells. ➤ Acycloguanosine (*left*) is a base analogue of guanosine without a pentose group. It is an unusual substrate for *viral* thymidine kinase. In infected cells, ACG-triphosphate is incorporated into new viral DNA and halts (truncates) replication. In healthy cells, ACG-triphosphate is not a substrate for the cellular enzyme as shown at right.

though it were deoxyguanosine triphosphate, and this immediately halts further replication since the polymerase cannot synthesize any new DNA past acycloguanosine. This is due to the lack of a 3′-OH group on the molecule (Elion, 1983). In effect, the infection is halted. Since uninfected cells do not catalyze acycloguanosine, the drug is nontoxic to healthy cells. Were it not for herpes entering a latent stage, corneal herpes could be completely cured.

Potential Gene Therapy of Retinal Disease

Currently, there are methodologies that may enable corrective gene therapy for some kinds of gene pathology. However, at present, the effects of the therapy can only last for several days to months (Fradkin, Ropp, Warner, 2000). In the future, artificial chromosomes may be able to be inserted into germline cells in which *cassettes* of corrective genes may be placed in order to permanently prevent disease (Campbell, Stock, 2000). Although there remain some tough technical questions yet to be answered about this approach, the human genome project has gone a long way to making this possible.

In the eye, much practical work also remains to try to describe the genes that are involved in ocular disease as well as to adequately show the control mechanisms that may fail to operate properly in those diseases. In addition, vehicles to deliver genes to be used for therapy are still being developed (Fradkin, Ropp, Warner, 2000).

As a practical example of the former, Ardell et al (2000) have described the organization of intron and exon genes for the human rod photoreceptor cGMP-gated, cation channel, β-subunit protein. This protein (already described in Chapter 6) is responsible for the transduction cascade of photoreceptors. Mutations in the genes that make polypeptides for the channel protein are potential candidates for describing the mechanism of development of *retinitis pigmentosa* (RP), a disease that involves degeneration of the retinal neuroepithelium. In fact, defects in the α-subunit gene have already been associated with one form of RP (Dryja et al, 1995). Ardell's group had previously located the gene at 13 *centimorgans* out on the *q arm* of chromosome 16. The entire gene was found to be quite complex having 33 exons with at least six independent promoters that may be functional within the gene locus. In addi-

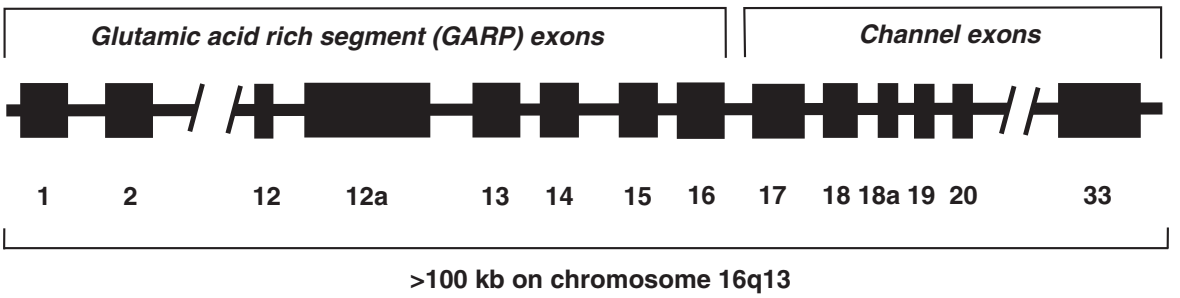
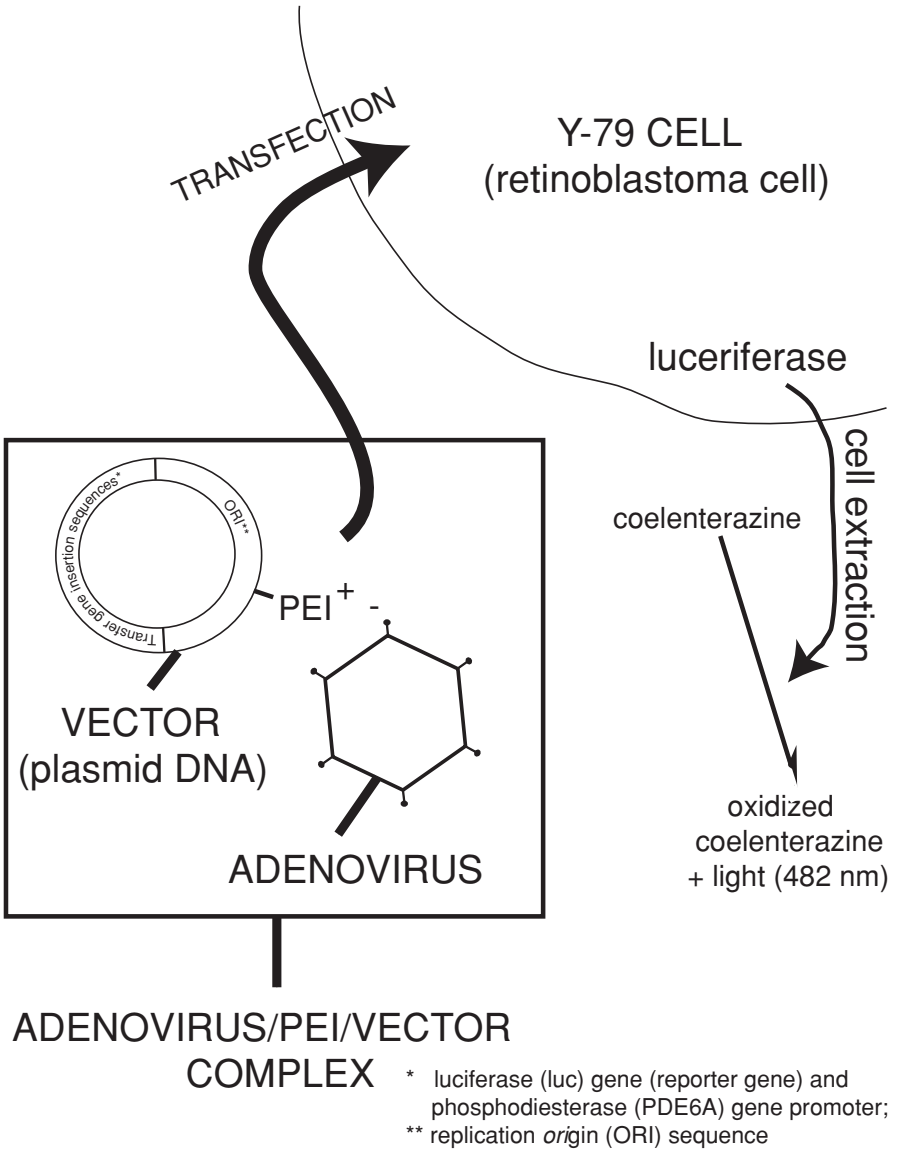


Figure 7–35
The gene map for the β-subunit of the photoreceptor cGMP-gated channel protein. ➤ Only those exons, indicated by dark rectangles and boxes, which are involved in alternative splicing, have been indicated. This alternative splicing could produce gene products that are involved in retinitis pigmentosa. Note that some of the exons produce the GARP domain of the protein (GARP = glutamic acid rich protein, a polypeptide that might stabilize the subunit in the photoreceptor membrane) while the remaining exons produce the channel portion of the protein that includes binding sites for calmodulin and cGMP (see Colville, Molday, 1996). Figure adapted from Ardell et al, 2000.



tion, the potential for alternative splicing was indicated. A simplified map of the gene is shown in Figure 7–35.

Another important retinal disease is retinoblastoma, described earlier in this chapter (see Mutations to DNA and Nucleic Acid Processing). A means to deliver corrective genes for therapy of this disease into retinoblastoma cells was studied by White et al (2001). This group conducted studies on cultures of Y79 retinoblastoma cells as an initial step in this direction. That is, the work demonstrates how it is possible to efficiently transfer genetic material into retinal cancer cells in order to differentiate the cells into photoreceptors that no longer have uncontrolled cell division. In the study, the investigators *transfected reporter* (test) genes into the Y79 cells using plasmid DNA bound to adenovirus all in a medium containing polyethylenimine, a polymer that aids the plasmid in penetrating the Y79 cells by partial membrane disruption (Boussif et al, 1995). This is shown in Figure 7–36. As mentioned above, transfection can only bring about a temporary solution to the problem, but it represents a means by which a permanent solution can be obtained by eventually inserting gene cassettes into germline cells.

SUMMARY

Nucleic acids exist in two forms: DNA and RNA. DNA preserves the cellular genome while RNA translates the code of the genome into proteins. The process of DNA synthesis is called replication while the process of RNA synthesis is called transcription. When RNA produces proteins, by a process known as translation, four kinds of RNA are involved: heterogeneous nuclear (hn), messenger (m), transfer (t), and ribosomal (r). The genetic code consists of specific three base sequences for each amino acid as well as some start and stop sequences. Protein synthesis involves the formation of peptides at ribosomes as the code sequence of mRNA is passed through each ribosome. A peptide bond is formed between each amino acid by the catalytic activity of rRNA after it is brought to the ribosome by tRNA. Several steps are involved in the process. Peptide and protein syntheses occur identically in both ocular and nonocular tissues. Damage to DNA in all cells can occur from both UV and ionizing radiation as well as from some types of chemicals. Such damage may cause cell death or uncontrolled cell division such as in retinoblastoma. However, reparative processes can fix some kinds of DNA damage.

In the eye, extensive investigations have shown the genetic location and some control mechanisms for the synthesis of different kinds of lens

Figure 7–36
Transfection and detection mechanisms for insertion of test genes into Y-79 (retinoblastoma cells). ➤ A vector of plasmid DNA bound to PEI (polyethylenimine) forms a charged complex with an inactivated form of adenovirus. The vector contains reporter genes such as the luc gene that can be used to confirm transfection. In this case the phosphodiesterase gene promoter was also included. The adenovrus/PEI/vector complex binds to the plasma membrane of the Y-79 cells allowing the vector to enter the cell. Here the binding/penetrating machinery of the adenovirus surface proteins is the active agent in cell penetration. Penetration is confirmed by testing for the presence of luciferase. The extracted luciferase enzyme catalyzes the conversion of the complex organic molecule coelenterazine into oxidized coelenterazine with the release of 482 nm blue-green light.

crystallins. The high synthetic rate of lens crystallins can explain how lens development takes place in embryos. Although complete information is lacking, the formation of defective crystallins in hereditary animal models of cataract formation has directed efforts to the investigation of human hereditary cataracts. Much more is known about congenital human cataract because of gene mapping. Mutations of the ocular development gene PAX-6 have also been associated with congenital cataract formation.

Viruses are relatively simple organisms consisting, essentially, of a protein capsid containing nucleic acids in one of several forms. Viruses enter cells and take charge of their genetic machinery in order to reproduce themselves. One example, herpesvirus, may invade corneal cells and reproduce itself on the corneal surface. Unfortunately, herpes and other viruses destroy the cells that they infect. In the case of herpes viruses, the virions may enter a dormant stage (latency) in nerve ganglia and then become reactivated after a traumatic stimulus. The replication of herpes DNA may be prevented by using base analogues that become incorporated into the viral DNA and prevent further synthesis. Recent interest in the molecular biology of retinal genes has progressed with possible gene therapy as its objective. Although this has been frustrated by the temporary nature of the present therapeutic applications, it is hoped that corrective gene cassettes, inserted into artificial chromosomes, may someday enact permanent cures. Presently, studies have been conducted on gene sequences possibly associated with retinitis pigmentosa as well as methods of delivering corrective genes into retinal cells.

PROBLEMS ●

1. If a segment of mRNA has the following sequence: AUGCUUGGUUUUUUUCAACAACCAAAGCCACGU what would the amino acid sequence of its peptide product be? What would you estimate the molecular weight of peptide to be? What physiological effects would you expect to be produced if a larger than normal supply of the mRNA existed (hint: identify the peptide first)?

2. What kind of an enzyme is a ribozyme and what does it accomplish?

3. Explain how retinoblastoma produces cancer in the eye.

4. Predict what might occur to a lens if the wild type Cryga gene were point mutated at its GAC sequence (see Fig. 7–30) to GAA rather than GGC? How might you experimentally confirm your prediction?

5. What is currently the most vexing problem with herpesvirus infections? Explain your answer. What can be done to minimize this problem in the short term? What might be done to achieve a permanent solution?

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CHAPTER 8

Ocular Neurochemistry

Neurotransmission represents a highly efficient and rapid mechanism for sending messages to and from the brain and other body tissues (Hille, Catterall, 1999). In the eye, it facilitates such functions as tear secretion, miosis, generation of IOP, and the very act of seeing. Just outside the eye, it mediates the movement of eyes, blinking, and lid closure. The transmission of nervous signals throughout the body is a combination of two biochemical processes: the transport of cations through membranes (creating the *action potential* in nerves) and the diffusion of *neurotransmitter* molecules from one neuron to the next neuron or to a muscle cell (as occurs at the synaptic cleft). Neurotransmitters function just like discrete hormones. That is, they have hormone action, but only within the area of a *synaptic cleft*. Like hormones, each neurotransmitter binds to a receptor protein that produces either a continuation of an action potential (stimulation), prevention of an action potential (inhibition), or muscle contraction (stimulation). The details of this will be explained further on. It is also important to understand the nature of the action potential that leads up to neurotransmitter release.

An action potential, unlike electricity flowing through a wire by means of electron movement, results from the rapid, sequential flow of Na^+ into a cell and K^+ out of a cell. A cell normally has a relatively high *internal* concentration of K^+ (140 mM vs. 5 mM) and a relatively high *external* concentration of Na^+ (145 mM vs. approximately 10 mM). The combination of these concentration differences creates a potential difference across cell membranes of approximately 60 mV (in some neurons the potential may be as high as 90 mV) with the inside of the cell being negative. The action potential begins when separate gate proteins for sodium and potassium open in sequence to allow the cations to flow through the membrane. This can be understood by referring to Figure 8–1. The *potential difference*, shown here as –64 mV (at time = 0), is established and maintained by the constant pumping activity of Na,K-ATPase (Chapter 3) and the very small leakage of both ions across the membrane. At time = 0, a wave of *depolarization* reaches the membrane from either an adjacent section of nerve or by some transduction

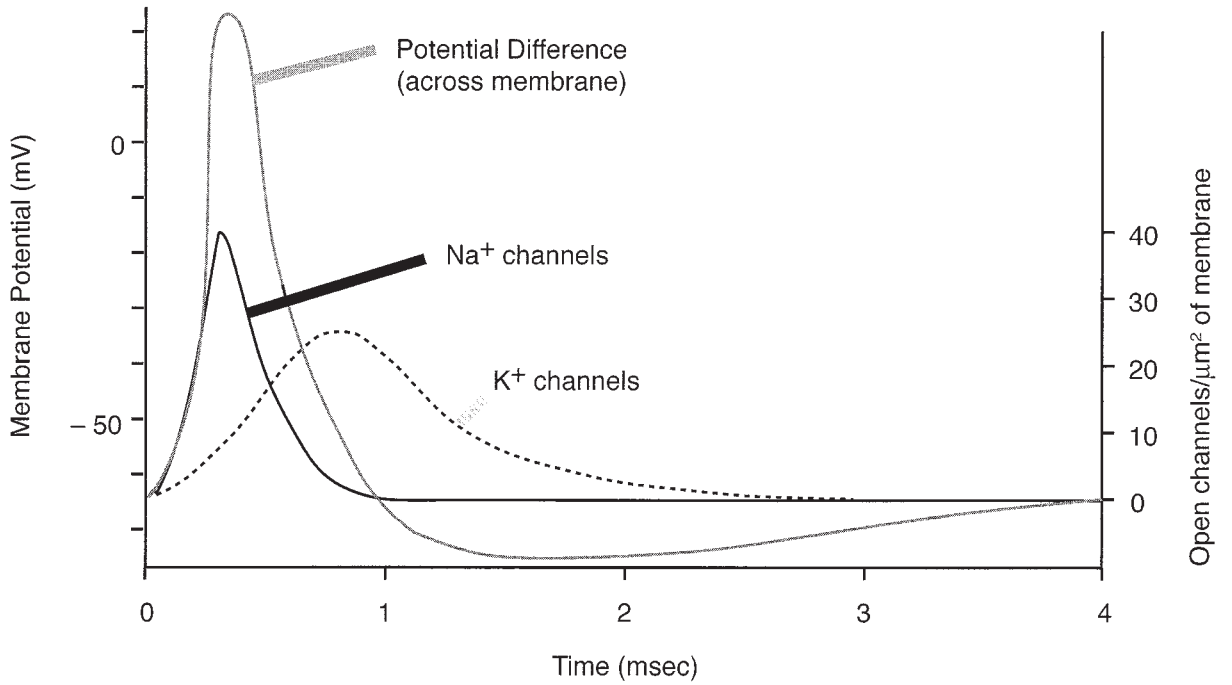


Figure 8–1
Graphical representation of an action potential and ion transport at a point along a nerve axon. ➤ At time = 0, depolarization from adjacent tissue reaches the point of initiation. Within 0.5 msec all the Na^+ channel proteins (*black line*) have opened and the K^+ channel proteins (*dotted line*) begin to open. In this period, the negative potential *(gray line)* decreases as Na^+ ions pour into the cell so that the potential difference actually becomes positive and remains so until 0.6 msec have elapsed. However, the exiting of K^+ ions (which reaches a maximum at approximately 0.9 msec) causes the potential to drop back to and past its original value. Recovery to the original potential is delayed until 4 msec have elapsed. During the recovery (refractory) period, additional action potentials cannot take place. (Adapted from Hodgkin, 1976.)

mechanism (such as a pain receptor on the skin). Depolarization is begun by small, local cation changes (from the adjacent membrane) on both sides of the membrane, which cause the opening, initially of Na^+ *channel proteins*. Although not many channel proteins open (at first), each opening triggers the opening of more channels and each open channel allows 4000 Na^+ ions to *enter* the cell per msec. Therefore, for each μm^2 of membrane, there is an influx of some 32,000 Na^+ ions for the 200-μsec period when the peak of depolarization is maximal between +10 and +22 mV. This represents the action potential. It is important to realize then that the opening (as well as the closing) of the channel proteins is sensitive to the local concentration of adjacent cations. As the Na^+ ion channel proteins open, the K^+ ion channel proteins also begin to open at a significantly slower rate and K^+ ions begin to *leave* the cell. This action of K^+ ions then causes Na^+ ion channel proteins to close. As the closing of Na^+ ion channel proteins continues, the potential voltage across the membranes decreases to a value somewhat below the resting potential (at time = 0). When all the Na^+ ion channel proteins have closed, the K^+ ion channel proteins begin to close also. The period during which the potential difference is lower than normal is described as being *refractory*. During this period (about 3 msec), no Na^+ ion channel proteins can be reopened by a succeeding wave of depolarization. This limits the rate of succeeding pulses of action potentials.

Action potentials can travel along nerves at rates between 1 to 100 meters per second (Mathews, van Holde, 1990). The higher rates of

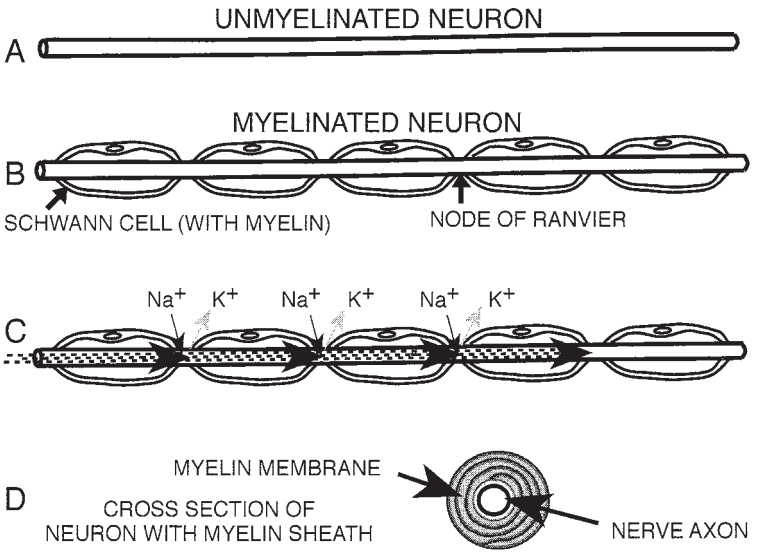


Figure 8–2
The differences between an unmyelinated nerve (*A*) and a myelinated nerve (*B*) are the presence of myelin insulation (provided by Schwann cells) and the restriction of ion-gated channel proteins to regions known as nodes of Ranvier (between myelin sheaths). ➤ The action potential of myelinated nerves (*C*) involve the entrance of Na^+ into and the exiting of K^+ just at the nodes. In this way depolarization leaps from node to node since hyperpolarization cannot redevelop along the myelinated (insulated) portions of the nerve until the depolarizing wave has passed. The myelin membrane itself (shown in cross section at *D*) is wrapped around the nerve axon numerous times.

travel are realized by the inclusion of layers of *myelin* lipid along a nerve axon. Myelin decreases the *capacitance* of an axon membrane. That is, myelinated nerves have a high potential with very little ability to leak cations to either side of the membrane. The areas of myelination have regular interruptions (*nodes*) of nonmyelination where the nerve membrane concentrates its channel proteins. Waves of depolarization in such nerves leap from node to node by causing local flows of cations (Na^+ and K^+) on both the inside and outside of the nerve membranes (Figure 8–2). Depolarization takes place at the nodes. The advantage of a myelinated nerve is that local cation flow moves faster than the continual, connected depolarization of a nonmyelinated nerve. Although myelin lipids insulate the nerve membranes, the lipid composition of myelin is not unique compared to most plasma membranes. That is, there are no lipids that, of themselves, confer special insulating properties to the myelin (Morell, Quarles, 1999). It is simply that the myelin is wrapped around the nerve many times to impart extra insulation.

Ion channel proteins themselves are relatively large proteins whose ability to transport ions is dependent upon the relative ion concentration in their vicinity of the nerve plasma membrane. That is, the gating (opening/closing) of the channel is controlled by the cations themselves. A sodium channel protein from rat brain, for example, has a molecular weight of 320 kD (Catterall et al, 1990). It consists of three glycoprotein chains (α, 260 kD; β_1, 36 kD; and β_2, 33 kD). As can be seen in Figure 8–3, Na^+ ions enter neurons through a channel (pore) in the α-chain. One of the transmembrane segments of the α-chain (S-4) has been designated as being the voltage sensitive (cation sensitive) region that opens the pore.

When an action potential reaches the terminal region of an axon, where the synaptic cleft is located, it opens a channel protein specific for Ca^{+2} ions rather than Na^+ ions. This channel is, therefore, also sensitive to local cation concentration. (See Figure 8–4 for a general diagram of a neural synapse.) The inflow of Ca^{+2} ions brings about the fusion of vesicles in the cytoplasm with the presynaptic membrane and the subsequent release of the vesicle contents, which are neurotransmitters. The neurotransmitters diffuse across the narrow space of the synaptic cleft (approximately 20 nm or 200Å) and bind briefly to receptors on the postsynaptic membrane. Activation, through binding of the receptors, causes

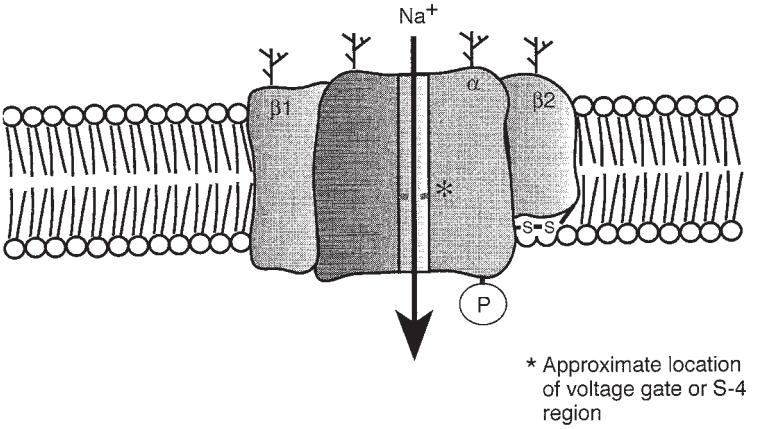


Figure 8–3
Ion or voltage-gated ion channel protein for Na^+ ions. ➤ The α-polypeptide provides the energy (phosphate group), the channel and the gate (S-4 region) for ion transport. The S-4 region is sensitive to the local concentration of cations such that a decrease in negative potential (i.e., increase in cation concentration) will trigger its opening.

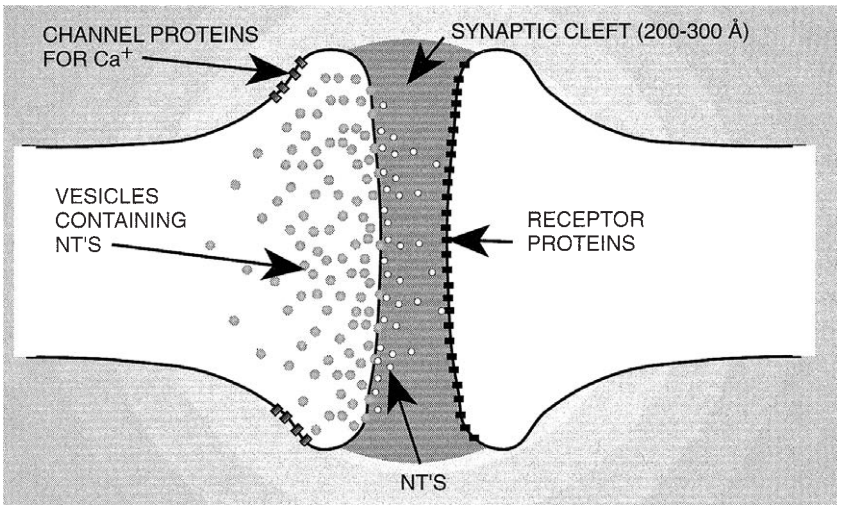


Figure 8–4
Diagram of a typical synaptic cleft. ➤ When a wave of depolarization arrives in the synaptic region, channel proteins for Ca^{+2} open and Ca^{+2} ions enter the presynaptic nerve. This causes vesicles containing neurotransmitters (NTs) to fuse to the presynaptic membrane facing the cleft. Upon fusion, the vesicles release their NT bundle into the cleft where the NTs diffuse to the postsynaptic membrane and bind to receptor proteins. The binding activates a particular postsynaptic biochemical event resulting in either an excitatory or an inhibitory postsynaptic potential.

a postsynaptic response (both biochemical and physiological) that mechanistically may be similar to the action of hormones. The response, as previously noted, may consist of either a depolarization, or a hyperpolarization of postsynaptic neurons, or a contraction of muscle tissue. The biochemistry of these responses has been already partially described and will be detailed further on.

Neurotransmitters and Receptor Proteins and Their Properties (Including Ocular Autonomic Functions)

Neurotransmitters fall into four chemical classes: *acetylcholine*, *catecholamines*, *amino acids*, and *amino acid derivatives*. Some peptides have also been described as neurotransmitters, but might be better labelled as *neuromodulators* (Kandel, Schwartz, Jessell, 2000). A neuromodulator (or neurohormone) is regarded by some as a substance with neurotransmitter properties for which experimental evidence is incomplete. Others refer to them as molecules that alter or "fine tune" the responses of neurotransmitters at the same synaptic cleft. Table 8–1 lists major neurotransmitters and their properties while Figure 8–5 shows some of their structures. Acetylcholine has been the most heavily studied of all the neurotrans-

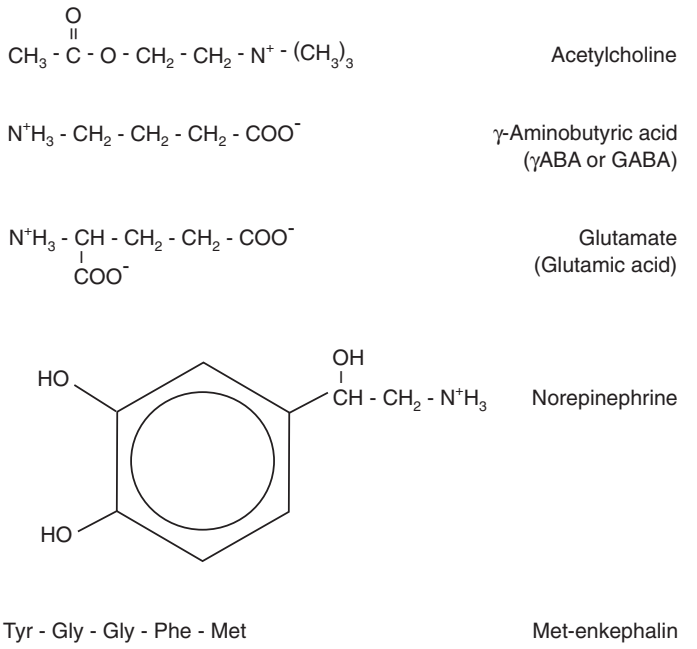


Figure 8–5
The molecular structures of four neurotransmitters (acetylcholine, γ-aminobutyric acid, glutamate, and norepinephrine) and one neuromodulator: met-enkephalin. ➤ Neurotransmitters usually function as shown in Figure 8–4. Neuromodulators are generally able to increase or decrease the effect produced by a neurotransmitter. Their effects are similar to those produced by painkillers and mood-altering substances although much weaker. All have their own receptor proteins.

TABLE 8–1 ➤ SOME NEUROTRANSMITTERS AND THEIR PROPERTIES[1]

Class	Example	Postsynaptic Response	Locations/ Characteristics
Acetylcholine	Acetylcholine	E/I	PNS, ANS. Fast nicotinic response, slow muscarinic response
Catecholamine	Norepinephrine[2]	E/I	CNS, ANS, retina
	Dopamine	I	CNS, retina
Amino acid	Glutamic acid[2]	E	CNS, retina
	Glycine	I	Spinal cord, retina
Amino acid derivative	Serotonin	I	CNS, retina
	γ–Aminobutyric acid	I	CNS, retina

[1]Key: PNS, peripheral nervous system; ANS, autonomic nervous system; CNS, central nervous system; E, excitatory; I, inhibitory.
[2]Epinephrine and aspartic acid may also act as neurotransmitters.

mitters. It is synthesized in the terminal axon bulb from acetyl-CoA (Chapter 4) and choline (an essential dietary component) by the enzyme *choline acetyltransferase*. When synthesized, it is incorporated into terminal vesicles. After acetylcholine has been released from its vesicle into the synaptic cleft and bound to its postsynaptic receptor protein, it is inactivated by hydrolysis with the enzyme *acetylcholinesterase*. The enzyme is located within the synaptic cleft. Inactivation is necessary to prevent excessive stimulation of the receptor proteins. The metabolism of acetylcholine is shown in Figure 8–6. Acetylcholinesterase is a very fast catalyst. Its *turnover number* of 25,000 molecules per second may be compared with lactate dehydrogenase (Chapter 3), which catalyzes only 1000 molecules per second (Stryer, 1988). Clinically, the esterase is important since it can be inhibited by agents such as physostigmine to produce

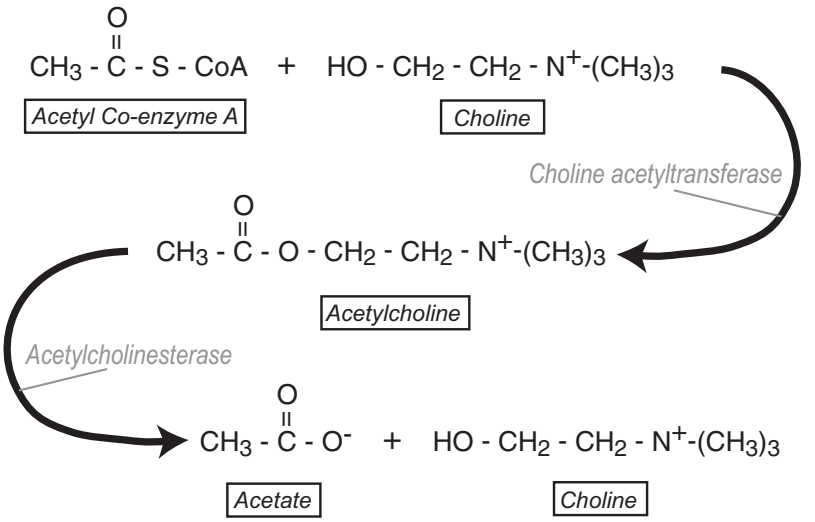


Figure 8–6
The synthesis and degradation of acetylcholine. ➤ Synthesis occurs in the presynaptic neuron while degradation occurs in the synaptic cleft. See text for other information.

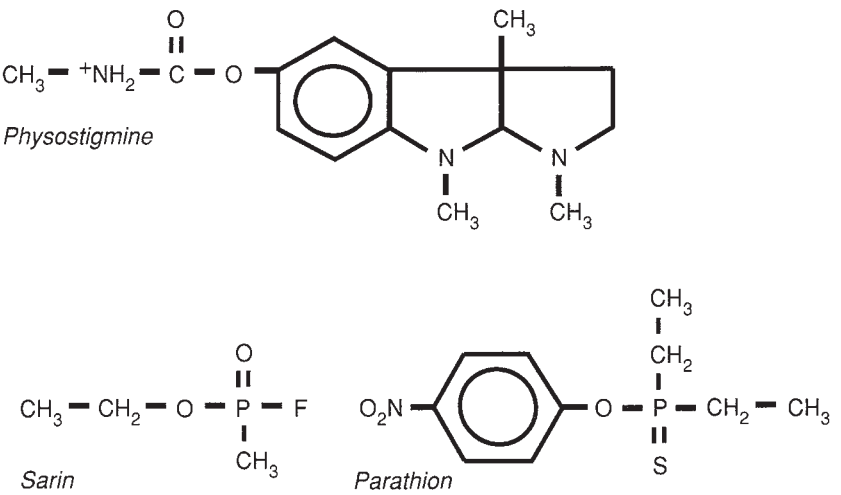


Figure 8–7
Three inhibitors of acetylcholinesterase. ➤ Physostigmine is an alkaloid (i.e., basic organic compound that contains nitrogen). It was first found in Colobar beans (from an African climbing plant). Its inhibition is temporary and controllable. It is used as a pharmaceutical agent. Sarin is a dangerous nerve toxin, which completely inhibits acetylcholine esterase by covalently binding to its active site. It forms a phosphate ester there. Parathion is a less toxic variant of sarin. It has been used as an insecticide. The phosphoryl containing inhibitors cause their destructive events by bringing on respiratory paralysis.

a controlled, temporary increase of acetylcholine available to stimulate receptor proteins. In the ocular autonomic nervous system, physostigmine has been useful in lowering the intraocular pressure of glaucoma patients. Other, more powerful agents have been used to totally inhibit the enzyme such as insect sprays (e.g., parathion) and nerve gases (e.g., sarin). The agents are shown in Figure 8–7.

Receptor proteins for acetylcholine consist of two different membrane proteins known as the *nicotinic receptor protein* and the *muscarinic receptor protein*. The nicotinic receptor, with a molecular weight of 282 kD (Mathews, van Holde, 1990), is somewhat similar to the sodium channel protein in size and function. However, this channel protein is not opened by a change in cation concentration, but by having a neurotransmitter bind to it. It is composed of five glycoprotein subunits that, together, form a pore for cation transport. It is named after the natural substance *nicotine* (Figure 8–8) that can also bind to it and stimulate the opening of its channel. Substances that imitate neurotransmitters are known as *agonists* (Greek: *agōnistēs,* competitors). On the other hand, *curare* (*tubocurarine*, a well-known neurotoxin shown in Figure 8–8), binds to and blocks the receptor. Substances that block or prevent

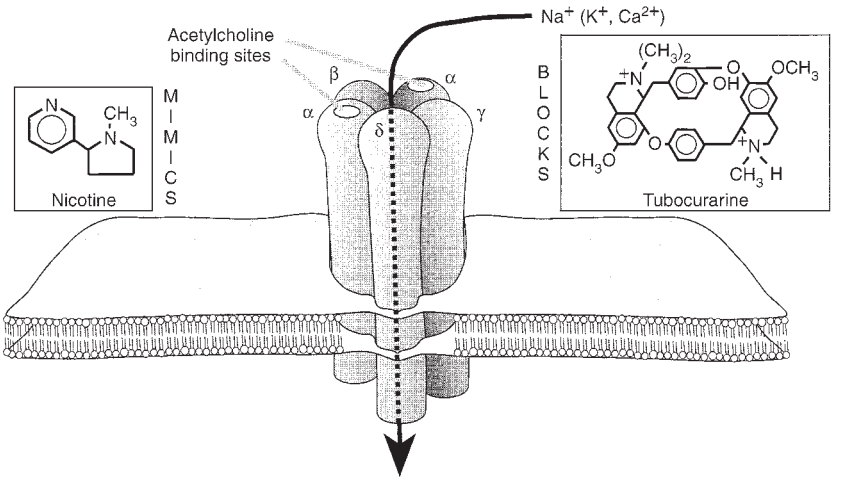


Figure 8–8
The receptor protein for acetylcholine. ➤ This protein is a channel protein for Na^+ predominately (although some K^+ and Ca^{+2} ions may also pass through). It is "gated" or opened by acetylcholine rather than by local changes in cation concentration. Nicotine mimics or binds the receptor in place of acetylcholine. Tubocurarine blocks the acetylcholine binding sites and prevents the channel from opening. The Greek letters label the different polypeptide chains that constitute the molecule (here shown imbedded in its plasma membrane).

receptor function are known as *antagonists*. The nicotine receptor molecule is also shown in Figure 8–8. Those synaptic junctions that use nicotinic receptor proteins are considered "fast" (operating in about 1 msec) and are commonly found between nerve axons and muscles in the *peripheral nervous system* and between ganglionic synapses of the *autonomic nervous system*. The latter would include *parasympathetic* and *sympathetic* activity of the iris and ciliary body muscles in the eye. In effect, pupil size and lens focus (*accommodation*) are controlled by the use of these receptor proteins.

Muscarinic receptor proteins, by contrast, are considered "slow" receptors (operating over a range of sec to min). Instead of directly opening a cation channel in the receptor, the receptor is linked to a G protein (see text in Chapter 6 and Figure 6–6) whose activation may ultimately cause any of the following biochemical events: stimulation of potassium channel proteins, inhibition of adenylate cyclase, stimulation of phospholipase C, and modulation of the activity of phospholipase A_2 (Hulme, Kurtenbach, Curtis, 1991). In this sense, these receptors resemble hormone receptor proteins. The end effect of the enzyme activity changes is to increase or decrease K^+ ion transport, decrease Ca^{+2} transport or increase general cation transport in the postsynaptic cell (Nicoll, Malenka, Kauer, 1990). These events occur over a period of approximately 100 msec, but may be much longer (Taylor, Brown, 1999). Although the actual structure of the receptor has not been described, it is thought to resemble most G-protein receptors in which a single polypeptide passes through the plasma membrane seven times in the form of an α-helix (see, for example, the structure of the rhodopsin molecule, Figure 2–21). Muscarinic receptors occur in all the effector cells (smooth muscles) stimulated by the parasympathetic nervous system. In the eye, only pupil and ciliary constriction are affected to decrease light intake and focus on near objects respectively.

The effector cells of the sympathetic system are stimulated by *norepinephrine* and have one or more of its receptor proteins on their postsynaptic membrane. The neurotransmitter norepinephrine (NE, shown in Figure 8–5) is derived from the amino acid tyrosine (Kuhar, Couceyro, Lambert, 1999). Three enzymes are required for its synthesis along with molecular oxygen, three vitamins, and copper (cupric) ions. The complete synthesis takes place in the presynaptic region of the neuron that releases NE. However, the synthetic pathway is compartmentalized or divided

between the cytoplasm and the vesicles that store NE. Figure 8–9 illustrates this. Norepinephrine is predominately conserved (saved), after binding to its receptor, by an active transport process constituting a reuptake mechanism. This process, involving a transport protein and Na,K-ATPase, moves the neurotransmitter back into the presynaptic cytoplasm where it is taken up again into presynaptic vesicles. A smaller amount of the neurotransmitter is lost from the synaptic cleft and broken down enzymatically. There are four receptor proteins for NE that are designated α_1, α_2, β_1, and β_2.

The reasons for the existence of so many norepinephrine (and epinephrine) receptors are their anatomical location, the option for stimulation or inhibition, and the need for a variety of sensitivities to neurotransmitter binding (Harrison, Pearson, Lynch, 1991). The receptors are found on the postsynaptic membrane except for the α_2-type, which occurs on the presynaptic membrane (Hoffman, 2001). All the catecholamine receptors have a general structure similar to that shown in Figure 8–10 for a β_2-receptor. The proteins characteristically have seven α-helices that pass through the membrane with a binding site deep in the

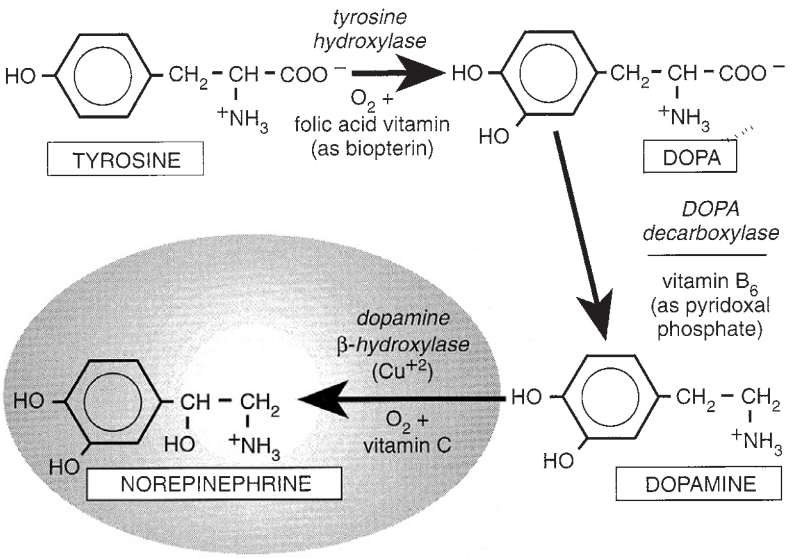


Figure 8–9
The synthesis of norepinephrine. ➤ Three enzymes catalyze the synthesis from tyrosine. Norepinephrine also acts as a feedback inhibitor of tyrosine hydroxylase. This limits the synthetic rate of norepinephrine. Note the vitamin requirements in each step. The final stage, (*in gray oval*) takes place in the presynaptic vesicles whereas the preceding stages occur in the presynaptic cytoplasm. Norepinephrine is preferentially conserved after use by re-uptake (i.e., transport) to the presynaptic neuron.

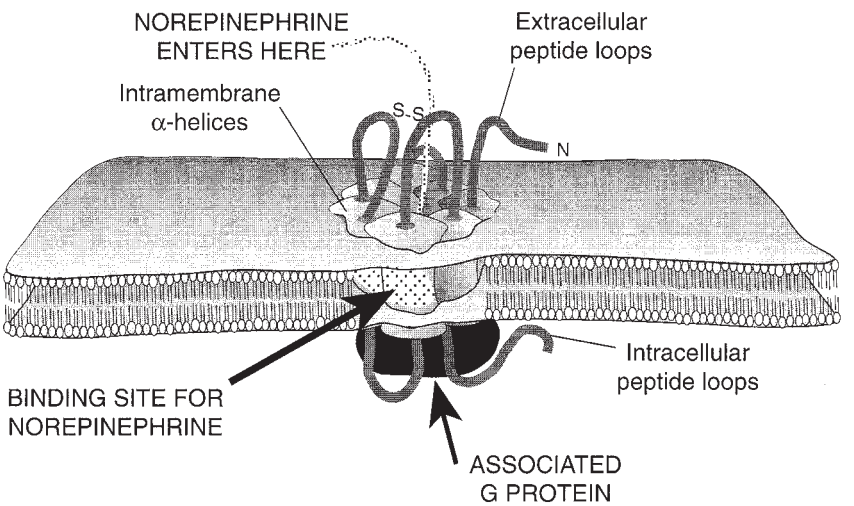


Figure 8–10
Receptor protein for norepinephrine. ➤ The neurotransmitter binds to its receptor deep within the plasma membrane region of the receptor α-helices. Like rhodopsin and other hormone receptor proteins, this receptor is associated with a G protein that the receptor activates. Such receptor proteins typically have seven α-helices traversing the cell membrane.

plasma membrane portion of the protein. They function by activating a G protein and, therefore, are similar to the structure of rhodopsin and other hormone receptor molecules that use G proteins (see Figure 6–6). It is interesting that norepinephrine and epinephrine use the same class of receptor proteins in both a hormonal and a neurotransmission mode. Norepinephrine has wider use as a neurotransmitter while epinephrine is used more often as a hormone. In both cases, the G protein associated with the receptor either stimulates adenyl cyclase (with β_1 and β_2 receptors) or inhibits it (with α_2 receptors) (see Figure 6–6). The postsynaptic muscle response parallels the stimulation or inhibition of cAMP production. Receptors that constitute the α_1 type, however, cause stimulation of muscle response by using Ca^{+2} ions as a second messenger instead of cyclic nucleotides (Kandel, Schwartz, Jessell, 1991; Kuhar, Couceyro, Lambert, 1999; Murray et al, 2000). The responses produced by the catecholamines are relatively slow and correspond to the responses produced by muscarinic receptors for acetylcholine. Table 8–2 shows the receptors and mechanisms used with acetylcholine and norepinephrine.

Note that α_2 receptors are located in the presynaptic membrane and serve in a feedback function to alter neurotransmitter release.

The autonomic nervous system makes extensive use of acetylcholine and norepinephrine. In the anterior segment of the eye, this system is responsible for pupil diameter, accommodation (distance focusing), modulation of the intraocular pressure, and the production of tears. A diagram of its basic features, neurotransmitters, receptors, and muscle connections are shown in Figure 8–11.

Neurochemistry of the Retina

The transduction of light in the retina has been previously discussed (Chapter 6: text and Figure 6–9 through 6–12). However, in order to understand how the light signal is transferred to area 17 of the brain biochemically, some knowledge of the anatomical and physiological characteristics of the retina are necessary. Figure 8–12 shows a simplified scheme of most of the functional neurons in a mammalian retina. Photoreceptors send electrochemical signals to the brain by both direct (cone) and indirect (cone and rod) synaptic mechanisms. In addition,

TABLE 8–2 ➤ RECEPTORS AND MECHANISMS USED BY ACETYLCHOLINE AND NOREPINEPHRINE

Neurotransmitter	Receptor	Mechanism
Acetylcholine	Nicotinic	*Fast;* causes postsynaptic depolarization primarily by acting as a gate for Na^+
	Muscarinic	*Slow;* G protein linked *via* either cAMP or Ca^{+2}
Norepinephrine	$\alpha_{1D,1B,1A}$	*Slow;* Ca^{+2} mechanisms for 1D and 1B; unknown for 1A.
	$\alpha_{2A,2B,2C}$	*Slow;* all three types inhibit adenyl cyclase and are presynaptically placed
	$\beta_{1,2,3}$	*Slow;* all three types stimulate adenyl cyclase

Modified from Kuhar, Couceyro, Lambert, 1999.

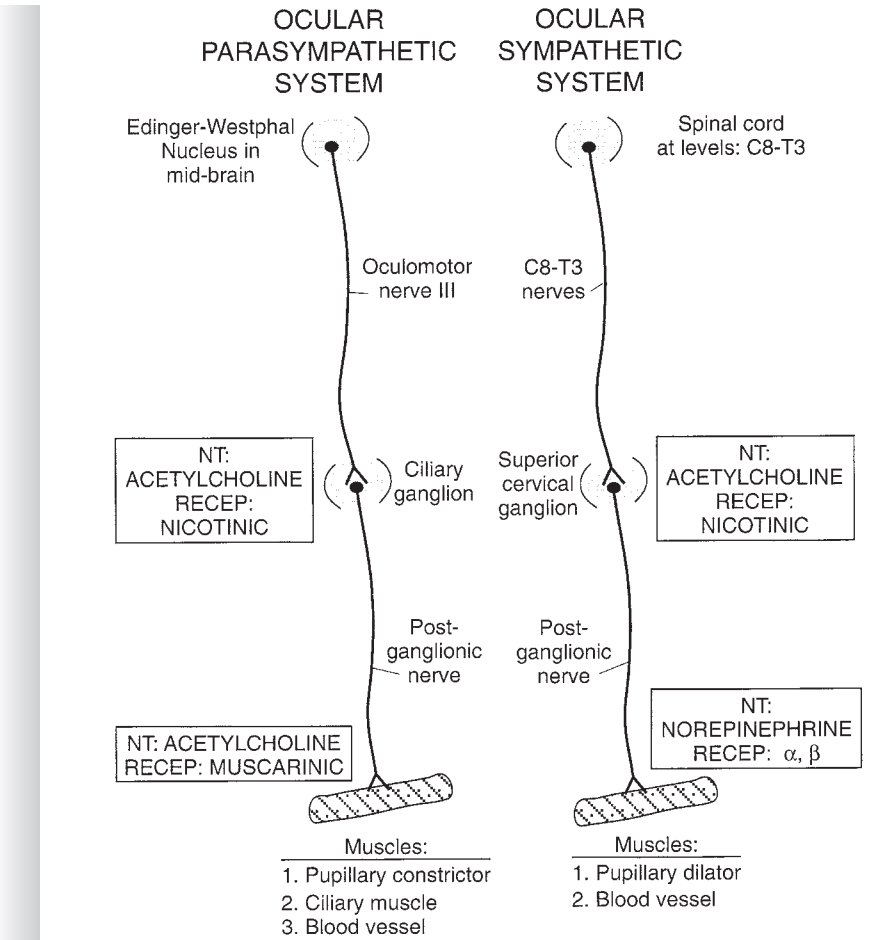


Figure 8–11
Basic anatomical and neurochemical properties of ocular autonomic nerves. ➤ These nerves also supply innervation for tear production (especially the parasympathetic division) and eyelid tension. Note the difference in neurotransmitters and receptors in the terminal nerves of each division.

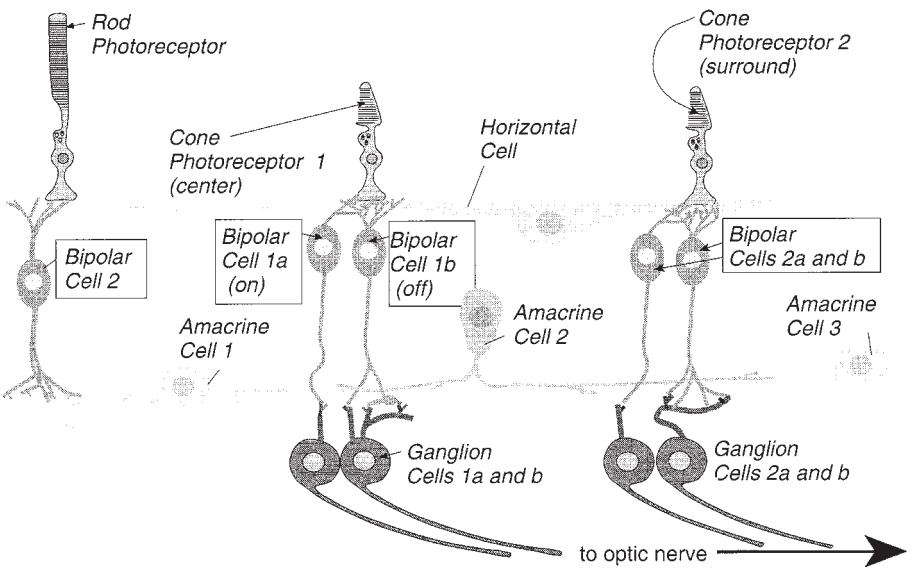


Figure 8–12
Schematic outline of the principal neurons of the retina. ➤ Although most neurons are connected by classical synaptic junctions using neurotransmitters, some make use of direct chemical ion transfer by use of gap junctions (not shown). These junctions include rod-to-rod, rod-to-cone and cone-to-cone connections. Also not shown here are interplexiform cells and the glial cells of Müller. The former transfer signals from amacrine cells to horizontal cells. The latter act as cell insulators, retina boundaries, and maintenance cells for the photoreceptors. See text for other cell functions.

there exists very sophisticated modulation systems that are facilitated by horizontal, amacrine, and interplexiform cells. The retinal cell-to-cell synapse itself has a complex structure that has some similarity to the synaptic structures found in the hair cells of the ear. A typical *cone* photoreceptor *triad synapse* is shown in Figure 8–13. This synapse has three important features to note: (1) several postsynaptic nerve processes share the synapse; (2) the neurotransmitter, glutamate, serves the postsynaptic receptors of all the processes; and (3) vesicle fusion into the presynaptic membrane is enhanced by a *synaptic ribbon*. It is quite common for several nerves to receive input from a single cone photoreceptor. In fact, the *pedicle* of a cone photoreceptor contains many *triad synapses* such that the photoreceptor may serve several bipolar cells and communicate with a number of adjacent photoreceptors by way of horizontal cell processes. The *rod* photoreceptor is more succinct and has only a single triad synapse at the end of its presynaptic process (*spherule*). The glutamate neurotransmitter in these synapses is unusual in that its constant release is necessary to maintain the synapse in the *inactive state*, that is, to prevent the postsynaptic fibers from depolarizing. A view of a typical photoreceptor synapse is instructive (see Figure 8–13). The figure shows a triad synapse for a cone photoreceptor. Here three nerve processes can be seen buried in the synaptic cleft: two horizontal cell processes and one bipolar cell process. In the figure, glutamate neurotransmitters are being constantly released in the dark-adapted state. This constant release of neurotransmitters is being facilitated by a synaptic ribbon apparatus whose structure and function has only recently begun to be understood (Schmitz, Königstorfer, Südhof, 2000). A protein named RIBEYE (synaptic *rib*bon protein of the *eye*), thought to make up an essential part of the ribbon structure, binds to synaptic vesicles that hold the neurotransmitter. RIBEYE transports the vesicles to the synaptic membrane at a rapid rate in order to facilitate their release. This protein is composed of four domains with a molecular weight of approximately 120 kD. Two identical A domains are considered essential to the formation and stabilization of the ribbon structure. Two identical B

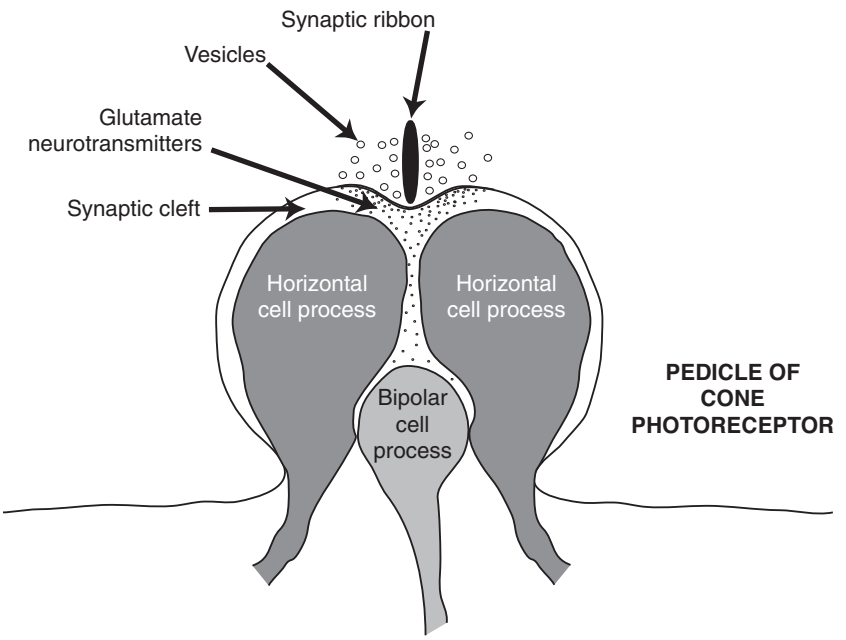


Figure 8–13
Triad synapse found on the pedicle of a cone photoreceptor. ➤ The name "triad" indicates that three cell processes synapse with the photoreceptor. However, many synapses of this type have more than three processes in the synaptic invagination. The synaptic ribbon is a protein complex that facilitates the constant release of glutamate into the synaptic cleft. (Adapted from Oyster CW: *The Human Eye. Structure and Function.* Sunderland, MA, 1999, Sinauer.)

domains are considered responsible for actual binding to the presynaptic vesicles in an undetermined manner. An active zone, at the bottom of the ribbon adjacent to the cleft membrane (not shown in the figure) is the location where vesicle fusion with the membrane takes place. In fact, many different proteins are known to be involved in both the function of the ribbon synapse and the presynaptic membrane itself (Morgans, 2000). As with other synapses, calcium channels facilitate the process of vesicle fusion and neurotransmitter release. However, the channels are not the typical channels found in conventional synapses and may not represent a channel type found in any other part of the nervous system (Taylor, Morgans, 1998). The function of these channels is to induce a partial *decrease* in the release of glutamate with light. That is, they slow the ribbon fusion device described above.

The most direct route of a light signal passing through the retina (*on-center mechanism*) would proceed from a *cone photoreceptor* onto a *bipolar cell* and then onto a *ganglion cell*. From the ganglion cell, the signal leaves the retina and proceeds to the *lateral geniculate nucleus* where nerves (optic radiation fibers) depart that nucleus and send their processes to the *primary visual cortex of area 17*. Such a pathway, here limited to just the retina, is shown in Figure 8–14. The pathway proceeds from the cone photoreceptor via synapse 1 (all synapses are circled) to bipolar cell 1a and on to the ganglion cell by way of synapse 2. The neurotransmitter of synapse 1 (and of all rod and cone photoreceptors) is glutamate (glutamic acid) (Masland, 2001). In the dark, or in dark-adapted conditions, both photoreceptor types continuously release their neurotransmitters to receptors located on bipolar cells. As previously mentioned, this is rather unusual since nerve presynapses generally release little or no neurotransmitter when inactive. This situation is the direct result of the continuous flow of Na^+ ions into photoreceptor outer segments and is referred to as the "dark current" (refer to Figure 6–10A and B). When the Na^+ ion flow is interrupted by light transduction, the resulting hyperpolarization (i.e., build-up of net negative charge) in the photoreceptor decreases the release of glutamate by opening the Ca^{+2} ion channels mentioned. Note that this role of Ca^{+2} ions at the synapse

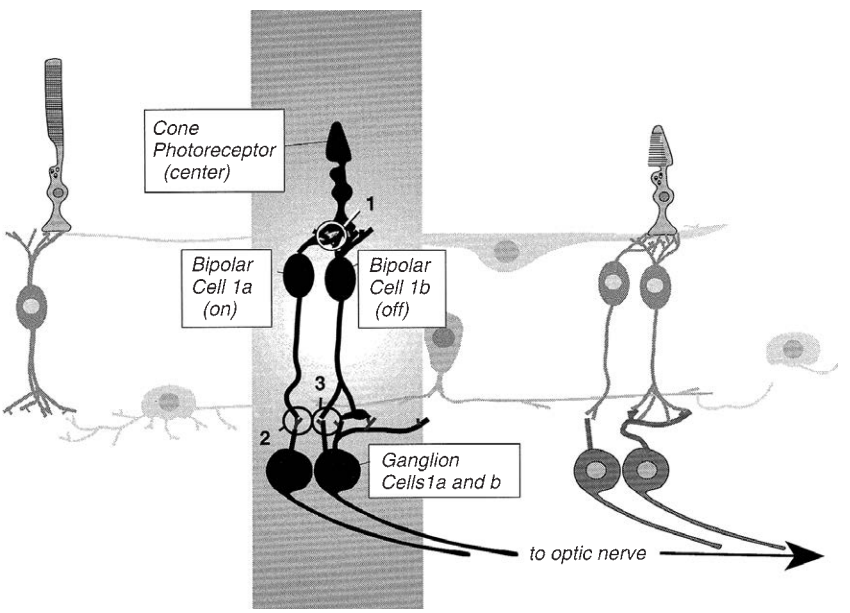


Figure 8–14
Principal cells and synapses involved in cone center *on* and *off* light reception. ➤ Area outlined in gray. The signals are sent in sequence to the blackened cells as described in the text.

should not be confused with the multiple roles that Ca^{+2} has in the outer segments (see Chapter 6)

The resulting *decrease* in glutamate release causes cation channel receptor proteins for Na^{+} to open indirecty in the bipolar *postsynaptic* membrane via a cGMP channel protein. This, in turn, allows the release of bipolar cell neurotransmitters to ganglion cell receptors at synapse 2 (Kandel, Schwartz, Jessell, 2000). The specific neurotransmitter in all bipolar cells is also Glu and is not released when the cell is not stimulated contrary to the condition for photoreceptors (Euler, Masland, 2000).

The *off signal mechanism* is a second direct route that occurs when light is turned off or suddenly decreased. It proceeds by synapse 1 to bipolar cell 1b (see Figure 8–14). When light is present, the receptor maintains closed channel proteins for Na^{+} (see Figures 6–9 and 6–10). As a result of this, the bipolar cell hyperpolarizes and decreases its release of glutamate to its ganglion cell at synapse 3 just as a photoreceptor would do in the presence of light. When light is turned down or turned off, the release of Glu from the cone presynapse, opens Na^{+} channels directly in the *postsynaptic* membrane that, in turn, depolarizes the bipolar cell and causing it to release Glu tonically (temporarily) in its synapse with its ganglion cell. This action *activates* the appropriate ganglion cell (3 in the figure) just as depolarization of a bipolar cell occurred with the on center mechanism. The mechanisms and neurotransmitters involved are summarized in Figure 8–14 and Figure 8–15.

An indirect deactivation mechanism also exists and involves partial inhibition of activated ganglion cells that are controlled by a so-called "surround" physiology. This includes light signals received by neighbor cone photoreceptors. One purpose of an indirect mechanism is for the operation of visual acuity; that is, the ability to distinguish borders or edges of a visualized object. In Figure 8–16, when a *greater amount of light* is obtained by an adjacent (or surround) cone photoreceptor, the synapse from that cone photoreceptor sends the signal in two directions. The most direct route is to bipolar cell 2a as usual. There the signal will produce the same action as the cone photoreceptor in Figure 8–14 using the same neurotransmitters and receptors. In addition, the inhibition of glutamate release from a surround cone photoreceptor (i.e., the neighbor with the stronger light signal) activates a horizontal cell that will release GABA as a neurotransmitter onto a center cone photoreceptor (the one having weaker light reception at synapse 2). The receptor protein for GABA (whose characteristics are unknown) then allows a continued release of glutamate from the center cone photoreceptor (as though a normal dark current existed) and, therefore, *prevents* release of neurotransmitter from the bipolar cell 1a at synapse 3 (on). In other words, it maintains an off signal in the center on ganglion cell (Ehinger, Dowling, 1987; Berson, 1992; and Kandel, Schwartz, Jessell, 2000). What is "center" and what is "surround" is relative to the strength of the light signals received by adjacent photoreceptors.

Rod photoreceptors, whose physiology is more concerned with detection of low levels of light, are indirectly connected to ganglion cells by way of amacrine cell synapses. This is shown in Figure 8–17. This is an indirect pathway, in which rod photoreceptors piggyback on to cone bipolar cells. It suggests that rod photoreceptors are a more recent evolutionary development of the retina (Masland, 2001). The sequence of connections is: rod photoreceptor → rod bipolar cell (synapse 1) using glutamate as a neurotransmitter; rod bipolar cell → amacrine cell (synapse 2) using glutamate as a neurotransmitter; amacrine cell → cone bipolar cell (on synapse 3)

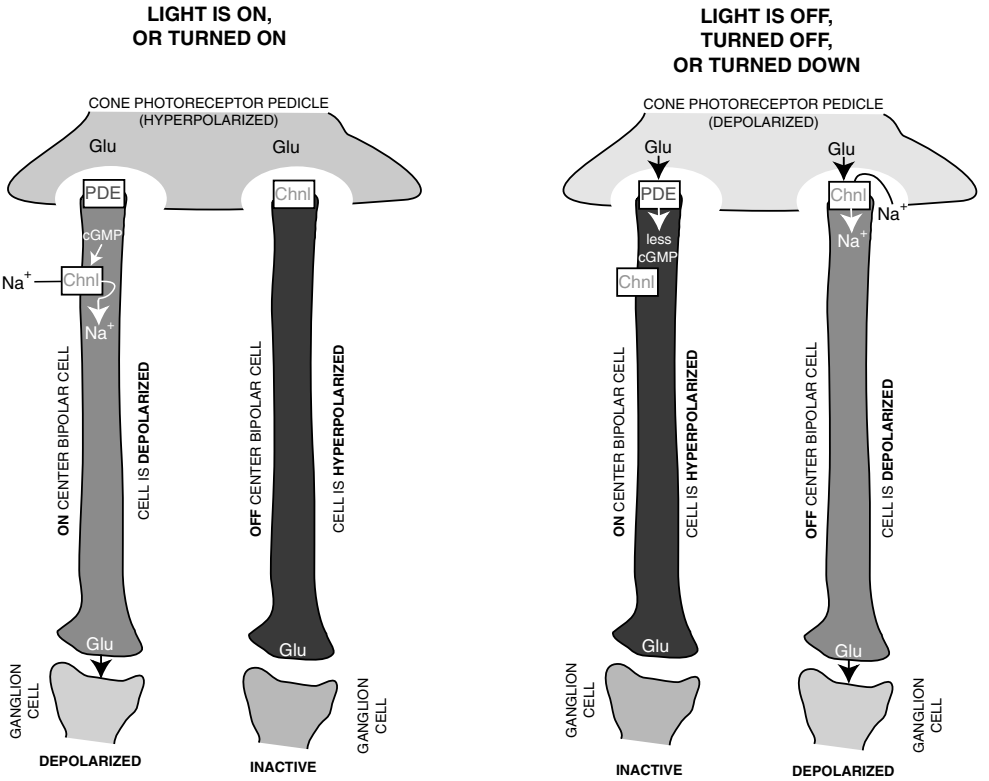
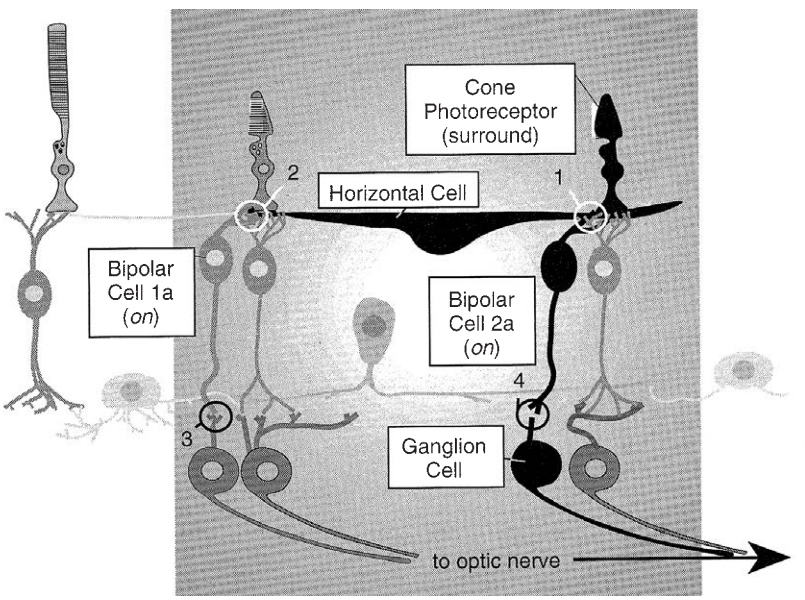


Figure 8–15
Biochemical and physiological diagram of neurotransmission differences between "on" and "off" mechanisms when light is turned on or off. ➤ Note, in particular, how the release (light off) of Glu affects the two classes of bipolar cells differently and, likewise, how the nonrelease (or decreased release) of Glu differentially affects the two classes of bipolar cells. This is largely due to whether the Na^+ channel protein is present as a NT receptor for Glu or whether the channel protein function is mediated by cGMP binding (i.e., phosphodiesterase, PDE, functions as a receptor for Glu).

Figure 8–16
Principal cells and synapses involved in cone surround "on" light reception. ➤ Area outline in gray. Signals are sent by two pathways (blackened cells) in which the pathway *via* the horizontal cell will inhibit the signal from an adjacent photoreceptor. See text for explanation of biochemistry and physiology.



possibly using indoleamine as a neurotransmitter (see Ehinger, Dowling, 1987) and, finally, cone bipolar cell → ganglion cell (synapse 4) using glutamate as a neurotransmitter. All of these pathways, transmitters, and receptors are summarized in Table 8–3.

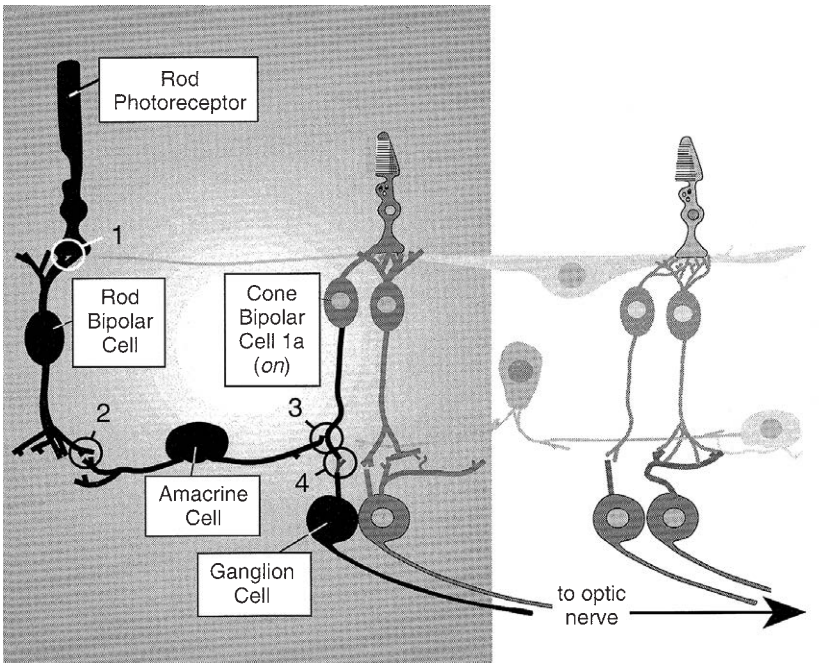


Figure 8–17
Principal cells and synapses involved in rod light reception. ➤ Area outlined in gray. Signals sent through blackened cells. It is important to note that there is no direct connection of rod bipolar cells to ganglion cells. See text for explanation and description of neurotransmitters involved.

TABLE 8–3 ➤ SOME BIOCHEMICAL SYNAPTIC PATHWAYS OF THE RETINA

Type	Synapse (Neurotransmitter)	Receptor Mechanism
Cone, center, on	Photoreceptor → bipolar (less Glu) Bipolar → ganglion (Glu)	Opening of Na^+ channels via cGMP Depolarization; unknown mechanism
Cone, center, off	Photoreceptor → bipolar (Glu) Bipolar → ganglion (Glu)	Closing of Na^+ channels via PDE Depolarization; unknown mechanism
Cone, surround, on	Photoreceptor[1] → horizontal (less Glu) Horizontal[1] → photoreceptor (γABA) Photoreceptor[2] → bipolar (no Glu) Bipolar[2] → ganglion (Glu)	Opening of Na^+ channel proteins Maintenance of Glu release Opening of Na^+ channel proteins Depolarization; unknown mechanism
Rod, low light	Photoreceptor → rod bipolar (less Glu) Rod bipolar → amacrine (Glu) Amacrine → cone bipolar (Indoleamine?) Cone bipolar → ganglion (Glu)	Closing of Na^+ channel proteins Opening of Na^+ channel proteins Opening of Na^+ channel proteins Depolarization; unknown mechanism

[1]This is the indirect pathway to the adjacent cone photoreceptor.
[2]This is the direct pathway to the ganglion cell that is equivalent to the center ON type shown above.

Ocular Neurochemical Pathology

In general, neurotransmission in the eye functions quite efficiently except when disrupted by degenerative conditions (such as *retinitis pigmentosa*) or by nerve lesions outside of the eye (such as *Horners syndrome*). *Parkinsons disease* has also been found to affect the visual system at both the level of the brain and the retina (Hunt, Sadun, Bassi, 1995). In the retina, Harnois and DiPaola (1990) have found a correlation with retina levels of dopamine and visual disturbances of patients with Parkinsons disease. These patients lose some contrast-sensitivity ability that, among other things, decreases their ability to read. Compared to normal individuals with an average retina concentration of 1ng dopamine per mg of protein, untreated Parkinsonian patients were found to have levels of

approximately 0.52 ng dopamine per mg of protein. Dopamine is a neurotransmitter found in some amacrine cells. Although its function was not described in any previous discussion in this text, evidence indicates that amacrine cells (some of which use dopamine) serve as intermediate cells for the lateral transfer of signals across the retina. In short, they appear to be part of an auxiliary system for visual acuity. Still another retina cell type, known as an interplexiform cell, may also use dopamine and have a similar function (Ehinger, Dowling, 1987; Vigh, Banvolgyi, Wilhelm, 2000). Moreover, recent studies, as explained by Nguyen-Legros, Versaux-Botteri, Vernier (1999), suggest that receptors for dopamine (D_1 and D_2 proteins) are widespread throughout all retinal cells. The implications of this in respect to retinal function have yet to be fully understood. However, it implies that dopamine may diffuse and cause effects beyond the confines of synaptic clefts. Therefore, the role of this neurotransmitter may also include neuromodulator functions. This is a role previously unsuspected and one that may be quite subtle in visual functions.

SUMMARY

Neural transmission occurs with the depolarization of nerve plasma membranes. The transmission results with the entry of Na^+ ions into the neuron and ceases with the sequential loss of K^+ ions to the outside of the nerve. This activity is facilitated by cation-gated, channel proteins and is initiated by an incipient, vicinal depolarization. In myelinated nerves, ion movement is limited to nerve nodes such that ion movement causes depolarization to leap from node to node. This causes the transmission rate to increase greatly. At nerve synapses, depolarization is converted to the release of neurotransmitters that diffuse across a small space (cleft) to bind to receptor proteins. This produces either a depolarization or a hyperpolarization in the postsynaptic cell and imitates, in some cells, a hormone mechanism in which G proteins are intermediates producing a physiological response. There are four classes of neurotransmitters: acetylcholine, catecholamines, amino acids, and amino acid derivatives. Of these, acetylcholine and norepinephrine have been very well described.

Acetylcholinesterase is used to degrade acetylcholine in the synapse and inhibitors of this esterase have been used as therapeutic agents to prolong the concentration of acetylcholine in synapses where they occur. In the eye, the inhibitors have been used to control glaucoma and to restore normal pupil size after an eye examination. The two ocular autonomic nervous systems use acetylcholine as a neurotransmitter at their ganglia. Nicotinic acetylcholine receptors are present there and produce a “fast” response. In the ocular parasympathetic division of postganglionic nerves, acetylcholine is also the neurotransmitter, but a “slower” acting muscarinic receptor protein is found on the muscle receptor membranes. These receptors use G proteins. In the ocular sympathetic division of postganglionic nerves, norepinephrine is the neurotransmitter.

There are nine kinds of receptor proteins present: six α-classes and three β-classes. All are "slow" acting and make use of G proteins.

In the retina, a variety of neurotransmitters may be found. However, photoreceptor cells that use glutamate possess an unusual mechanism by which they transmit signals. These cells normally release glutamate when they are inactive. When they detect light, they hyperpolarize and decrease their release of glutamate. Their postsynaptic cells are either hyperpolarized themselves or depolarized depending on their receptor mechanisms. There are several mechanisms by which the retina responds to light and brings about a cascade of neurotransmission in amplified, inhibited or modulated fashions: cone on/off, cone center/surround, and rod dominated. Each system uses a specific sequence of neurotransmitters and receptors through a variety of cells. Dopamine has been found to function in the retina as both a neurotransmitter and a neuromodulator. Its levels are decreased in Parkinson's disease and this has been linked to losses of visual acuity.

PROBLEMS

1. What is the location of the Na^+ ions that trigger the opening of the cation channel protein (as pictured in Figure 8–3)? Explain your answer.

2. Both norepinephrine (produced as a neurotransmitter in the iris) and epinephrine (produced as a neurohormone in the adrenal medulla and released into the circulation) will increase the diameter of the pupil by stimulating the dilator muscle of the iris. The process is called mydriasis. Phenylephrine is a chemical analogue of epinephrine and is used as a topical agent on the cornea to produce mydriasis for ocular examinations. Unlike norepinephrine, the effect of phenylephrine lasts for several hours. Why might this occur?

3. If the gene for the RIBEYE protein, present in ribbon synapses, were defective, what might be the biochemical effect produced at the pedicles of cone photoreceptors? In the same case, what might be the physiological effect on the visual system?

4. When a light is turned off in a room, why is glutamate released from the off center bipolar cells to the off center ganglion cells? What effect does the release of glutamate have on the ganglion cells?

5. What retina effects occur to dopamine in Parkinsons disease? How is overall vision affected?

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CHAPTER 9

Ocular Immunochemistry

Immunology is a major scientific discipline that deals with the manner in which organisms defend themselves against foreign invasion. In respect to humankind, this is usually thought of in terms of bacterial and viral infections. However, any foreign substance may provoke an immune reaction. In fact, under unfortunate circumstances individuals may reject some of their own tissue in what is known as an autoimmune reaction. In the eye, immune reactions are normally limited to the region of the anterior segment. Here, the discussion will be focused on immunochemistry and, in particular, the biochemical substances: *immunoglobulins* and *complement* with a short treatise on *inflammation.*

Review of Immunoglobulins

Immunoglobulins are relatively large proteins with a minimum number of four polypeptide chains in each protein. Their function is to bind to a foreign substance in order to identify it and initiate an immunological defense. On the biochemical level, the foreign substance is either a molecule or a portion of a molecule. More will be discussed about this later. The history of the discovery of immunoglobulins goes back to 1847 when H. Bence Jones isolated parts of immunoglobulins from the urine of a patient who had high levels of urinary proteins (Day, 1990). What Bence Jones had found were the lighter of the four polypeptide chains of immunoglobulins and, subsequently, these became known as Bence Jones proteins. Immunoglobulins (also known as antibodies) have an overall conformation similar to the letters "Y" or "T". More complex forms are variations of these basic shapes. Figures 2–5 through 2–9 in Chapter 2 show the primary, secondary, tertiary, and quaternary structures of immunoglobulins. However, it is now necessary to explain the conformation of immunoglobulins in greater detail. Figure 9–1 shows such a detailed figure of immunoglobulin G (IgG). The four polypeptide chains are shown in various shades of black and gray. The heavier chains have four domains, each connected by random coil sequences, while the lighter chains have just two domains apiece. Each domain is partially held together by an intrachain disulfide bond (S-S) of the type discussed in Chapter 2. Both heavy and light chains are associated by interchain disulfide bonds. Note that the two heavy chains are also bound to oligosaccharides (see Chapter 4).

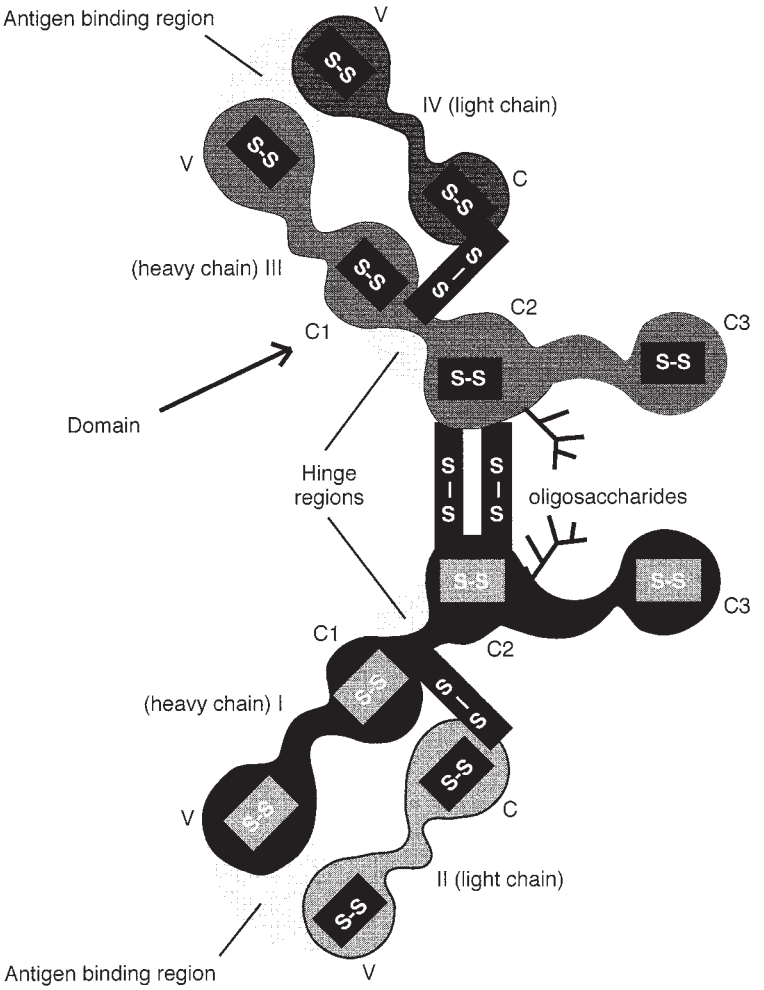


Figure 9–1
A detailed diagram of Immunoglobulin G. ➤ Shown here is the subtype: IgG1. The other subtypes vary in number and position of disulfide bonding. Subtype IgG3 has a large C2 domain on each heavy chain. See text for explanation.

Each domain is labelled as "C" (for *constant*) or "V" (for *variable*). The amino acid sequence in each C domain is always the same for the particular immunoglobulin that it constitutes. However, the amino acid sequence in each V domain is determined by the *antigen* (foreign substance) with which the *antibody* must bind. The antigen binding region denotes the space between two variable domains where binding takes place. For simple immunoglobulins, there are two binding regions per molecule. The hinge regions represent sequences of amino acids between C1 and C2 of the heavy chains that can twist or flex (Nisonoff, 1985 and Day, 1990). In this way, the immunoglobulin molecule can twist back and forth from a "Y" to a "T" shape in order to bind to one or two antigenic molecules. All the domains of an immunoglobulin have specific roles in addition to the binding role of the V domain (Figure 9–2). For example, the C1 and C2 domains can bind to complement (to be discussed) while the C3 domain can bind to receptor proteins on *macrophages* and *monocytes* (white blood cells). The light chain C domain, however, seems to be primarily structural in function (Roitt et al, 1998). Three roles have been assigned to the oligosaccharides attached to immunoglobulins: increase in water solubility; protection against enzymatic degradation; and facilitation in secretion from cells producing them (Nisonoff, 1985).

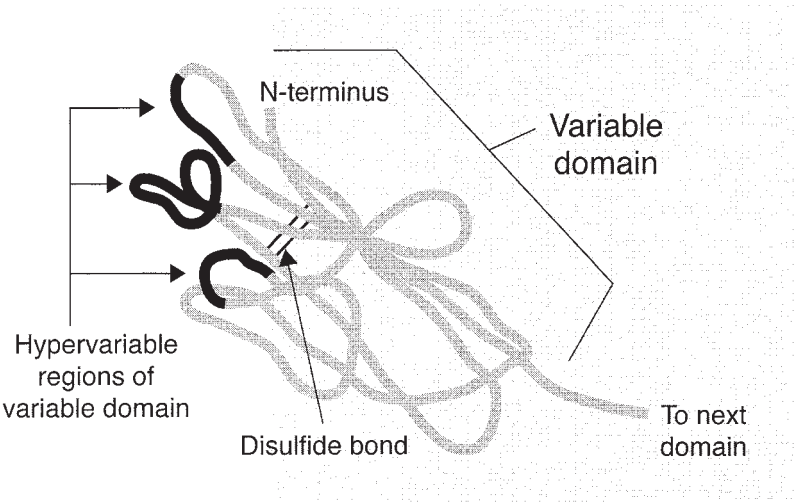


Figure 9–2
The variable domain of an immunoglobulin. ➤ Two classes of sequences are found here: hypervariable (*dark shading)* and occasionally variable or conserved (*light shading*). The hypervariable regions are specifically synthesized to bind to one or a very few antigens. The conserved sequences are synthesized primarily to maintain the conformation of the domain and are occasionally varied to help the hypervariable regions align optimally with an antigen. (Adapted from Alberts et al., 1989.)

There are five major classes of immunoglobulins: A, D, E, G, and M. Their characteristics are summarized in Table 9–1 and are described in detail below.

Immunoglobulin A tends to form a two molecule unit (dimer) when secreted by cells that make it. As shown in Figure 9–3, secretory IgA is joined stem-to-stem by a polypeptide called a "J" chain (15,000 daltons) where J stands for "joining." Another polypeptide, termed an "S" chain (60,000 daltons) where S stands for "secretory," is wrapped around the two immunoglobulin tetramer units. The S chain prevents proteolytic degradation of the immunoglobulin over and above the protection provided by the oligosaccharides. This is necessary as the precorneal tear film and other external secretions are particularly rich in proteolytic enzymes.

Immunoglobulin D is considered to be significant in connective tissue defense. It makes up less than 1% of the total plasma immunoglobulins, but is present on the membranes of many *B cells*. As such it may play a role in lymphocyte differentiation (Roitt et al., 1998). It is not normally found in the anterior ocular fluids.

TABLE 9–1 ➤ THE MAJOR IMMUNOGLOBULIN CLASSES: CHARACTERISTICS AND CONCENTRATIONS

Characteristics	IgA	IgD	IgE	IgG	IgM
Subclasses (No.)	4	None	None	4	None
Molecular weight (kD)	160[1]	170	190[2]	146[3]	970[2]
Carbohydrate (%)	7–11	10–12	12	2–3	9–12
Primary location	Secretions	Blood vessels	Mast cells	Most tissues	Blood vessels
Serum[4,7] (μg/mL)	760–3900[5]	40	≪1	6500–15,000	900–3450
Tear film[4,8] (μg/mL)	1930[6]	0	0	4	18
Aqueous[4,9] (μg/mL)	10	0	0	70	0
Lens[9] (μg/g)	0	0	0	0	0

[1]Secretory IgA, 405 kD.
[2]H has a C4 domain on its heavy chains.
[3]IgG3, subclass, 165 kD.
[4]Normal valve, but increases with foreign invasion.
[5]Secretory IgA, ≤0.05 μg ml.
[6]Value for secretory IgA; dimeric and monomeric IgA also are present.
[7]Data from Silverman et al. (1986).
[8]Data from Fullard and Tucker (1991).
[9]Data from Allansmith et al. (1973).

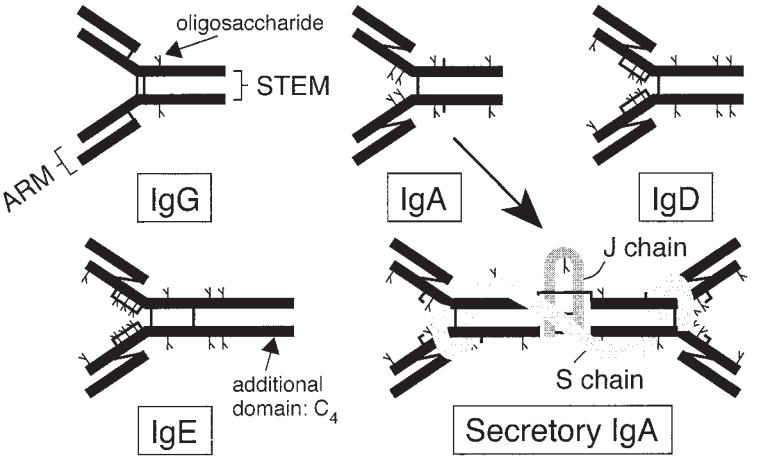


Figure 9–3
Comparison of immunoglobulins A, G, D, and E. ➤ Note the variations and numbers of disulfide bonds (lines connecting the chains) and oligosaccharides (small y shaped lines). IgE has an additional domain on each heavy chain with which it binds to mast cells. In the precorneal tear film, IgA is found principally as secretory IgA. (Adapted from Roitt et al., 1998.) See text for explanation.

Immunoglobulin E seems to occur in very small quantities in the precorneal tear film. However, its role there is imperfectly understood. It is known that it will cause the release of histamine from mast cells after binding to their surface. In other parts of the body this activity is associated with hypersensitivity reactions in asthma and hay fever as well as the eventual rejection of internal parasites (Roitt et al., 1998).

Immunoglobulin G forms about 80% of all immunoglobulins found in blood serum and is found in significant quantities in both precorneal tear film and aqueous humor. IgG is the smallest of the immunoglobulins and generally is found in interstitial fluids in the highest quantities. It is even able to diffuse through the cornea itself. After a second exposure to a particular antigen, IgG is made in high quantities to respond to an infection. Accordingly, IgG is considered to be the second line of defense while IgA is the first line of defense to a specific antigen. IgG can bind to surface receptor proteins of B lymphocytes in order to stimulate those cells (B lymphocytes are the cells that synthesize immunoglobulins). Immunoglobulin G is the second most important antibody molecule in ocular tissues and is found in both precorneal tear film and aqueous humor.

Immunoglobulin M is a very large molecule composed of five tetrapeptide units (a pentamer) as well as a J polypeptide, the same molecule as found in secretory IgA. Its structure is shown in Figure 9–4. One IgM unit has ten antigen binding sites and is one of the first immunoglobulins to be synthesized after the immuno "recognition" of an antigen, that is, after IgA binds to the antigen. IgM tends to agglutinate, that is, to form large molecular complexes around foreign bacteria. This immunoglobulin is found in the precorneal tear film.

At the center of all immunoglobulin function is the antigen-antibody reaction that takes place between the ends of the variable domains of the heavy and light chains (the antibody binding region as seen in Figure 9–1). An *antigen* (Greek: αντιγενειν antigenein "to make against") can be any substance that will produce an *antibody* (immunoglobulin) to which it will bind. This includes, of necessity, binding to antibodies that are part of the surface of B cells and T cells of the immune system. It is important to emphasize that for most immune antigen-antibody reactions, the immunoglobulin binds to only a *portion* of the antigen. That portion that is bound is called an *antigenic determinant* or an *epitope*. The epitope may be a short peptide, oligosaccharide,

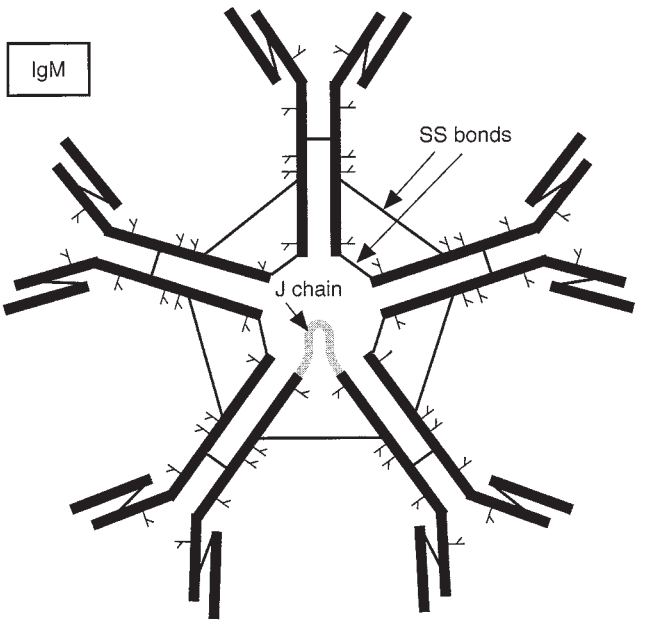


Figure 9–4
The large IgM molecule. ➤ This figure is suggested by some electron photomicrographs. However, the individual Ig units may not be in the same plane around the disulfide bond ring that connects them. (Adapted from Roitt et al., 1998.)

a lipid molecule, or even a nucleic acid fragment. This is usually the case with bacteria and viruses in which an antibody binds to a portion of the prokaryotic surface. In addition to such organisms, polypeptides themselves (>1000 daltons) and large polysaccharides (>1,000,000 daltons) may be antigenic (Bach, 1982). Some substances may also become antigenic when combined with a larger carrier molecule. These substances are called: *haptens* (Greek: ‘απτειν “to fasten”) and include a number of small organic chemicals. Among these are substances containing *azo groups* (–N=N–) that form colored *diazonium complexes* with the aromatic amino acids of their carrier proteins. These colored antigens can be used to determine the number of haptens present (spectrophotometrically) when studying antigen–antibody reactions. Such assays were a precursor to the extremely sensitive radioimmunoassays that were later developed by Berson and Yalow (see Yalow, 1978). In radioimmunoassays, antigen–antibody reactions are coupled to radioactive compounds to give extremely sensitive assays (to 10^{-15} *M*) for hormones and other substances present in low concentrations in tissues. The basic method for such assays is shown in Figure 9–5.

The nature of the antigen–antibody reaction is complex and involves all of the noncovalent forces described under protein structure formation (see Chapter 2) as well as the correct conformational requirements as needed, for example, between enzyme and substrate (see Chapter 3). For review and reinforcement, these factors are given in Table 9–2. It is emphasized that the antigen–antibody reaction is the sum of all these factors. Naturally, some substances will produce better reactions than others. Just like the nature of substrate–enzyme binding, each antibody is specific for only one or a few antigens. The kinetics of binding are similar, therefore, to the kinetics of substrate–enzyme affinity.

Genetically, it is of great interest to understand how an antigen (or antigenic determinant) can specify the synthesis of immunoglobulins that bind to one or very few, similar antigens. This process is presently only

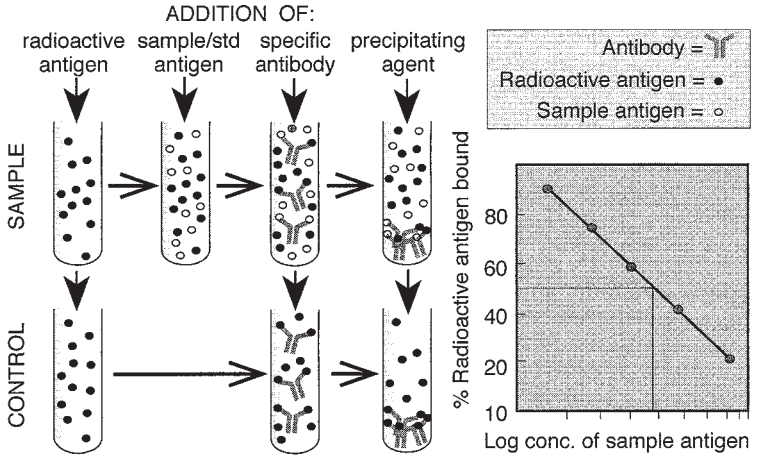


Figure 9–5

A radioimmunoassay combines the sensitivities of both radioactive and immunochemical reactions. ➤ In the assay, a radioactive antigen competes with a nonradioactive antigen for the possession of a binding site on an antibody. The assay ultimately measures the radioactivity on the bound antibody. Accordingly, the greater the binding of a nonradioactive antigen on an antibody, the lower the level of radioactivity measured. The control gives the reference radioactivity for 100% binding of radioactive antigen. In the assay, standard, known amounts of nonradioactive antigens are reacted in the upper row and compared with the control. From this, a standard curve is constructed (shown on right). One or more unknown samples are run. The percent of radioactivity in the antibodies are measured and intersected with the standard curve to obtain the concentration of antigen. This method, for example, is very useful in determining very low concentrations of hormones present in blood.

partially understood (Hay and Westwood, 1998). As mentioned, B cells or B lymphocytes are the site of immunoglobulin synthesis. In the adult human, B cells are formed in the bone marrow and then transported to lymphoid tissues prior to full development as B plasma cells. When they leave the bone marrow they can only produce nonspecific IgM and IgD that binds to the cell surface membrane at the stem side of the molecule (Banchereau and Rousset, 1992). In the lymphoid tissues, the B cells are induced to make specific immunoglobulins by the binding of antigens to

TABLE 9–2 ➤ FACTORS THAT CONTRIBUTE TO ANTIGEN-ANTIBODY REACTIONS AT THE ANTIGEN-BINDING REGION OF AN IMMUNOGLOBULIN

Factor	Type	Characteristic
Chemical bond	Hydrogen	Sharing of a hydrogen atom due to partial charges between two atoms
	Electrostatic	Full positive and/or negative charge(s) on antigen and/or antibody, respectively
	van der Waals	Induced partial charge between two atoms (not involving hydrogen)
Nonpolar bond	Hydrophobic	Association of molecules away from a water (polar) environment
Conformation	Lock and key	Complementary shape of antigen and hypervariable part of V domain

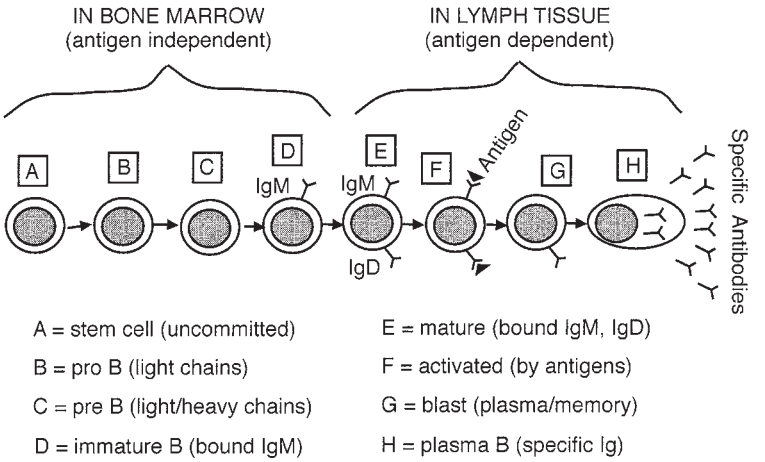


Figure 9–6
The development of plasma B cells from uncommitted stem cells. ➤ The cells begin by making surface bound antibodies which induce (by antigen binding) the production of large quantities of specific antibodies. (Adapted from Banchereau and Rousset, 1992.) See text.

the IgD and IgM that is attached to the B cell plasma membrane surface (Figure 9–6). The synthesis of immunoglobulins by these developing B cells, nonetheless, begins in the bone marrow when the cells become "committed" to the synthesis of light chains initially, then heavy chains to produce IgM, then IgD immunoglobulins. These immunoglobulins, as mentioned, have hydrophobic tails (the tips of the stems) that bind to the membranes of the B cells. When antigens encounter IgM and IgD bound to the nascent B cells, they bind to these antibodies and transmit a signal to begin specific antibody synthesis. Such short-lived, high output B lymphocytes are called *plasma cells*.

Figure 9–7 shows the current understanding of how the selection process functions, at the genetic level, for the synthesis of so many different kinds of antibodies. This process, which represents the *antibody diversity hypothesis,* is shown in the figure just for the light chains of immunoglobulin G. The top diagram represents uncommitted B cells present in the bone marrow (or in the fetal liver). In this case the DNA has present the codes for 300 separate sequences of the variable domain (V genes). It also contains, downstream, the codes for four separate peptides that will connect or join the V and C domains (J genes). It should

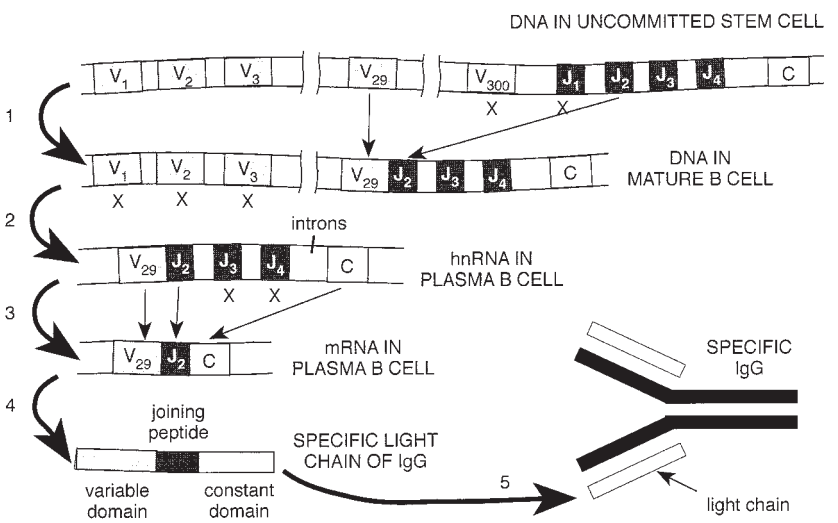


Figure 9–7
The genetic component of the antibody diversity hypothesis for the production of light chains of specific antibodies. ➤ In the determination of a specific antibody, the cell deletes unwanted exons as in steps 1, 2, and 3. In this case exons: V_{29}, J_2, and C have been joined as mRNA. In step 4, the specific light peptide is synthesized and incorporated into IgG (step 5). See text for explanation.

be emphasized that the J genes do *not* code for the J protein found in secretory IgA and IgM, but for a joining peptide between the V and C domains. Lastly, and still further downstream, is the code for the constant domain (a single code known as the C gene). When the stem cell becomes committed to B cell development, the DNA containing all these codes recombines by excision (cutting out) of part of the DNA. In one example, as shown by the next lower diagram of Figure 9–7, all of the V genes 30 through 300 have been excised and V gene 29 has been directly joined to J gene 2. J gene 1 was also excised. Even though V genes 1 through 28 are still present, they are not directly connected in sequence to the J2 gene and are, therefore, inactive. This is also true of the J3 and J4 genes. This is the form of DNA that exists in a B cell that is ready to begin IgG synthesis. In the transcription state of forming hnRNA, only V gene 29, J genes 2, 3, 4, and the C gene are transcribed. When hnRNA is processed to mRNA, all the introns, as well as the inactive J3 and J4 exons are spliced out of the gene sequence. This leaves the V29, J2, and C codes for the specific light chain of IgG to be translated and assembled into an IgG, which will bind to a specific antigen. The processing of the heavy chains is similar, but more complex.

New information about the antibody diversity hypothesis also suggests that: somatic mutations of the V genes may occur; *pseudo V genes* may become incorporated into a V gene (creating a new V gene); and extra nucleotides may become incorporated into V genes during a splicing mechanism (Hay, Westwood, 1998). In the consideration of all the possible combinations of heavy and light chains, as well as three variations of splicing V and J genes, it may be possible for cells to make over 10^{20} different kinds of antibodies from committed and activated B cells (at least in mice)! We truly do not know the variability in humans.

Ocular Immunoglobulins

As can be seen from Table 9–1, there are three antibodies that occur in significant amounts in ocular tissues: IgA, IgG, and IgM. The IgA in the precorneal tear film is primarily the secretory type (see Figure 9–3). It is assembled from dimeric IgA in the lacrimal gland epithelial cells. These epithelial cells synthesize the S chain polypeptide that surrounds the IgA molecules and protects them from degradation (Smolin and O'Connor, 1986). IgM is limited in its penetration of the cornea due to its large size. IgE is present, but since it normally is tightly bound to mast cells, its occurrence in free form is unlikely in the normal cornea (note also its low concentration in free form in blood serum, Table 9–1). It is emphasized that concentrations of ocular immunoglobulins, as shown in the table, are for the eye when antigenic presence is not pathological. The occurrence of immunoglobulins in the eye is fairly limited to the precorneal tear film, the aqueous, the ocular blood vessels, and the outermost tissues such as the cornea and the sclera. In the deeper ocular tissues, such as the lens, vitreous and nonvascular parts of the retina, no antibodies are normally found. In the case of tears, the determination of both normal and infected immunoglobulin concentrations has been frustrated in the past by ignorance of the variations that occur with resting and stimulated (reflex) tear secretions. In general, the more that tear production is stimulated (e.g., by emotional or chemical means), the lower the content of immunoglobulins and other protein contents due to reflex dilution. The manner of collection of tears for assay of immunoglobulins is important:

TABLE 9–3 ➤ COMPARISON OF IgG SUBTYPES IN THE TEARS OF INDIVIDUALS WITH AND WITHOUT HERPES INFECTIONS

	Concentration of IgG[1] (μg/mL tears)	
Individual	IgG1	IgG4
Control (uninfected, n = 5)	2.01	0.08
Herpes (infected, n = 9)	2.22	0.56

Data from McBride and Ward (1987).
[1]The tears in this study are presumably stimulated when compared with the unstimulated tear data contained in Table 9–1. IgG2 and IgG3 subtypes are not found in the tears of individuals.

(1) tears collected with small sponges tend to be contaminated with conjunctival secretions; and (2) tears obtained with filter paper (Schirmer strips) may cause reflex stimulation. However, tears that are drawn into glass capillary tubes do not have these drawbacks if the collection is done carefully.

As antigens invade either the ocular surface or enter the tissue itself, immunoglobulin concentration is considerably altered. For example, when the corneal surface is exposed to an antigen such as herpes virus, IgM is the first antibody to respond from the precorneal tear film. Its levels increase. This is followed by increases in IgG and secretory IgA (Smolin and O'Connor, 1986). As the infection progresses, herpes antigens become present not only in the cornea, conjunctiva, and tear film, but also in the iris and the trigeminal ganglion. It may be recalled from Chapter 7 that nerve ganglia are sites of storage of inactive forms of this virus. In the cornea, infected cells may have some of the viral antigens incorporated into their plasma membranes. This action will trigger antigen–antibody reactions against the cells themselves. Those viruses that are still extracellular are also attacked by antibodies, especially by secretory IgA, which hinders the virus from attaching to uninfected cells. IgG causes *phagocytosis* of the antigen virus as discussed in the next section and in the section on inflammation. McBride and Ward (1987), were able to measure the quantities of IgG subtypes that respond to herpes simplex invasion. Their findings are shown in Table 9–3 and indicate a sevenfold increase in the subtype IgG4. A second example of immunoglobulin level alteration occurs with patients having *acute hemorrhagic conjunctivitis*. There the level of IgG can reach 1300 μg/ mL versus a normal level of approximately 4 μg/ mL as seen in Table 9–1 (Langford et al., 1995). In a third example, Aghayan et al (2000), have shown that free IgE itself can be detected in the tears of individuals susceptible to pollen-born allergens.

Complement

Complement is a collection of related proteins of which most are either proteolytic enzymes or membrane binding proteins. Complement has two principal immunological functions: (1) the direct destruction of foreign organisms by membrane lysis, and, (2) the activation of phagocytosis (cell engulfment) following *chemotaxis* (cell movement induced by chemicals). As such, complement is an immune biochemical mechanism that is capable of destroying Gram negative bacteria and some

TABLE 9–4 ➤ THE PROTEINS OF COMPLEMENT ACTIVATED BY THE CLASSICAL PATHWAY[1]

Protein	Molecular Weight (kD)	Number of Chains	Characteristics
C1q	410	18	Ig stem-binding
C1r	83	1	Proteolytic zymogen
C1s	83	1	Proteolytic zymogen
C2	110	1	Proteolytic/chemotactic
C3	190	2	Proteolytic/chemotactic
C4	206	3	Proteolytic zymogen
C5	190	2	Membrane/chemotactic
C6	95	1	Membrane-binding
C7	120	1	Membrane-binding
C8	163	3	Membrane-binding
C9	79	1	Membrane-channel

[1]The classical pathway requires antibodies for activation; the alternate pathway does not.

parasites as well as neutralizing some viruses. Compare this with the activity of lysozyme as discussed in Chapter 3. The functions of complement will be presented in detail after a discussion of the molecular properties of complement and *complement activation*. Complement in the classical pathway (started by antigen–antibody reactions) is constituted by 11 separate and distinct protein types known as: C1q, C1r, C1s, C2, C3, C4, C5, C6, C7, C8, and C9. However, since more than one protein of the same type is used in the activation of one complement complex and its byproduct formation, a very large total number of proteins are involved. The mechanism is a cascade amplification of a magnitude similar to that caused by cyclic nucleotides. The properties of each protein are given in Table 9–4.

The sequence of *complement fixation* (or activation) begins with the binding of the C1 complex (C1q, two C1s, and two C1r molecules held together by Ca^{+2} ions) to the stem portion of either IgG or IgM molecules, which are themselves bound to the membrane antigens. This is shown in Figure 9–8. Upon binding to the immunoglobulin, a conformational shift of the C1 complex induces *autocatalytic properties* in C1s and C1r. That is, the proteins cause their own activation and C1s molecules ultimately become a proteolytic enzyme for the next substrate proteins in the sequence: C4 and C2 (Figure 9–9). The C4 is broken into fragments C4a and C4b. C4b binds to the membrane in the vicinity of the antigen–antibody complex while C4a does not participate in the sequence. The C2 is also broken into a C2a and C2b by C1s. The C2a binds to the C4b molecule and becomes an enzyme (C3 *convertase*) to split C3 into C3a and C3b. The C3b fragment associates with C4b and C2b to form still another enzyme: C5 convertase. The C3a fragment has a different role to which we shall return. C5 is split into C5a and C5b fragments. The C5b fragment binds to a nearby region of the antigen membrane and initiates the formation of a complex that contains bound C6, C7, C8, and several molecules of C9. The C9 proteins are *channel forming proteins*. In order to be completely effective, the C9 protein channels must eventually pierce the cytoplasmic membrane of the bacteria (Figure 9–10). This complex forms a channel in the antigen membrane through which Na^{+} and water molecules quickly pass. The formation of the channel and rapid movement of water and ions causes the lysis of Gram negative bacterial membranes due to the osmotic

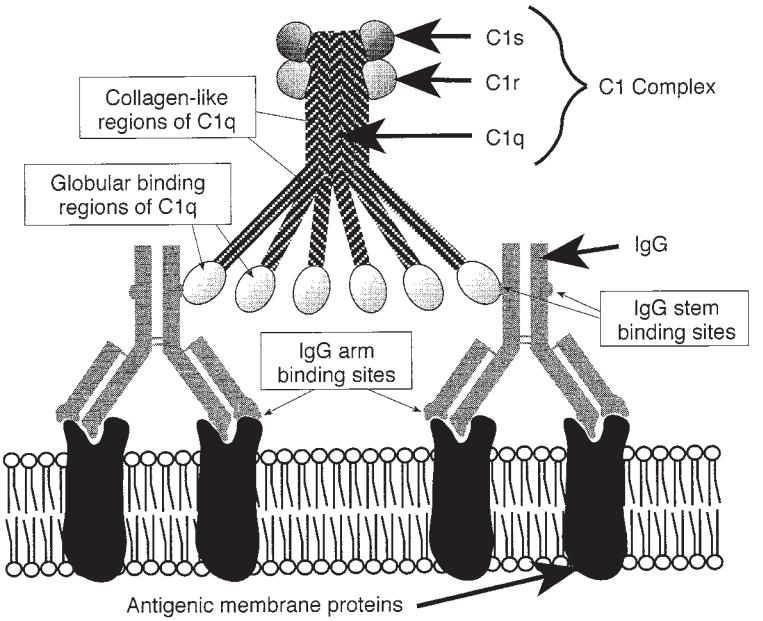


Figure 9–8
The initial reactions of antibody induced, complement formation. ➤ When either IgG or IgM binds to the antigenic membrane proteins of an organism (such as a bacterium), C1q binds to the stem region of at least two antibodies. C1q is part of the C1 complex. Binding causes a conformational shift of the C1q molecule that is communicated to C1r and C1s. As a result, C1s becomes an esterase enzyme following the activation of C1r as an enzyme (C1s is a substrate for C1r). No peptide chains are broken. (Adapted from Roitt et al., 1998.)

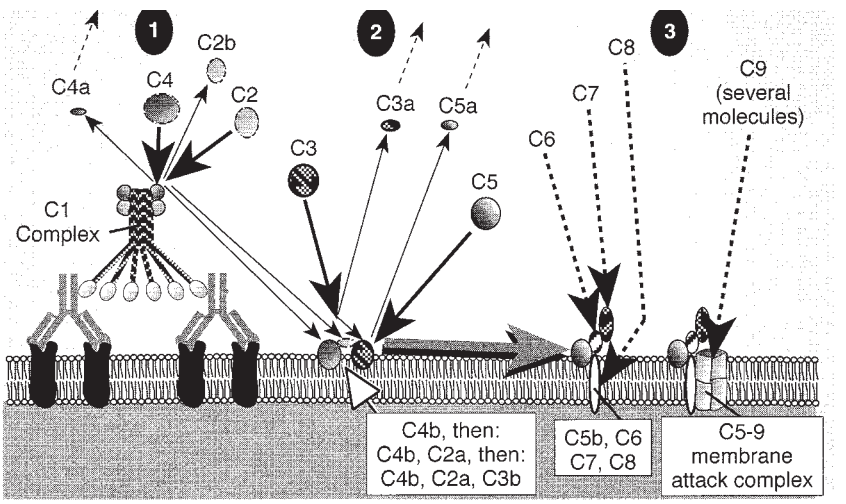


Figure 9–9
The complete diagram of complement activation by the classical pathway. ➤ In stage 1 (number on black background at top of figure), the C1s proteolytic enzyme is activated to split C4 and C2. In stage 2, the activation of C3 and C5 convertase (in sequence) begins the ultimate formation of the membrane attack complex, stage 3. The membrane attack complex causes the lysis of bacterial organisms. In the meanwhile, C4a, C3a, and C5a peptides released during stages 1 and 2 begin the inflammatory process. See text for other details. (Adapted from Roitt et al., 1998.)

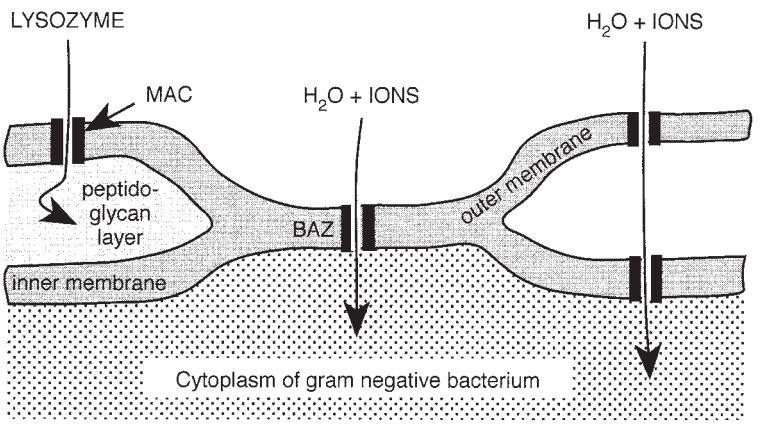


Figure 9–10
The possible action of the membrane attack complex (MAC) on bacterial cell membranes. ➤ In Gram-negative bacteria, lysis of the outer membrane by MAC (shown on the left) allows lysozyme to enter and initiate hydrolysis of the peptidoglycan layer. Afterwards, MAC formation takes place at the inner membrane (shown on the right). This allows water and ions to enter the bacterium and cause its lysis. Alternately, MACs may form at limited regions where the outer and inner membranes are fused, the Bayer adhesion zones (BAZ). Since there is no intervening peptidoglycans, membrane perforation here directly brings about bacterial lysis. The exact mechanism is unknown. (Adapted from Morgan, 1991.)

shock produced by the lesion. An additional mechanism is that the channel allows the entrance of lysozyme which attacks the thinner peptidoglycan wall between the outer lipoprotein cover and the plasma membrane of the bacteria (Morgan, 1991). Viruses exposed to complement are neutralized, but osmotic stress is not a factor. That is, complement prevents a virus from attaching to host cells by covering the viral surface with complement proteins. The fate of the C5a fragment, mentioned before, is similar to that of the C3a fragment and is discussed under Inflammation.

Complement in the Eye

The effective distribution of complement in the eye is limited by the high molecular weight of the C1q component. This component is only able to partially penetrate the cornea (Bielory, 2000). None of the complement proteins are normally transported to the aqueous chamber and the interstitial fluids of the retina. Complement activation is also limited by the presence of regulatory (i.e., inhibitory) proteins that may protect the cornea from unwarranted complement activation (Bora et al., 1993), but not in all cases. The destruction of *Staphylococcus aureus* (a Gram positive organism) is enhanced by complement fixation. It should be pointed out that the destruction of Gram positive organisms require lysozyme to destroy peptidoglycan coverings prior to direct complement destruction. Other bacterial organisms that may elicit complement fixation include: *Neisseria gonorrhoea* (a Gram negative bacteria) and *Haemophilus influenzae* (which is also Gram negative). The former is associated with gonorrhea infections and occurs in both the conjunctiva and the cornea. The latter is commonly associated with the flu in young children and neonates in the conjunctiva (Asbell, Alcaraz-Micheli, 1998). Aizuss et al (1985) demonstrated that complement has a protective effect against *Pseudomonas aeruginosa,* a Gram negative bacterium that frequently infects the cornea. In viral infections, *Herpes simplex,* oddly enough, seems to cause a complement fixation only when the virus penetrates to the stromal level. In that case, the amount of antibodies present must have a high enough concentration (or *titer*) to elicit the complement reaction.

Significant pathological processes in which complement participates also include: *cicatricial pemphigoid* and *Mooren's ulcer*. Cicatricial pemphigoid is an autoimmune disease in which immune mechanisms, including complement, are directed against basal epithelial cells of the conjunctiva. Mooren's ulcer is a wasting away of corneal tissue from the exterior and is possibly also of autoimmune origin. The involvement of complement in the latter is associated with the appearance and activation of mast cells by C5a and other complement peptide fragments. See Robin et al. (1998) for more information.

Inflammation

Inflammation is a physiological reaction to an injury or an invasion by foreign antigens, but it uses biochemical mechanisms. The process of inflammation consists of a series of biochemical reactions that include the following three physiological events: (1) an increased blood supply to the affected area; (2) an increased capillary permeability that allows white blood cells (leucocytes) and many large molecules to enter the

interstitial spaces; and (3) the migration of leucocytes to the exact site of the injury of foreign invasion (chemotaxis). The latter event is accompanied by phagocytosis of foreign matter by the leucocytes. *Complement fixation commonly provides the biochemical agents for this process to occur*, but other biochemical substances can also control this process. Three complement peptides, which are formed during complement fixation (or activation), are involved: C3a, C4a, and C5a (see Figure 9–9). These peptides, which are formed by the protein hydrolytic reactions mentioned previously, are also referred to as *anaphylatoxins* since their injection (in pure form) into animals can produce a lethal immune reaction similar to *anaphylactic shock*, an extreme immunomechanism that can occur in hypersensitive individuals (Bach, 1982). Of the three peptides, C5a is the most powerful. The anaphylatoxins are inactivated by the enzyme serum carboxypeptidase N (SCPN), an enzyme that removes a critical C-terminal Arg from each peptide (Plummer, Hurwitz, 1978).

The C3a, 4a, and 5a peptides diffuse away from the site of complement fixation until they encounter nearby blood vessels and *mast cells*. Mast cells are small white blood cells that, like basophils, contain numerous granules. The granules hold inflammatory agents including: histamine, serotonin, prostaglandins, leucotrienes (substances related in structure to prostaglandins, Chapter 6), and *platelet activating factor*. The complement peptides or anaphylatoxins bind to the mast cell surface at receptor proteins that themselves inhibit the activity of adenylate cyclase *via* a G-protein (see Chapter 6). The primary mechanism by which this occurs in mast cells points to the C5a peptide and its receptor protein: *C5aR* (Ember et al., 1999). Figure 9–11 shows the protein binding complex of C5a and C5aR with its associated G_i protein. This mechanism is very similar to that used by local hormones (see Chapter 6). Internally, the decreased activity of adenylate cyclase lowers the levels of cAMP (since the phosphodiesterase is unaffected). Lower levels of cAMP cause the mast cell granules to fuse with the plasma membrane and release their contents into the tissue environment (Figure 9–12).

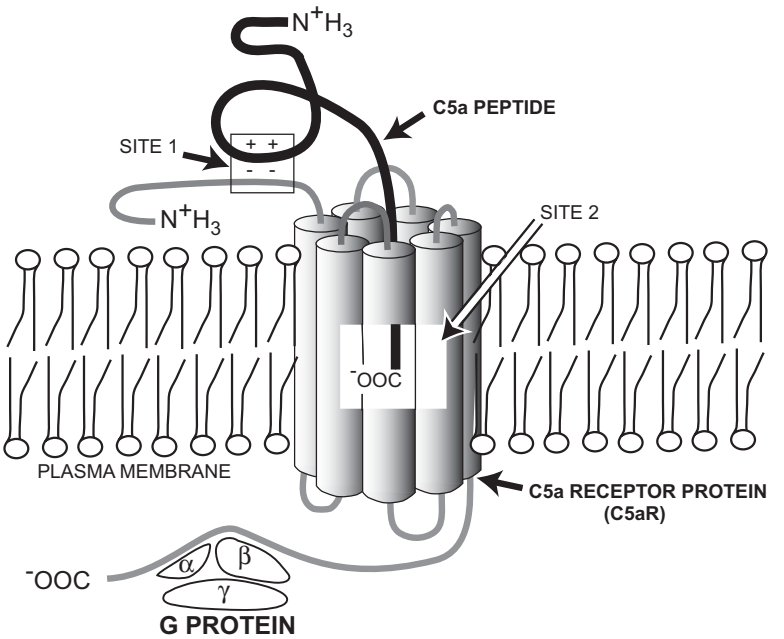


Figure 9–11
Peptide binding complex C5a bound to its receptor protein C5aR. ➤ The figure shows the complement protein fragment, C5a, bound to its receptor protein (C5aR) and associated G_i protein on the plasma membrane of a mast cell. The figure demonstrates two significant binding sites: one ionic (site 1) and the other hydrophobic (site 2).

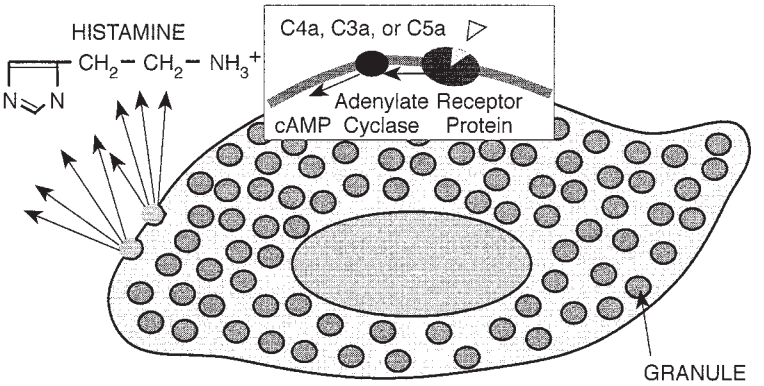


Figure 9–12
Mast cell degranulation. ➤ C4a, C3a, or C5a peptides bind to receptor proteins on the cell's plasma membranes. This action inhibits adenylate cyclase activity and lowers cAMP levels. Lowered levels of cAMP cause granules to fuse with the cell membrane and release their contents of histamine and other inflammatory reactants.

At this point the released histamine as well as other C3-4-5a peptides (that were not involved in binding to mast cells) diffuse toward local blood vessels where they induce vasodilation (increase in the diameter of blood vessels). This increases the local blood supply, allows fluid to leak from the vessels, and causes white blood cells to adhere to the blood vessel walls (pavementing) and to squeeze through the wall itself (diapedesis–from the Greek: "to jump through") into the surrounding interstitial fluid. The biochemical mechanism for this latter process is not well understood. However, two kinds of cell surface proteins known as *selectins* and *β(2)-integrins* are known to cause the white blood cells to bind to the walls of the blood vessels prior to their passing through them (Diez-Fraile et al., 2002). Once released, the leucocytes (white blood cells) are guided to the site of the immune reaction (complement fixation) by the chemical gradient created by the diffusing C3-4-5a peptides. This is the process of *chemotaxis* as shown in Figure 9–13.

Although no chemotactic mechanism has yet been rigorously described, it is fairly well understood that the direction in which the leucocytes travel is determined by the relative density and location of C3-4-5a peptides bound to receptor proteins on a given region of the leucocyte membrane (Figure 9–14). The mechanism by which this occurs is somewhat complex, but it involves the familiar process in which a substance (the chemoatractant such as C5a) binds to a cell receptor protein attached, in turn, to a G-protein that activates or inhibits a phosphate

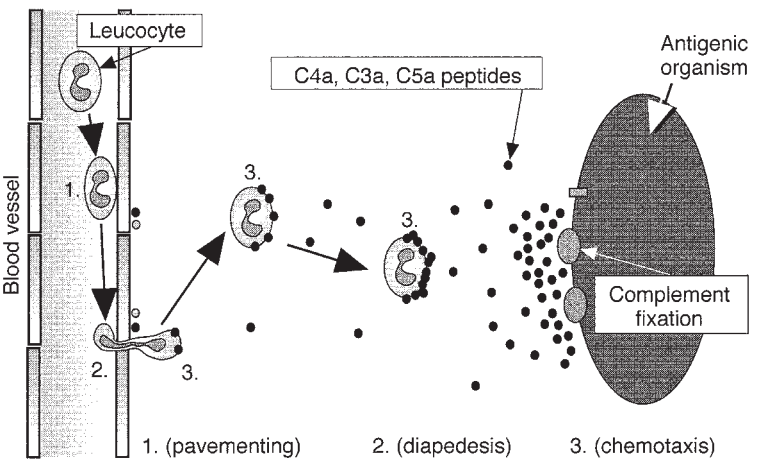


Figure 9–13
Leucocyte migration toward an antigen during the inflammatory response. ➤ Histamine and complement inflammatory peptides cause white blood cell (leucocyte) adhesion to blood vessel walls (pavementing) followed by transport through the wall (diapedesis) and migration to the antigen (chemotaxis).

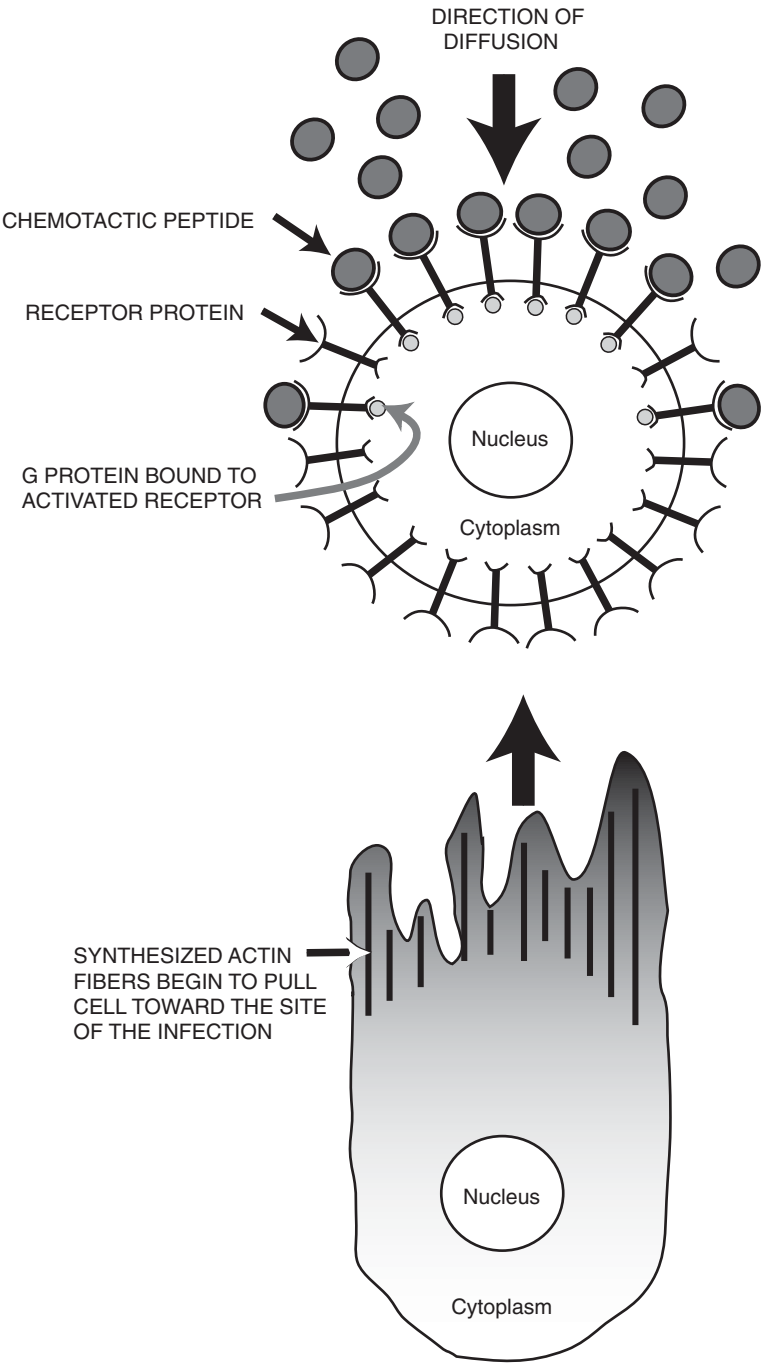


Figure 9–14
Diagram of the chemotactic process. ➤ Chemotactic peptides, such as C5a, bind to receptor proteins preferentially on the side of the leucocyte where their concentration is the highest (i.e., origin of direction). Each receptor protein is bound to a G protein. The G protein sets off a cascade that results in the synthesis of actin proteins (on the side of chemotactic protein binding) to cause cell motion in that direction. The cascade mechanism is shown in the next figure. (Adapted from Weiner, 2002.)

transferring enzyme (see, for example, Figure 6–8 in Chapter 6). In this case, the G-protein activates a phosphoinositol-3 kinase γ that sets off a biochemical cascade to produce cellular motion-setting actin fibers. This is shown in schematic form in Figure 9–15. What is noteworthy about this mechanism is the orientation density of the participants based upon the density of the chemoatractants at the receptors giving the actin fibers directionality.

Upon arrival at the site of complement fixation where the offending antigens are present, it is necessary for the white blood cell (leucocyte) to

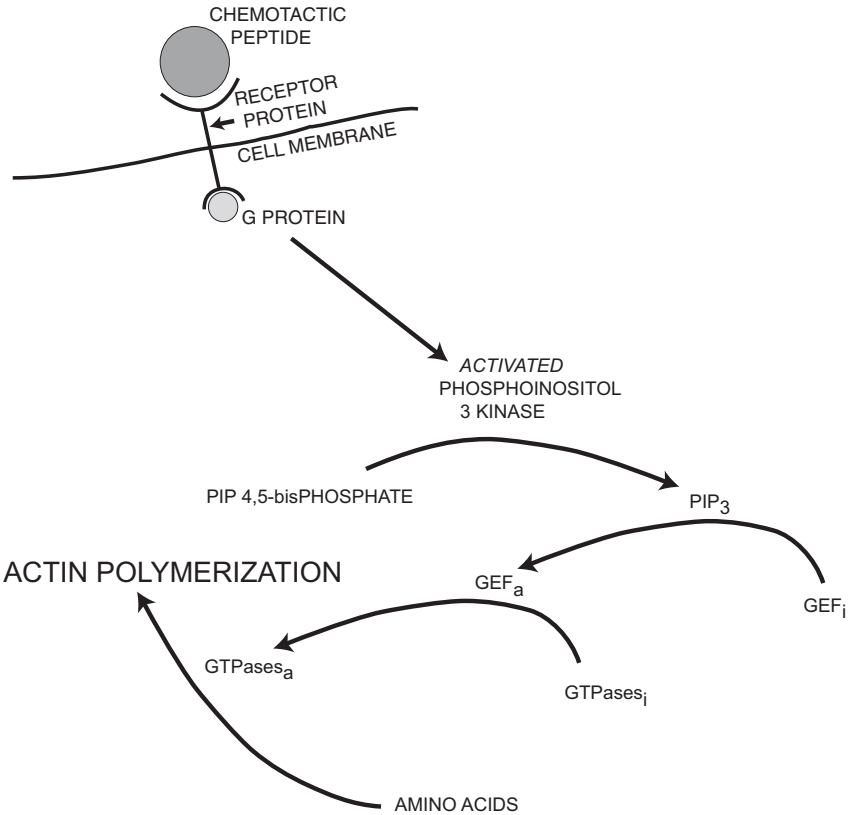


Figure 9–15
The G protein that is dissociated as Gα-GTP causes the activation of phosphotidylinositol 3 kinase γ on the cell plasma membrane (see Chapter 6). ➤ This releases phosphoinositol 1,4,5 trisphosphate (PIP_3). However, instead of being involved with Ca^{+2} release, PIP_3 activates a guanine nucleotide exchange factor (GEF, inactive [i] → active [a]) that, in turn, activates rhoGTPases. These enzymes cause localized polymerzation of actin that, in turn, provides a directional framework along which cytoplasm can flow. (Adapted from Weiner OD. Regulation of cell polarity during eukaryotic chemotaxis: the chemotactic compass. *Curr Opin Cell Biol* 14:196–202, 2002.)

be able to recognize the antigen (or antigen particles if the MAC complex has destroyed any bacteria). The coating, which these antigens receive from the binding of antibodies (IgG) and complement protein components, represents recognition labelling. The process is called *opsonization* (from the Greek "prepared for dinner") and by it the antigens are marked as targets for the leucocytes. Leucocytes possess receptor proteins for these opsonization components and, after binding to them, begin an interesting transformation process (Figure 9–16).

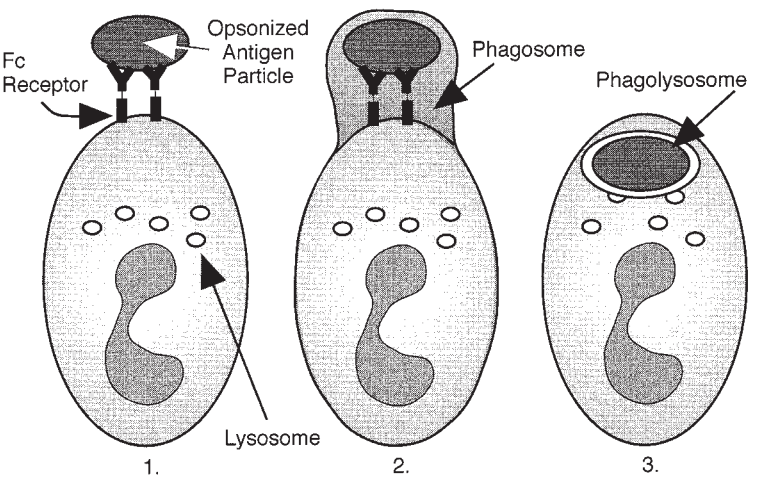


Figure 9–16
Phagocytosis of opsonized antigens by leucocytes. ➤ See text for some details. The Fc receptor protein is one that will bind to the stem of an antibody. In stage 1, the leucocyte binds to the antigen. In stage 2, it forms a compartmentalized organelle around the antigen (phagosome) and moves the phagosome inwards. In stage 3, lysosomes fuse with the phagosome contributing their lytic enzymes to form a phagolysosome.

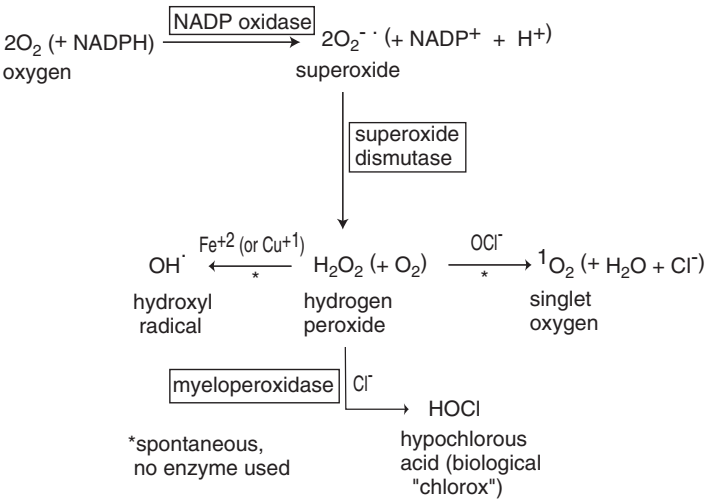


Figure 9–17
Formation of free radicals and highly reactive oxygen compounds: superoxide (O_2^- or $O\text{-}O^-$), hydroxyl radical ($OH^\bullet$) and hypochlorus acid ($HO\bar{C}l$) by respiratory burst. ➤ These compounds readily donate their electrons to membrane lipids and proteins.

The antigens become surrounded by the leucocyte membranes forming a *phagosome* (Greek: φαγοσομα- phagosoma—literally: a body for eating). The phagosome itself is internalized and fused with *lysosomes* (Greek λυσοσομα: literally: a body for breaking) where the antigenic particles or even whole bacteria or viruses are destroyed. Note that the fused bodies are this point are called *phagolysosomes*.

The destruction of antigenic organisms begins in the phagosome with the formation of highly active forms of oxygen. This process is initiated with a *respiratory burst* in which leucocytes take up large quantities of oxygen (O_2) to form superoxide radicals ($O_2^{-\bullet}$). This is shown in Figure 9–17. Superoxide radicals have unpaired electrons that are highly reactive and unstable. The radicals are rapidly converted to other compounds (*hydrogen peroxide*, *hydroxyl radicals,* and *singlet oxygen*) that are themselves highly reactive with tissue proteins and membrane lipids. Some examples are shown in Figure 9–18.

In the initial reaction of Figure 9–17, superoxide is *dismuted* or *disproportioned* (one molecule is reduced while the other is oxidized) to hydrogen peroxide by the enzyme *superoxide dismutase*. The need for the enzyme, in spite of the highly reactive species, has been shown to be necessary due to the relatively low concentrations of superoxide that are present (Babior, 2000). Some of the hydrogen peroxide is also converted to hypochlorous acid (commercially known as "chlorox"), a very highly reactive and destructive chemical for nearly any living organism (see Figure 9–17). All these oxidative species attack the membranes of the organism (see Figure 9–18) within the phagosome and then spill out into the surrounding cellular environment to cause collateral damage to nearby cells or even the phagocytes themselves eventually. The enzyme that catalyzes the formation of hypochlorous acid, *myeloperoxidase*, has a heme group at its active site that has a green color. The green color is the cause of the green hue that is associated with pus formation. Pus formation itself is the buildup of cellular debris from the oxidative destruction (described here) and enzymatic onslaught (described below) that has taken place.

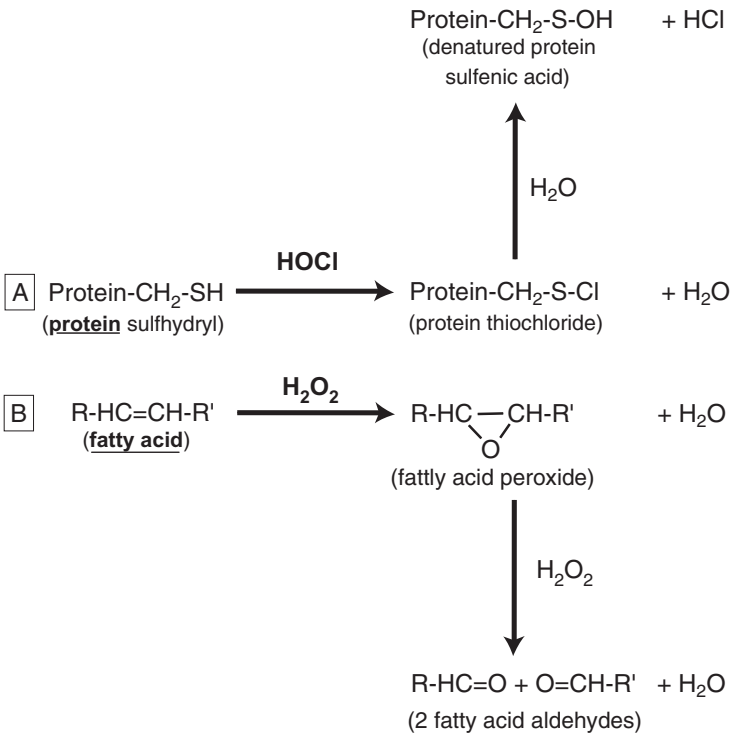


Figure 9–18
Examples of reactive species of oxygen with membrane proteins and lipids. ➤ [A] Hypochlorus acid reacting with a protein sulfhydryl group forms first a protein thiochloride, then a sulfenic acid derivative that denatures the protein. [B] Hydrogen peroxide reacts with a membrane fatty acid to form initially a fatty acid peroxide, then two fatty acid aldehydes. This eventually brings about the rupture of the membrane.

Several mechanisms are employed in the subsequent processes that occur after the oxidative attack at the phagosome. When the phagosome has been taken into the leucocyte and fused with a lysosome organelle, approximately 40 different kinds of hydrolytic enzymes act to break down the viral or bacterial structures (Alberts et al, 1989). These enzymes include: proteases, nucleases, glycosidases, lipases, phospholipases, phosphatases, and sulfatases. All these enzymes function maximally at approximately pH 5.0 and the interior of the phagolysosome is maintained near that pH by a proton pump (H^+-ATPase) at the organelle membrane. Prior to the acidification of the membrane, however, it has been suggested that the pH environment inside the phagolysosome is temporarily made alkaline so that certain cationic polypeptides, such as *defensins*, may continue to damage the outer lipid layer of Gram-positive and negative bacteria (Raj and Dentino, 2002; Roitt et al, 1998; Griffin, 1988). Afterwards, the pH drops to 5 so that the hydrolytic enzymes may attack the bacterial contents.

Ocular Inflammation

Inflammation in the cornea may normally result from external infections or internal infections such as *uveitis*. If sufficiently involved, blood vessels will grow out from the limbal blood vessels superficially to support the inflammatory response (Robin et al., 1998). In some diseases, complement fixation followed by phagocytosis completely fails. An ocular example is *histoplasmosis*, a disease caused by *Histoplasmosis capsolatum*, a yeastlike fungus. Fungi are plant organisms. In histoplasmosis, anterior segment inflammation is absent and neutrophils are

unable to kill the fungi, although it is known that the organisms become covered with antibodies. Friedlander, 1993, has indicated that the neutrophils do not become activated. It may be that the organism secretes molecules that either block inflammation and chemotaxis or prevent phagocytosis. Such anti-immune mechanisms are known to occur in certain species of bacteria (Roitt et al, 1985).

SUMMARY

Molecular mechanisms of immunity are principally involved in antigen–antibody reactions, complement formation, and the inflammatory response. Although there are other molecular interactions, those others are generally considered to be cellular mediated events. There are five major classes of immunoglobulins: A, D, E, G, and M. Generally, only classes A, G, and M are involved in ocular defense. Of these, IgA functions predominately in the secretory forms and IgM is limited to the ocular surface due to its large size. The immunoglobulins are made by B lymphocytes and are specifically designed to interact with an antigenic region of one or very few invading substances or organisms. The immunoglobulins coat an antigen's surface and prepare it for complement activation and/or phagocytosis. They may also prevent the antigen from attaching to or invading host cells. In the eye, immunoglobulins are not found normally in the deeper regions (i.e., lens, vitreous, and retina). Complement is a series of proteins that interact to coat bacterial, viral, and fungal surfaces in order to either destroy them or prepare them (opsonization) for engulfment by white blood cells (phagocytosis). Complement fixation releases peptides in the interstitial fluid (or precorneal tear film) in order to attract white blood cells to the site of antigen invasion (chemotaxis). Some bacteria are known to activate complement fixation in the eye. The inflammatory response in the cornea is a normal response to a superficial or a deep infection.

PROBLEMS

1. If IgA has the highest immunoglobulin concentration in the tear film prior to corneal infection, why does IgM actively respond first to an antigenic presence there?

2. Can the antibody diversity hypothesis completely explain how B cells supply an antibody for every foreign substance (antigen) that invades ocular tissues? Explain your answer.

3. Why is complement activation limited in ocular tissues? Give an example of such a limitation.

4. What biochemical explanation has been offered for the process of chemotaxis during inflammation?

5. How are membrane lipids of a bacteria destroyed by oxidation during inflammation?

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CHAPTER 10

Ocular Biochemical Degradation

AGING AND PATHOLOGICAL PROCESSES

This chapter is a sojourn into biochemical processes that degrade the eye. Some of these processes are a natural result of aging and some occur as a result of an accidental occurrence or a disease. For example, as a part of the aging process, the normal gel structure of the vitreous slowly is converted to a liquid. This conversion may ultimately result in a detached retina. Another example occurs in industrial settings when a strong alkali, such as sodium hydroxide that is used and is accidentally spilled on a worker's eyes resulting in opacification of the cornea.

There is great interest in being able to describe and find ways of curing corneal wounding of all kinds as a way of avoiding unnecessary corneal transplantation. Here, only a selected number of the biochemical processes of degradation will be discussed. Some other degradative processes have already been described in previous chapters.

Cellular Apoptosis

Apoptosis (from the Greek: αποπτοσις—a falling down or degradation) is a form of programmed cell death. In the eye, it occurs in photoreceptor cells as a result of excessive light exposure (Wenzel et al., 2001); in cultured conjunctival cells placed in contact with ocular preservatives (Debbasch et al., 2001); and in corneal epithelial and keratocytic cells after wounding (Wilson, Kim, 1998).

In general there are two forms of cell death that are acknowledged by scientists: *necrosis* and apoptosis (Cameron and Feuer, 2000). Necrosis (Greek: νεκροσις—a killing) usually occurs as a result of several conditions including: *hypoxia*, *ischemia*, tissue trauma, and complement-mediated cell damage. It is a process in which the cell initially appears swollen, the cytoplasm is reddish when stained with the dye eosin, and the nuclei's *chromatin* (genomic material) becomes condensed or clumped. Later the chromatin vanishes and the cells are consumed by phagocytes (see inflammatory reaction in Chapter 9). Biochemically, all the cell membranes become indiscriminately permeable as the various

transport proteins fail and this is followed by membrane destruction. The release of lysosomal enzymes then hastens the destruction of the remaining proteins, lipids, and nucleic acids.

Apoptosis, as a biochemically programmed feature of cell death, often occurs as a normal process to eliminate excess or unneeded cells during organizational development. This mechanism can, therefore, be used to control tissue size. However, in response to a pathological process, apoptosis may accompany the development of cancer, cellular immune reactions, and be initiated in the presence of toxins. This process has been described as metabolically active and is characterized by several anatomical and chemical features. Typically, an apoptotic cell decreases its size as its contents become more concentrated. The nucleus becomes fragmented and the cell separates into separate small bodies that are then phagocytosed without an inflammatory response. The gross anatomical changes for necrosis and apoptosis are shown in Figure 10–1.

Biochemically, the principal vehicle for cell destruction in apoptosis is the activation of *caspases,* which are *c*ysteinyl *asp*artate-specific pro*teases*. These are protein cleaving enzymes that require the presence of aspartate on the protein substrate and make use of cysteine at the enzyme's active site (Zimmerman et al., 2001). The mechanism is complicated by the fact that the cell makes use of several caspases (7 of the 14 known enzymes in this family) as well as the fact that the enzyme will

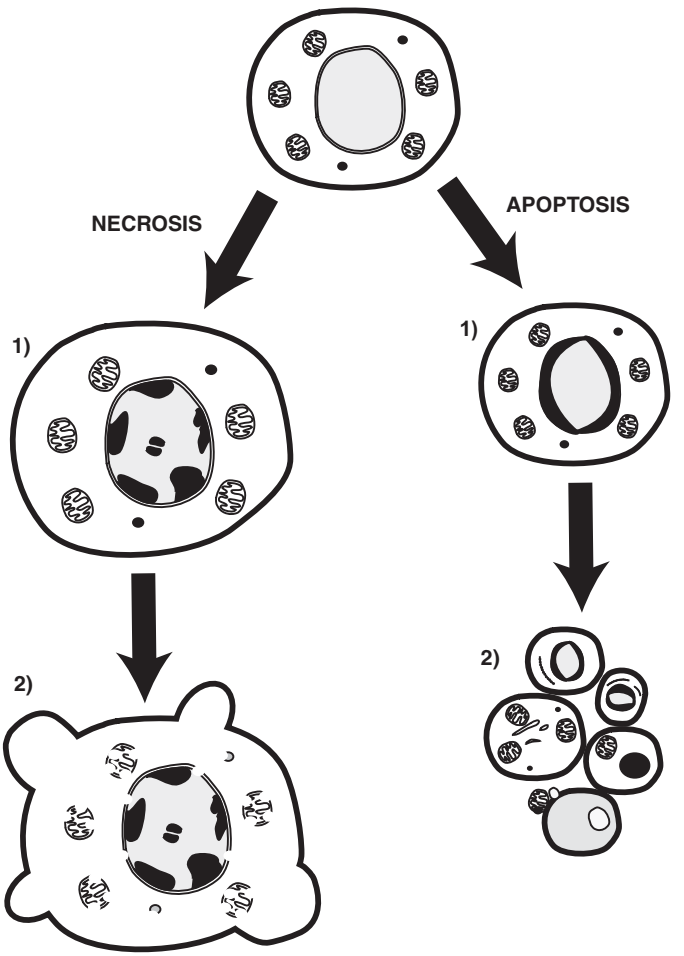


Figure 10–1
Anatomical features of cellular apoptosis compared to necrosis. ➤ A normal cell is shown on the top. In *apoptosis* (shown on the right at 1), the cell volume decreases while the contents condense, particularly the genomic contents. Mitochondrial structures remain intact. The cell then fragments into separate, smaller globular masses with nuclear material divided between several of the masses (shown on the right at 2). The masses are later phagocytosed. In *necrosis* (shown on the left at 1), genomic material condenses haphazardly following cell swelling. The cell then loses its subcellular boundaries (i.e., membranes) and breaks apart by blebbing (i.e., budding and breaking apart) as shown on the left at 2). Phagocytosis also follows afterwards. (Adapted from Cameron R, Feuer G. Incidence of apoptosis and its pathological and biochemical manifestations. In Cameron R, Feuer G, editors: *Apoptosis and Its Modulation by Drugs.* Berlin, 2000, Springer.)

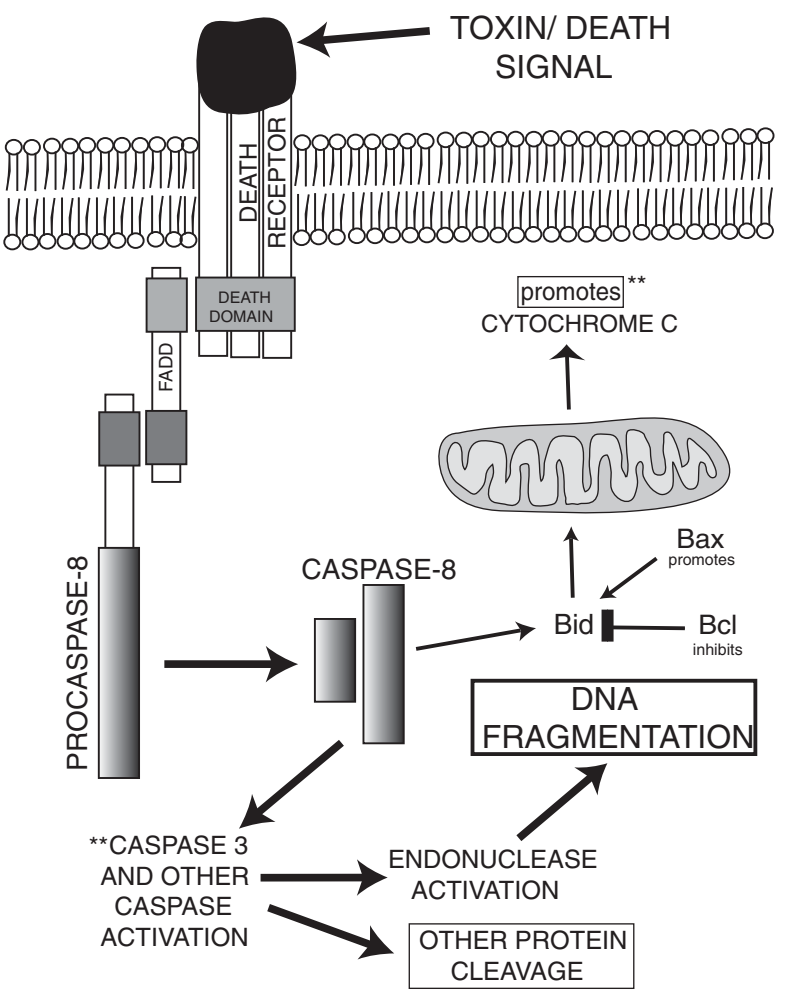


Figure 10–2
The Fas receptor mechanism for apoptosis. ➤ This represents a typical and the most simple mechanism for programmed cell death. A protein, such as FasL (ligand) binds to the Fas receptor protein (labeled in the figure as the "death receptor"). On the inside of the plasma membrane, the portion of the peptide known as the *death domain* (or DD) binds to an intermediate or adapter molecule as a result (in this case: FADD or *Fas-associated death domain protein*). In turn, at least two units of procaspase 8 bind to FADD where they are activated by auto-proteolysis to caspase 8. The active caspase 8 begins a cascade of activation/proteolysis of other caspases (such as caspase 3) and a member of the *B-cell lymphoma protein* known as Bid. The other caspases cause the significant breakdown of important cell-function proteins such as fodrin, a protein that holds the plasma membrane to the cell cytoskeleton. In addition, these caspases activate the endonuclease known as CAD (caspase-activated dexoxyribonuclease) bringing about the truncation of the cell's vital genome. The activated form of Bid binds to the surface of cellular mitochondria affecting the release of cytochrome C which augments caspase activation. (Adapted from Zimmerman KC, Bonzon C, Green DR: The machinery of programmed cell death, *Pharm Therap* 92:57–70, 2001.)

activate members of its own family by its catalytic activity in an overall amplification mechanism (cascade). A relatively simple and understandable explanation of this mechanism is that a signal (toxin or other molecule) binds to a receptor protein (also known as a death sensor or death receptor) in the plasma membrane of the cell. The death signal is then communicated to the cell *via* the caspase cascade system *provided* that it is properly promoted by other signals at the death receptor protein. However, it can be either further promoted or suppressed by still other signal proteins (e. g., *Bax protein* for promotion and *Bcl protein* for suppression). If the caspase cascade system has been given the "go ahead," it will cause the destruction of vital cell proteins and nucleic acids to execute the cell. This is diagrammed in more detail in Figure 10–2.

APOPTOSIS IN OCULAR CELLS

The mechanism in Figure 10–2 was shown, not so much for its "relative" simplicity, as for its inclusion as characteristic of immune privileged tissues such as the eye (Zimmerman et al., 2001). In the case of ocular tissues, the death signal is FasL (Fas ligand), which binds to the

Fas receptor (death) protein. FasL is known to exist as a surface protein component of ocular cells (Wilson, 1999). However, in some cases, it is not apparent that the Fas/FasL system is the only signaling system. In the eye, apoptosis can occur in cells of the retina, cornea, lens, conjunctiva, and Tenon's capsule.

In the cornea, the destructive processes of Fuch's dystrophy results in the loss of both endothelial and stromal cells by apoptosis (Li et al., 2001). Both Fas and FasL were found to be increased (i.e., upregulated) in corneal cells afflicted with the dystrophy to indicate triggering of the death signal. It was also found that Bax protein mRNA was much higher in keratocyte cells compared to levels of Bcl protein mRNA. This did not seem to be the case for endothelial cells and indicated that while Bax promoted apoptosis in keratocytes, some other supplementation/amplification process was at work to promote apoptosis in the endothelium.

In cataractous human lenses, epithelial cells have been found to express Fas, Bcl, and Bax (Nishi et al., 2001). Fas ligand was not detected. However, an antibody to Fas was able to induce apoptosis in these cells. It remains to be seen whether some other *in vivo* ligand may be able to induce apoptosis in these cells.

Much more work has been accomplished on mechanisms of apoptosis in the retina. For example, Sparrow and Cai (2001), have shown that *A2E* (a phosphatidyl-pyridinium *bis*retinoid a component of *lipofuscin*) can induce apoptosis in retinal pigment epithelial (RPE) cells. The structure of A2E is shown in Figure 10–3. The A2E related apoptotic mechanism that stimulates caspase 3 requires blue light (~480 nm) for activation. Therefore, this is apparently not a simple Fas-Fas ligand mechanism. A2E is a detergent with membrane solubilizing capacity and

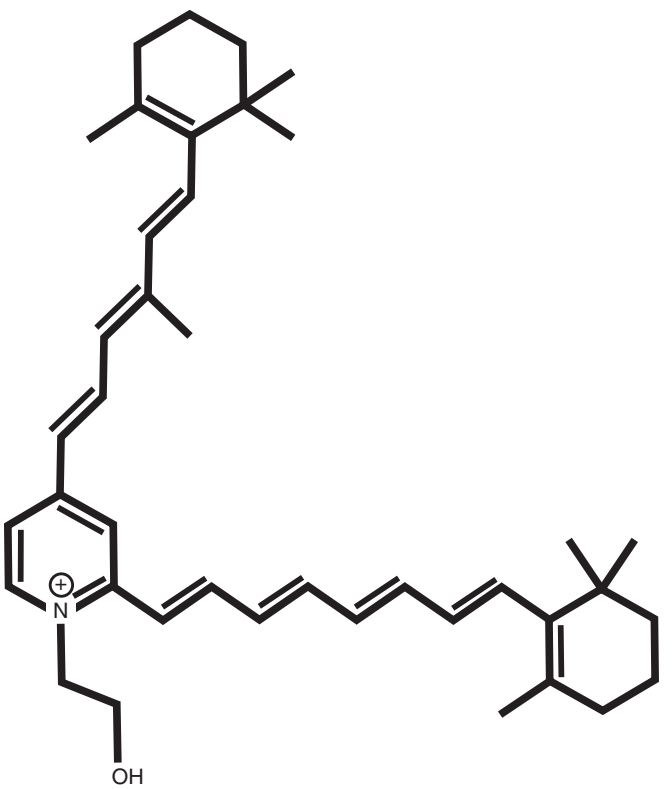


Figure 10–3
Molecular structure of A2E (pyridinium *bis*retinoid). ➤ This is a component of lipofuscin, an autofluorescent age-related pigment formed and deposited in older retinas. A2E is an undegraded fused ring form from two vitamin A molecules and has a role in initiating apoptosis in retinal pigment epithelial cells of the retina. See text for explanation. (Redrawn from Parrish CM, Chandler JW. Corneal trauma. In Kaufman HE, Barron BA, McDonald MB editors: *The cornea.* Boston, 1998, Butterworth-Heinemann.)

may possibly be released/activated itself by blue light. The membrane solubilizing capacity of A2E may be sufficient to activate the Fas receptors. The death of RPE cells by apoptosis has been related to *Stargardt's disease*, *Best's disease*, and some other forms of *macular degeneration* as well as some forms of *retinitis pigmentosa*.

An interesting discovery has been that insulin can spare retinal neurons from apoptosis by reducing the activation of caspase-3 (Barber et al., 2001). A cell line cultured from rat retina was deprived of serum for 24 hours to induce apoptosis. When these deprived cells were exposed to 10 nM insulin, it was found that caspase-3 activation was suppressed. The data suggests that insulin receptors prevent apoptosis by pathway activation of a serine/threonine kinase (Akt) that phosphorylates members of the apoptotic pathway and inhibits them. This would ultimately result in the inhibition of caspase-3.

Following glaucoma filtration surgery, for example, fibroblasts in Tenon's capsule often develop to produce scar tissue (Crowston et al., 2002). These cells may be induced to die by apoptosis when treated with mitomycin-C. In this case mitomycin-C, a heterocyclic antibiotic, antitumor agent synthesized by *Streptomyces caespitosus*, acts as a death signal for tenon capsule fibroblasts to prevent the development of scar tissue.

Liquefaction of the Vitreous

The bulk of the volume of the eye is composed of a liquid/gel containing a few cells near its border with the retina. This liquid/gel is known as the *vitreous* or *vitreous body.* The vitreous referred to here is actually the secondary vitreous as the primary vitreous is only the embryological remnant of the hyaloid artery while the tertiary vitreous is more properly called the *zonules*. The vitreous, whose chemical composition is given in Table 1–4, is actually ~98% water (Mayne et al., 1997). The major nonaqueous biochemical components: collagen and glycosaminoglycans (GAGs) form the vitreous into a gel that has important viscoelastic properties. These properties prevent mechanical shock from being transmitted to the retina while, at the same time, exert continuous intraocular pressure to the retina preventing detachment of its delicate structure of functional neurons.

Here interest is focused on the gellike nature of the vitreous. This is so inasmuch as the gel content decreases with age as the propensity for vitreal and retinal detachment increases (Sebag, 1989). In the human, liquefaction is already at 20% (volume) by around age 18 and progresses to greater than 50% by the 80th decade (Bishop, 2000). As liquefaction increases, GAGs become equally divided between the gel and liquid portions of the vitreous, while collagen is only associated with the gel portion. The liquefaction begins as small central pockets and then coalesces (becomes unified). It may develop as a posterior vitreous detachment (Figure 10–4). In time this could bring about a retina detachment. Posterior vitreal detachments occur in about 25% of the population.

IMPORTANT VITREAL GEL COMPONENTS

In general, the existence of a gel in the vitreous depends upon critical interactions between GAGs and collagen (vitrosin). Bishop (2000), has reviewed important biochemical structural components of the vitreous. In additon to hyaluron (hyaluronate, the negatively charged version of

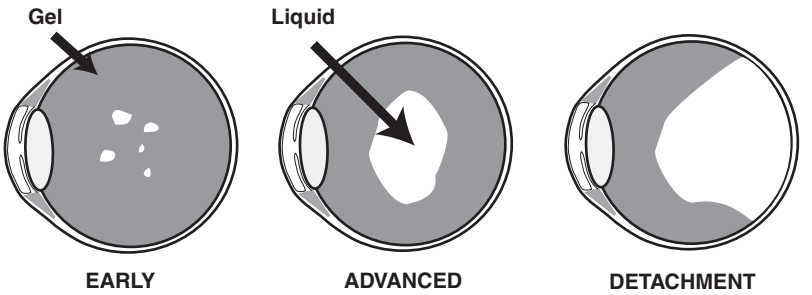


Figure 10–4
Liquefaction of the vitreous of the human eye. ➤ In the early stages as shown on the left, at around age 18, only small isolated pockets of liquid develop (white areas). By age 40, the liquid areas increase and unify (middle figure). In later years, liquefaction may increase to the extent that the vitreous detaches from the retina as shown on the right. (Based on Bishop PN. Structural macromolecules and supramolecular organization of the vitreous gel, *Progress Ret Eye Res* 19:323–344, 2000.)

hyaluronic acid), other GAG components that are found in the vitreous include: *Type IX collagen, versican,* and possibly *agrin*. Type IX collagen will be discussed further on as it represents a complex of both GAGs and collagen. Versican is the principal GAG (as a proteoglycan, see Chapter 4) found in the vitreous and is composed of chondroitin sulfate. The protein component of this GAG (see Figure 4–45 for comparison with a typical cornea proteoglycan structure) consists of three functional domains: a C-terminal domain (for binding to non-GAG sugars), a central domain (where GAGs bind), and an N-terminal domain that binds to hyaluron. Facts about the latter domain will be addressed later. Agrin, which has been located in chick viteous, will not be discussed here (see Tsen et al., 1995).

Type II collagen accounts for 75% of the total vitreal collagen (see Chapter 2 for a review of structural collagen) and is the principal "structural" collagen present. That is, its presence is essential to gel formation. Combined types V/XI collagen represent ~10% of the collagen present. Currently, the exact role of this collagen hybrid has not been determined. Type IX collagen, previously referred to, is a true collagen of which two domains seem to be important. First, a chondroitin sulfate chain is attached to the α2 chain of noncollagenous domain 3 (NC3, Figure 10–5). Due to this, type IX collagen is also classified as a proteoglycan since it has chondroitin sulfate bound to it. Secondly, COL2 (see Figure 10–5) has sites for crosslinking to type II collagen. Therefore, type IX collagen is a "go between" molecule linking type II collagen with the GAG: chondroitin sulfate. Some completely, noncollagenous proteins are also present, for example: *opticin*. Opticin is an extracellular matrix, leucine-rich repeat protein (ECM, LRR protein) that binds noncovalently

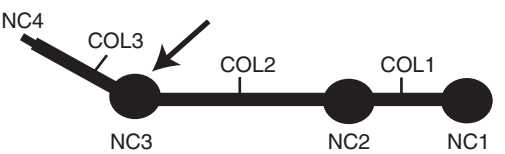


Figure 10–5
A "strand" of type IX collagen. ➤ COL stands for collagenous domain while NC stands for noncollagenous domain. This collagen is also classified as a proteoglycan due to its attachment of the GAG: chondroitin sulfate at NC3 (*arrow*). (Redrawn from Bishop PN: Structural macromolecules and supramolecular organization of the vitreous gel, *Progress Ret Eye Res* 19:323–344, 2000.)

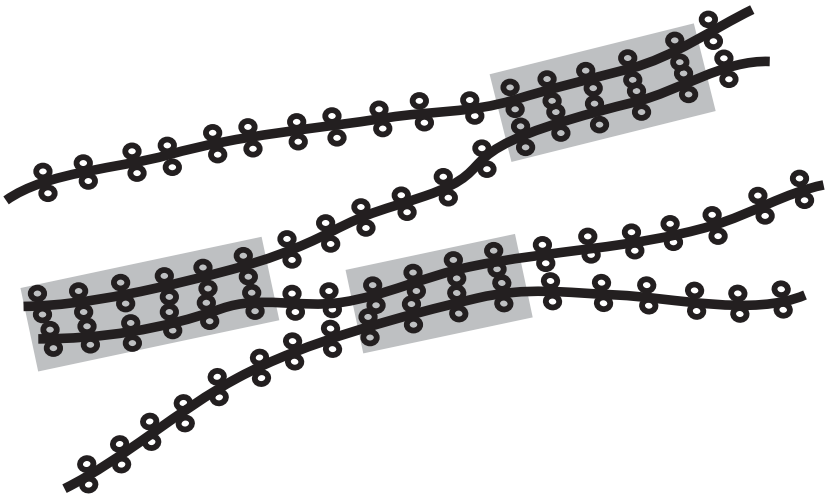


Figure 10–6
Representation of the molecular gel of the vitreous. ➤ Collagens types II and IX are represented by dark lines. Adhering chondroitin sulfate GAGs (attached to type IX collagen) and opticin are indicated by small spheres on each line. The GAGs allow the collagen fibers to maintain a flexible independence within the gel while the opticin prevents collagen aggregation. Gray areas represent molecular associations between collagen fibers that is facilitated by GAGs bound to type IX collagen. Hyaluronin fills the space between the fibers, but is not essential to gel formation. (Redrawn from Bishop PN: Structural macromolecules and supramolecular organization of the vitreous gel, *Progress Ret Eye Res* 19:323–344, 2000.)

to collagen and prevents the fusion of collagen fibrils. That is, the fibrils do not come together (aggregate), but remain independent.

So with all of these macromolecular components, one would wonder how the vitreous gel is assembled. Let's recall again who the principal players are thought to be: hyaluronan, collagens types II and IX (proteoglycans), versican (also a proteoglycan), and opticin. This is easier to describe by eliminating one of the principal players: hyaluronin. Bishop et al. (1999), showed that hyaluronin is not essential for gel formation although it increases its mechanical stability. That is, it is a passive, unnecessary partner. Versican seems to be another passive partner that binds to hyaluronin. It is currently proposed that the collagens (i.e., essentially collagen II and IX) are: (1) held *apart* by GAGs located on type IX collagen while (2) opticin prevents fiber aggregation. The combination of these molecules represents the vitreous gel and is shown in Figure 10–6.

MOLECULAR AGING EFFECTS AND LIQUEFACTION

Age related liquefaction of the vitreous appears to be the result of the breakdown of the spacing mechanisms in vitreal collagen. Such a breakdown would allow the formation of noncollagenous volumes within the vitreous; i.e., liquid areas. Research in this direction has been directed toward genetic abnormalities of opticin. No significant results have been obtained (Takanosu et al., 2001) although there do seem to exist four sequence variations of this protein (Friedman et al., 2002). Therefore, detailed knowledge of the degeneration of the coating molecules on vitreal collagens is lacking.

Chemical Burns of the Cornea

Exposure of the human cornea to caustic agents is a serious event that requires immediate medical attention. Generally, both mineral acids and alkalis cause the most frequent chemical damage to corneal tissues. Investigations of these events have centered on damage caused by alkali such as sodium hydroxide.

Exposure to alkali (depending on the concentration) will instantaneously change the cornea from a clear to a cloudy to white opaque tissue and represents the early and rapid chemical damage that is done. Subsequent events involve immune responses and even *keratomalacia* if the burn is sufficiently severe.

EARLY CHEMICAL DAMAGE

When alkali is exposed to the human cornea it produces the following effects: (1) destruction of corneal cells; (2) a two-stage reaction with corneal collagen; and (3) generally a single-stage reaction with proteoglycans (Whikehart, 1991). Generally, the cellular membrane damage to the cells (in sufficiently concentrated alkali) will bring about cell death upon *saponification* or lysis of lipid ester bonds in the cell membranes. The effects on collagen are twofold. Initially, alkali is sorbed onto the collagen macromolecules by the binding of hydroxide ions to basic amino acids and the formation of both *van der Waals* forces and *hydrogen bonding*. These events allow considerable swelling and distortion of collagen fibers to occur as water enters the enlarged spaces between the fibers. Secondarily, the peptide bonds of the exposed and distorted fibers can be hydrolyzed by the alkali (hydroxide groups) itself in what amounts to fiber polypeptide destruction. The proteoglycans are cleaved from their GAGs at the GAG-proteoglycan linkage (xylose-serine glycosidic bond) by β-elimination in the presence of the alkali (Neuberger et al., 1972). These reactions are shown in Figure 10–7. The destruction that occurs obliterates the regular collagen fiber-fiber distance and negates the mutual light interference that maintains a clear cornea.

IMMUNOCHEMICAL DAMAGE

When an alkali burn is severe (greater than 0.1 *M* alkali exposure), the initial chemical insult previously described is followed by a strong infiltration of *polymorphonuclear lymphocytes* (PMNs) to initiate the destructive elements of inflammation (Paterson et al., 1984). Some of this destructive process has already been described in Chapter 9 for bacterial and viral infections. However, in the case of alkali burns, the destruction is directed toward a continued degradation of corneal collagen with the possible perforation of the cornea. Pfister et al. (1995) and Haddix et al. (1999) found and characterized a degraded collagen peptide that acts as a chemoattractant for PMNs. This peptide is presumably generated by the early alkali protein lysis. It has been itdentified as the tripeptide: *N*-acetyl-Pro-Gly-Pro and its *N*-methylated analogue and was found only to be active when the *N*-terminal proline was blocked with a functional group such as acetate. How the chemoattraction occurs *in vivo* is not known, but is probably analogous to the events shown and described in Figure 9–14. Infiltration of PMNs occurs within the first two days of the burn. If the burn is severe, reparative processes cannot commence due to cellular destruction. Then ulceration of the tissue develops from the destructive activity of PMNs (Parrish, Chandler, 1998). Both the PMNs and any remaining undamaged cells produce and release matrix metalloproteinases (described in Chapter 3). These enzymes further lyse damaged corneal collagen and proteoglycan proteins. This is generally a severe and terminal situation for the cornea when the processes of protein degradation exceed the reparative synthesis of new collagen by any undamaged or newly formed cells from the periphery.

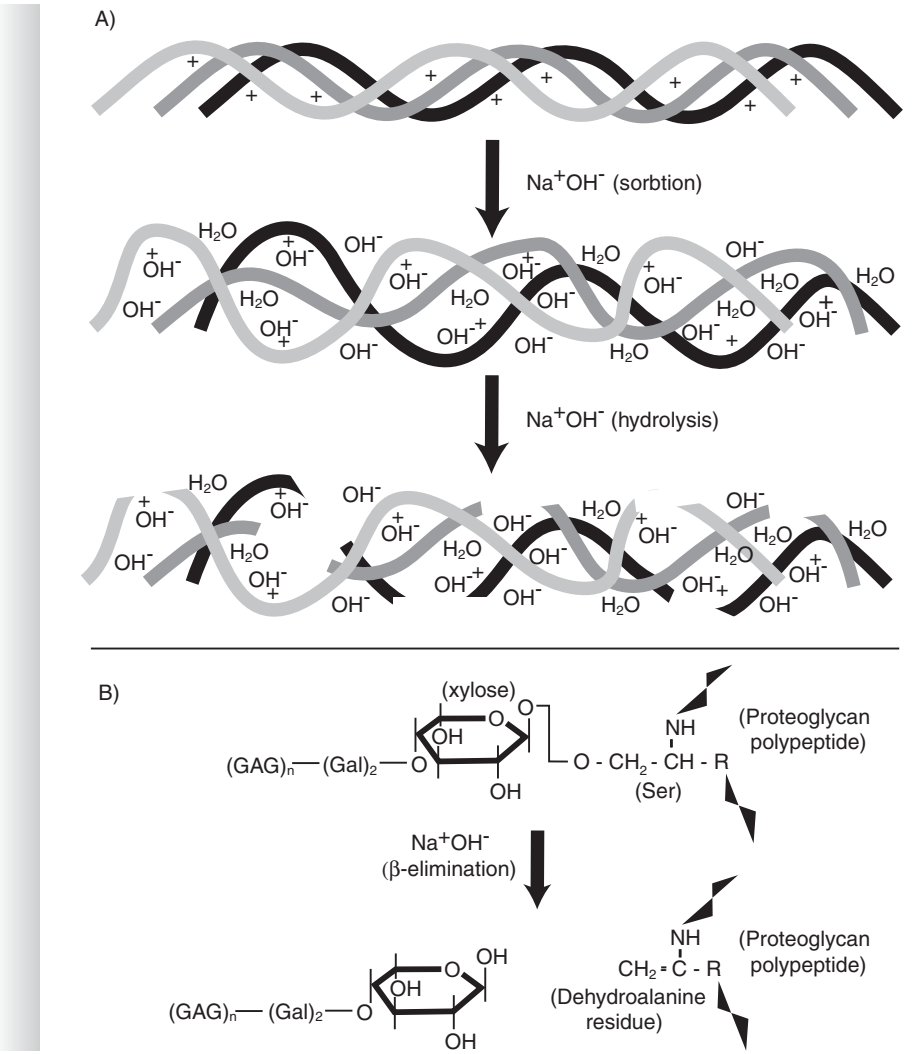


Figure 10–7
Chemical damage to the cornea in an alkali burn. ➤ A, When alkali makes contact with normal corneal collagen (shown at the top), it is adsorbed to basic amino acids and forms both van der Waals and hydrogen bonds with the collagen. This swells the collagen, distorting the normal helical structure. The distortion is reinforced by osmotic inclusion of water molecules as shown in the second figure down. Eventually, the alkali attacks the peptide bonds of the collagen (a hydrolysis reaction) and degrades the fibers as seen in the third figure. B, Alkali generally ruptures only the glycosidic bond between xylose and serine on the proteoglycan by β-elimination. This releases the GAG from its protein core. The serine is converted to a dehydroalanine residue.

S U M M A R Y ●

Biochemical degradation in the eye consists of both normal and pathological processes. Usually degradation occurs in concert with some reparative process and the winner of the competition determines whether a given population of cells survives or passes away. Commonly, cellular degradation occurs by the mechanisms of apoptosis in which a cell dies by a programmed series of biochemical cascades and necrosis in which the cell dies by the loss of its membranes. The mechanism of apoptosis has been heavily studied in the eye in more recent times. It has been found that a death signal (often FasL) binds to a death receptor (usually Fas) to initiate a series of amplification reactions to activate caspase enzymes. These proteolytic enzymes bring about catalytic hydrolysis of key cell proteins as well as the degradation of genomic DNA. Other proteins such as Bax and Bcl can, respectively, promote or inhibit the apoptotic

mechanism. The operation of apoptosis in several ocular cell types are known in detail. Liquefaction of the vitreous is a process in which the vitreous gel decreases while liquid volumes increase. This is a normal process in the aging eye. However, the mechanism tends to destabilize the retina and may lead to retinal detachment. The vitreal gel's stability is thought to depend upon the interaction of collagens II and IX in particular with the GAG component of type IX collagen serving as a molecular spring between collagen fibers. Opticin, a recently discovered protein, attaches to collagen and prevents the fibers from aggregating. Hyaluron, also a GAG, is not necessary for gel formation. The changes that occur in these molecular relationships during aging are currently not understood. Alkali burns in the cornea produce three immediate effects: cellular destruction; distortion and lysis of collagen fibers; and removal of GAGs from their proteoglycan protein cores. This is accompanied by varying degrees of cloudiness and opacification of the cornea. A later event is the invasion of PMNs into the cornea due to a chemotactic attraction of collagen peptide remnants. This inflammatory invasion is accompanied by the release of matrix metalloproteinases that bring about further destruction of collagen and may cause an ultimate perforation of the cornea upon the development of ulceration.

PROBLEMS

1. Distinguish between the cell death mechanisms of necrosis and apoptosis. Include both anatomical and biochemical differences.

2. What is wrong with this statement: "Bax protein supports and amplifies the apoptosis mechanism of all corneal cells in patients with Fuch's dystrophy?" Explain your answer.

3. What three proteins are essential for the formation of the vitreous gel and what GAG is not essential for gel formation? How can you explain this?

4. Given the information at hand, how might you conduct an investigation to determine how aging affects vitreal liquefaction? Consider that studies on opticin have, as yet, failed to reveal any clues.

5. Given the destructive mechanism for alkali burns of the cornea, explain how a mineral acid, such as sulfuric acid, might cause initial corneal damage.

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Glossary

A2E — Phosphatidyl-pyridinium bisretinoid. This derivative of vitamin A can induce apoptosis in retinal pigment epithelial cells.

Absorptivity — See *Beer's law.*

Acceptor stem — The portion of tRNA that binds to an amino acid.

Accommodation — This is a term that can be used with two different meanings. In reference to visual acuity, it specifies the ability to focus on near objects. In reference to neurophysiological phenomena, it specifies the ability of a neuron to adjust to increased or continuous stimuli by turning down or stopping its depolarization. In the retina, accommodation occurs whenever one is suddenly exposed to bright light or enters a dark theater.

Acetylation — The addition of an acetyl group (CH_3–COO^-) to another molecule.

Action potential — A moving wave of depolarization (from negative to positive across the cell membrane) through a nerve caused by the rapid, lateral transport of Na^+ and K^+ ions through its membranes.

Activation energy — The energy, in the form of heat or chemically stored energy, that is necessary to bring a reactant (substrate) to conversion to a new compound (product).

Active site — Location on an enzyme where the reaction takes place.

Acute hemorrhagic conjunctivitis — A corneal infection caused by coxsackievirus A-24 and enterovirus type 70 (single stranded RNA viruses) that includes subconjunctival bleeding. IgG levels are greatly elevated.

Adenosine triphosphate (ATP) — One of several high energy containing compounds found in cells. This one is the most practical for obtaining useful energy. The energy is contained in ATPs terminal phosphate groups.

Advanced glycation end products (AGEs) — The terminal products formed from reactions between proteins and glucose. See *Glycation*.

Aerobic metabolism — Any metabolic process that requires molecular oxygen.

Aggregate — A collection of proteins whose shape and bonding are not well defined.

Agonist — Any chemical substance that can act in place of a neurotransmitter; that is, it binds to the same postsynaptic receptor.

Agrin — A protein known to attach to heparan sulfate in the chick vitreous. Its role in human vitreous has not been studied.

α-Helix — A secondary protein structure in which the amino acid structure forms a helical form having 3.6 amino acids per turn. The helical structure is reinforced by hydrogen bonding.

Akt — A serine/threonine kinase that inhibits members of the apoptotic pathway by phosphorylating them.

Alkylating agents — Chemicals that form covalent alkyl bridges with nucleic acid bases.

Allosteric enzyme — An enzyme whose activity is influenced by substances that bind at alternate sites (see text).

Amadori rearrangement — Early stage binding of glucose to an amino group on a protein. The relatively stable product formed is a *ketimine*. These are the initial reactions of glycation.

Amino acid — An acid containing at least one amino group linked to the carbon adjacent to its carboxylic acid (α-carbon).

Amphipathic — Literally means *having two characteristics*. For lipids this means being both hydrophobic (i.e., not compatible with water) and hydrophilic (i.e., compatible with water).

Amylose/amylopectin — Polysaccharide components of starch. Amylose is unbranched while amylopectin is branched, but to a lesser degree than glycogen.

Anabolic process — One or more chemical reactions in which energy is used to carry out cellular functions. For example, the synthesis of proteins.

Anaerobic metabolism — Any metabolic process that does not require molecular oxygen.

Anaphylactic shock — Also known as anaphylaxis, is a strong immune reaction that results in a marked blood vessel dilation and smooth muscle constriction that may result in death.

Anaphylatoxins — Biochemical substances capable of causing severe immunological reactions.

Antagonist — Any chemical substance that can bind to the same postsynaptic receptor of a given neurotransmitter without causing any postsynaptic effect.

Antibody — A protein that bind to an antigen. Also known as an *immunoglobulin* (abbreviated: Ig), which usually reacts with a foreign molecule (i.e., antigen) within body tissues and fluids. (See Chapter 8.)

Antibody diversity hypothesis — A hypothesis that states that there are genes to produce antibodies for every known antigen. The hypothesis fails to account for the appearance of new antigens or antigenic determinants.

Anticodon — A three-base code on t-RNA that corresponds (= binds) to the codon on mRNA for a specific amino acid.

Antigen — Any substance that will cause an antibody to be produced against it and to which it will bind.

Antigenic determinant — The exact chemical site at which an antibody binds to an antigen (also known as an *epitope*).

Antiport — Transport of substances in opposite directions.

Apoprotein — The protein portion of a complete protein (holoprotein) which consists of a prosthetic group (non-protein portion) + an apoprotein (protein protion).

Apoptosis — A term from the Greek meaning *a falling down*. Apoptosis (pronounced: apo-tosis) is a form of biochemically programmed cell death. Fragmentation of cellular DNA and cell shrinkage are characteristics of the process.

ATP synthase — The enzyme in the mitochondrion that converts ADP to ATP and releases it when a proton passes through its structure.

Autocatalytic properties — Properties of an enzyme that can initiate its own activation.

Autocrine — Affecting the same cells. Autocrine hormones affect the same cells that produce the hormones.

Autonomic nervous system — Nerves under involuntary control (coming from either the brain or the spinal chord) that operate a variety of physiological functions. In the eye this includes control of pupil size, accommodation, intraocular pressure, and blood flow. The sympathetic and parasympathetic divisions tend to control opposing functions (such as increasing and decreasing pupil size), but there are exceptions.

B cell — A white blood cell (lymphocyte) that can produce any of a large number of antibodies. The label "B" originates from the chicken *b*ursa where these cells were orginially found.

β-Elimination — Chemical reaction in which functional groups (on adjacent carbons) are removed from the compound. Such reactions may be carried out in alkali.

Basement membrane — A tissue scaffolding or sheet that supports or separates cells from other noncellular parts of a tissue.

Bax protein — Stands for Bcl-2-associated protein X. This protein promotes apoptosis by causing (indirectly) the release of cytochrome C from mitochondria.

Bcl protein — Also known as Bcl-2 protein. This protein binds to Bax protein and cancels its effects. That is, it is anti-apoptotic.

Beer's law — Relationship of light to the concentration of a substance expressed as A = abc. "A" is the absorbance of light by a sample. "a" is the absorptivity of the sample that is an empirically determined quantity containing the physical-chemical characteristics of the molecule. "b" is the path length of light through the molecular sample and is usually 1 cm. "c" is the concentration of the sample molecule.

Best's disease — A congenital degeneration of the macula.

Bleaching of rhodopsin — Conversion of rhodopsin to opsin plus all-trans retinal.

Buffer — A combination of a salt and acid *or* salt and base that is able to resist changes of pH in solution.

Calmodulin — Calcium binding protein involved with the second messenger effects of calcium.

Cancer — Derived from the Latin word for *crab*. It is a cellular tumor composed of cells whose cell division is uncontrolled. The term *carcinoma* (Greek: crab) has a similar meaning.

Capacitance — The ability of a potential difference to be stored between two charged surfaces. If the density of charge per unit area is increased, the capacitance is increased. If the distance between the charged surfaces is increased, the capacitance is decreased.

Carbohydrate — A polyhydroxy compound with the general formula: $C_n(H_2O)_n$ that may have other functional groups such as a carbonyl group. *Sugar* and *saccharide* are alternate names.

Cardiolipin — A trigycerolic lipid complex variant of a phospholipid that is found significantly in mitochondria. Its function is unknown.

Cascade — A term borrowed from electronics that refers to any enzyme amplifying mechanism that increases the original biological signal made to a cell.

Caspase — A protease requiring Asp as part of its substrate and which itself contains Cys as part of its amino acid sequence.

Cassette — Any segment of DNA that is introduced into a host's DNA may be referred to as a DNA cassette.

Catabolic process — One or more chemical reactions in which energy is extracted from a chemical compound. For example, the formation of adenosine triphosphate from glucose.

Catalyst — Any chemical substance that can speed up a chemical reaction without itself being altered by the reaction. It achieves its goal by lowering the activation energy for the reaction.

Cataract — Any opacity in the lens that causes an alteration to perceived vision.

Centimorgan — A distance on a chromosome equal to approximately 1 million base pairs. It is computed on the basis of a distance at which a genetic recombination will occur by a given percentage.

Centromere — Central location of a chromosome that is an attachment point for proteins involved in chromosome segregation during cell division.

Ceramide — The compound formed when a fatty acid is esterfied to sphingosine (see Figure 4–12).

Cerebroside — A ceremide to which a single sugar molecule is bonded via an oxygen bridge.

Cervonic acid — Fatty acid, also known as *docosahexaenoic acid*. This highly unsaturated fatty acid (22:6) is a common constituent of photoreceptor discs and membranes where it imparts a high degree of fluidity to the membrane.

Chalazia — A granual-like inflammation of the eyelid margin.

Channel forming proteins — Proteins that are capable of initiating a passageway for transport of some substance(s) through a membrane (usually a plasma membrane).

Channel protein — A protein that conducts any substance across a cell membrane. Such substances are often ions. *Gate protein* has the same meaning.

Chaperone — A protein that tends to renature denatured proteins; that is, return them to their normal conformation.

Chemoattractant — Any substance that can cause a directional movement of a cell (usually a white blood cell) based on the concentration gradient of that substance.

Chemotaxis — A process in which white blood cells are drawn to the site of a complement reaction by complement fragments C3a, C4a, and C5a.

Chromatid — Two joined chromosomes.

Chromatin — The genetic material in a cell that is readily stainable in the nucleus.

Chromosome — A single double-stranded molecule of DNA and its associated proteins that are found in eukaryotic cells, viruses, bacteria, or even an organelle. However, at metaphase a "chromosome" actually consists of two chromosomes (chromatids) bound together.

Cicatricial pemphigoid — A presumed autoimmune conjunctivitis affecting the mucosal tissue on the ocular surface. An immune attack is made on the conjunctival epithelial cells.

Codon/anticodon — A codon is the portion of mRNA that contains the code for a specific amino acid (or start or stop signal). The anticodon is the portion of tRNA that binds to an mRNA codon.

Co-enzyme — Another name for a second substrate.

Collagen — An extracellular protein that gives structural support to tissues. Nineteen different types exist. (See description in Chapters 2 and 10.)

Collagen fiber — A structural edifice of collagen that is made up of several "fibrils" (10 to 300 nm diameter). Fibrils are composed of five rows of topocollagen units.

Committed B cell — A B cell in the process of development toward making a specific Ig.

Comparative metabolism — The process in which different forms of metabolism are contrasted and measured to note their advantage to each cell type.

Complement — A collection of proteins that initiate lysis of an antigenic organism.

Complement activation — The process in which complement proteins are induced to react in sequence to initiate a complement reaction. This is also known as *complement fixation*.

Condensation reaction — The formation of a larger compound from two smaller compounds (also known as *ligation*).

Cone transducing proteins — Proteins similar to rhodopsin that are found in cone photoreceptors.

Conformational structure — A three-dimensional structural representation of carbohydrates and other carbon chemical structures based on carbon as the center of a tetrahedron (see Figure 4–2). Term may also apply to any macromolecule.

Constant domain — A domain of an Ig that has a fixed sequence of amino acids. Constant regions, however, may vary between Ig classes.

Corneal dystrophies — A collection of inherited, bilateral, primary alterations of the cornea that are not associated with a prior disease or pathological condition. They may exist in the epithelium, stroma, and/or endothelium.

Cotransport — The simultaneous transport of more than one substance by the same transport mechanism.

Crystallins — Proteins unique to the crystalline lens. There are three major classes in the human adult eye.

Crystallography — The science of determining the three-dimensional structure of compounds using x-rays to produce a diffraction pattern from a crystal of a pure compound.

Cyclic nucleotides — Second messenger hormones that are either cyclized adenosine monophosphate and cyclized guanosine monophosphate.

Dalton (D) — A unit of mass nearly equal to that of hydrogen (1.000). One thousand Daltons = one kilodalton (kD).

Dark current — The flow of cations through photoreceptors that is maximal in the dark.

Deamidation — The removal of an amide group from a compound. In lens biochemistry, the conversion of asparagine/glutamine to aspartic acid/glutamic acid.

Death receptor — A protein receptor on a cell which when bound to a death signal initiates apoptosis. On ocular cells the protein is often Fas.

Death signal — Any substance that binds to a receptor on a cell and initiates apoptosis.

Decorin — A proteoglycan found in the cornea.

Defensins — Cysteine-rich, cationic polypeptides that form pores in bacterial membranes prior to their destruction by lysosomal enzymes in phagocytes.

Degradation — The breaking of peptide bonds in proteins.

Denaturation — In reference to proteins, the process in which proteins lose their function by an alteration in their structure.

Deoxyribonucleic acid (DNA) — A genetic molecule that preserves the genome of a cell. It consists of four kinds of bases, as well as deoxyribose and phosphate. The phosphate imparts acidity to the molecule.

Depolarization — The decrease in charge difference between the outside and inside of a cell. Usually, this means that the inside of the membrane becomes more positive while the outside of a membrane becomes more negative.

Detached retina — Separation of the retina from the pigment epithelial layer.

Deturgescence — A physiological and biochemical process that maintains the cornea in a clear state.

Diabetes — A disease that results in the unequal distribution of glucose inside and outside of cells as a result of the inability of certain cells to transport glucose sufficiently.

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Diabetic cataract — In the widest sense, a cataract is any obstruction to light in the lens. A diabetic cataract is one whose cause can be associated with a diabetic state. Diabetic cataracts may occur rapidly in the subcapsular region as a so-called "snowflake" cataract (in more severe diabetes) or as a senescent-like cataract (as is more common in type 2 diabetes).

Diacylglycerol (DAG) — In the diabetic state, this compound is synthesized from glyceraldehydes 3-phospate and dihydroxyacetone phosphate (E-M pathway metabolites). DAG stimulates protein kinase C and, ultimately, the synthesis of endothelin-1. See *Protein kinase C*.

Diester — Literally means any compound having two ester bonds. In the precorneal tear film, this refers to any combination of three precursor molecules—hydroxy fatty acid, long chain alcohol, and cholesterol.

Diffusion — The even dispersal of molecular particles from an area of higher to lower concentration. In transport, *simple diffusion* is the movement of lipid soluble substances across a plasma membrane to an area of lower concentration.

Disaccharide — A carbohydrate composed of two sugar units. Maltose is an example.

Dismutation — Also known as *disproportionation* is a chemical reaction in which one molecule is reduced while the other is oxidized.

Dissociation constant — The ratio of dissociated to nondissociated components of a buffer.

Disulfide bond — Two sulfur atoms that are bonded together and act as a bridge between the same or different polypeptides (i.e., crosslink). The bond occurs between two cysteine amino acids.

DNA enhancers — Regions of DNA that can modify the rate of specific RNA synthesis.

DNA helicase — An enzyme that forms single-stranded DNA by breaking the hydrogen bonds between bases on opposing DNA strands.

DNA mutation — Any alteration in DNA (such as the formation of a pyrimidine dimer) that causes an aberrant function of DNA.

Domain — A sequence of amino acids in a protein that consists of more than one secondary structure. It may include disulfide bonds. Generally, a domain has some specific function in a protein. (See also *Motif*.)

Double-helix destabilizing proteins — Proteins that temporarily prevent the joining of newly formed DNA strands during replication.

E2F — Cyclin *E2* transcription *f*actor. A protein that promotes the transcription of mRNAs to promote the cell cycle. See Figure 7–24.

Eicosanoid — Cyclic lipids derived from eicosanoic acids. They act as short-term hormones.

Embden-Meyerhoff pathway — The carbohydrate glycolytic pathway ending at pyruvate that was first described by Gustav Embden, Otto Meyerhoff, and five other investigators in 1940.

Endocrine cells — Cells that secrete chemical substances into the bloodstream. These cells participate in the neuroendocrine system since the first order hormones in the system originate from the hypothalamus.

Endothelin-1 — See *Protein kinase C*.

Enhancer — A region of DNA that binds to steroid and thyroid protein complexes in order to influence a promoter site.

Enzyme — A protein that is capable of catalytic activity. The term means *in yeast* where enxymes were first discovered. (See Chapter 3.)

Enzyme-substrate complex (ES) — An enzyme having a substrate present at its active site.

Epitope — The portion of an antigen with which an Ig will bind.

Esters — A lipid class in which a carboxylic acid and an alcohol are joined by an oxygen bridge (i.e, "ester bond"). It is often a constituent of another lipid class such as a phospholipid.

Exon — A segment of a DNA gene that carries part of the code for polypeptide synthesis.

Exophthalmos — An abnormal extension or proptosis of the eyeball from its orbit.

Extension peptides — Nonhelical portions of collagen that are lyzed or broken off in some collagen types with the formation of tropocollagen.

Facilitated transport — The process of moving substances across a membrane with the aid of one or more proteins. *Passive facilitated transport* requires no energy and moves substances to a side of lower concentration. *Active facilitated transport* requires energy and moves substances to a side of higher concentration.

Fat — A lipid ester of fatty acids and glycerol.

Fat soluble vitamin — A lipid that acts as a vitamin.

Fatty acid — A carboxylic acid of varying chain length.

Feedback inhibition — The inhibition of an enzyme by a product formed along a metabolic pathway in which the enzyme participates.

Fiber — Used in reference to collagen. It is a collagen structure made up of several "fibrils." A fibril has a diameter of 10 to 300 nm and is composed of many "microfibrils." Each microfibril is formed from five staggered lengths of tropocollagen units.

Fisher structure — Essentially a staggered ball and stick formation used to represent carbohydrate and other chemical structures (see Figure 4–2).

Furan — Five-membered carbon ring in which the fifth member of the ring is oxygen. Five-membered carbohydrate compounds are based on the structure of this compound.

G protein receptor — Any receptor of an hormone or other effect or substance that interacts with a G protein.

G proteins — Proteins that normally bind GDP when inactive, but bind GTP when stimulated by a receptor protein. They are intermediates between receptor proteins and enzymes that synthesize or degrade second messengers. G proteins either activate or inhibit these enzymes.

Galactitol — The polyol that is formed from galactose in galactosemia via the polyol pathway and is osmotically more active than sorbitol since polyol dehydrogenase cannot use it as a substrate. See *Galactosemia*.

Galactosemia — A disease involving the inability of cells to metabolize galactose as a result of a deficiency of one of three enzymes.

Galactose-phosphate uridyl transferase (GALT) — An enzyme that converts galactose 1-phosphate to uridyl diphospho-galactose and is the primary enzyme that is deficient in galactosemia.

Ganglioside — The compound formed when more than one carbohydrate is bound to ceramide (see Figure 4–13).

Gate protein — A type of transport protein that allows only specific substances (usually ions) to cross a plasma membrane by facilitated diffusion. During action potentials two different gate proteins for Na^+ and K^+ ions are operative. These proteins are also called *channel proteins* or *voltage-dependent gate proteins*. In the postsynaptic membrane, a Na^+ ion channel, receptor protein (nicotinic receptor) belongs to a group of channel proteins that are ligand or neurotransmitter activated.

Gel — A homogeneously dispersed solid within a liquid that is viscous in nature.

Gene — A DNA sequence that will determine a distinct polypeptide chain (protein) or code for a member of a set of closely related polypeptides (protein isoforms).

Gene transcription — The process of copying a specific code onto RNA from DNA by synthesis of the RNA.

Genetic code — Three-base sequences in nucleic acids that code for amino acids as well as the initiation and halting of protein synthesis.

Genome — The entire genetic information contained within a cell.

Glucocorticoid activity — Ability of steroid hormones to affect glucose metabolism.

Gluconeogenesis — The formation of glucose from lactate. This usually takes place in the liver.

Glucose transport proteins — (Abbreviated as GLUT) are proteins that transport glucose into cells. The GLUT associated with insulin is GLUT-4.

Glucuronic acid (glucuronate) — A glucose molecule having a carboxylic acid group attached to carbon #4 (in the up position in the Haworth structure convention) (see Figure 4–41). *Iduronic acid* is an isomer of glucuronic acid in which the carboxylic acid group is down in the Haworth structure convention as shown in Figure 4–42.

Glycation — A mechanism of complex binding of glucose with proteins that may cause loss of protein function (denaturation). Early forms of this binding are called *ketimines* while late forms are referred to as *advanced glycation end products* (AGEs).

Glycerol phosphate shuttle (malate-aspartate shuttle) — Reaction mechanisms by which extra electrons are transported into the mitochondria to obtain additional ATP.

Glycocalix — Literally means "sugar coat." Refers to sugars that are attached or associated with a cell membrane.

Glycogen — The polymer storage form of glucose in animal cells. It is characterized by extensive branching of the main chain.

Glycogen phosphorylase — See *Glycogen synthase*.

Glycogen synthase — Final enzyme in the glycogen formation pathway that adds glucose onto glycogen. Its kinetics are tightly regulated in the opposite direction with glycogen phosphorylase, the enzyme that breaks down glycogen.

Glycolysis — Literally the splitting of carbohydrates. The term is synonymous with the Embden-Meyerhof pathway that ends with the formation of pyruvate. Glycolysis can be both anaerobic and aerobic depending upon the metabolic fate of pyruvate.

Glycoprotein — Any protein to which one or more oligosaccharides are bound. "Mucins" are glycoproteins found on the surface of the cornea.

Glycosaminoglycans (GAGs) — Carbohydrate polymers composed of disaccharide units of one amino sugar and one nonamino sugar. They have a high density of negative charges.

Glycosylation — The addition of one or more sugars to a compound by enzyme catalysis.

Guanylate cyclase binding proteins (GCAPs) — Proteins that, when bound to calcium, inhibit guanylate cyclase.

Hapten — A substance that has become antigenic when combined with a larger molecule.

Haworth structure — A representation of carbohydrates in a ring structure (named after Sir W.N. Haworth, who won the Nobel prize for his contribution in establishing glucose as predominately a ring form in solution). (See Figures 4–2 and 4–3.)

Hemi-desmosome — Buttonlike, intracellular complex that is anchored to an extracellular material via an anchoring collagen rod. It is differentiated from a desmosome that maintains cell-to-cell contact.

Henderson-Hasselbalch equation — An equation that can be used to determine pH, the dissociation constant, or the amounts of dissociated and nondissociated components of a buffer. See text for explanation.

Herpesvirus (or herpes virus) — In reference to eye infections, the form that usually infects the eye is *Herpes simplex type I or type II*. This is a virus whose capsid is filled with double stranded DNA. See text for more information.

Histamine — A chemical derivative of the amino acid histidine that causes white blood cells to be released from blood vessels as part of an inflammatory mechanism.

Histone — A basic protein that binds to DNA in order to compact and store it within the cellular nucleus.

Histoplasmosis — A disease of the reticuloendothelial system that is concerned with blood formation and destruction as well as other functions. In the eye, the disease can affect the retina.

Hormone — A chemical messenger, belonging to several classes, released from cell-to-cell in the blood stream (or the interstitial fluid) or released within the confines of a single cell. Originally, a hormone was only considered to be a substance that was released from a cell and delivered via the blood stream to another cell. That concept is now outdated.

Hormonal response — The physiological response to a hormone is brought about either by a biochemical receptor—second messenger cascade or by a biochemical receptor—enhancer interaction. The first mechanism activates several enzymes in sequence while the second either promotes or inhibits protein synthesis at the DNA level.

Horner's syndrome — A lesion in a sympathetic autonomic nerve that results in reduced pupil size and causes the eyelid to droop on the affected side.

Human leukocyte antigens (HLA) — Proteins critical for distinguishing between host and foreign cells.

Hurler's syndrome — A mucopolysaccharidosis characterized by a deficiency of α-iduronidase.

Hyaluron — Hyaluronic acid. Also known as hyaluronate to refer to its ionized form as it exists in the vitreous. A long chain and principal GAG in the vitreous.

Hydrogen bond — A relatively weak bond between a hydrogen atom and an atom of oxygen or nitrogen. Such bonds are temporary, but their number can be quite large and influential. They occur very often between water and their solutes or within protein structures themselves.

Hydrolysis reaction — Cleavage of a compound by the addition of a water molecule.

Hydrophobic — The property of being lipid soluble or soluble in organic solvents such as hexane. The name literally means *water hating* or *fearing*.

12-Hydroxyeicosatetraenoic acid (12-HETE) and 12-hydroxyeicosatrienoic acid (12-HETrE) — Eicosanoid metabolites of arachidonic acid via cytochrome P-450 or lipoxygenase. They have been found to have defined roles in the cornea.

Hyperpolarization — Increase in the negative charge (usually) within a cell.

Hyperthyroidism — Any condition in which the thyroid gland releases more than the normal amount of T_3 and T_4. One form of hyperthyroidism is Graves' disease.

Hypothalamic function — A brain function attributed to the hypothalamus, the region of the brain that forms the floor and part of the wall of the third ventricle. Nucleated cells of the region control body housekeeping functions such as temperature, sleep, and water balance.

Hypoxia — A biological condition in which cells suffer from insufficient amount of oxygen.

Iduronic acid — An isomer of glucuronic acid. See *Glucuronic acid*.

Immunoglobulin — A protein that binds to an antigen at its epitope or antigenic determining site.

Indoleamine — Another name for serotonin. A neurotransmitter derived from the amino acid tryptophan.

Infectivity — The ability of a virus to infect other cells in addition to the cell in which it is present.

Inflammation — A process in which a local area of tissue becomes swollen, reddened and, possibly, pus-filled. See text for immunological and biochemical mechanisms.

Inhibition — The process of decreasing the normal velocity of an enzyme catalyzed reaction with an inhibitor substance.

Insulin resistance — The process in which circulating insulin is not able to cause glucose uptake into insulin-dependent cells properly. This is usually associated with type 2 diabetes.

Integral protein — A protein that exists in a bilipid membrane. Also known as an *intrinsic protein*.

Intraocular pressure (IOP) — The pressure generated inside the ocular globe that averages approximately 16 mm Hg.

Intrinsic membrane protein — A protein whose structure crosses the lipid membranes of cells.

Intron — A segment of a DNA gene that acts as a noncoding spacer and is removed from hnRNA as mRNA is formed.

Ischemia — A biological condition in which cells suffer from an insufficient blood supply.

Isomerization — Relocation of a portion of a molecule to a different site on the same molecule. The alteration of a compound by the movement of one or more of its functional groups to new positions.

Isoprenoids — A lipid class that contains isoprene units. Cholesterol and its derivatives are important members of this class.

Isozyme — Different polypeptide forms of the same enzyme. These forms alter the kinetic properties of the enzyme. *Isoform* is an alternate term.

J chain — A polypeptide that will link immunoglobulins (found in IgM and secretory IgA). J stands for "joining."

$K_{apparent}$ — Also known as K_{app} or $K_{0.5}$ and is equal to the concentration of substrate at which V_{max} is one-half its normal value. It is used for enzymes that do not follow Michaelis-Menten kinetics and have no absolute K_m value and for Michaelis-Menten enzymes in the presence of an inhibitor.

Keratomalacia — Literally corneal melting. A condition in which the cornea is in the process of degeneration possibly leading to perforation.

Ketimine — See *Amadori rearrangement*.

Ketone bodies — Acidic metabolites of fatty acids formed from their excessive catabolism (as occurs in more severe forms of diabetes).

Kinetics — Discipline referring to enzyme activity and the rates (velocities) at which enzymes operate and the conditions that influence the rates.

K_I — Inhibitor constant that is equivalent to the K_m for a substrate. It is an indirect measure of the affinity of an inhibitor for an enzyme.

K_m — Michaelis-Menten constant that is equivalent to k_2/k_1 (with k_3 sufficiently small) or the concentration of a substrate at which V_m is one-half its normal value.

Lagging strand / leading strand — Forms of daughter DNA made during replication. Leading strands are made in a simple fashion $5' \rightarrow 3'$ while lagging strands require the initial formation of Okazaki fragments prior to joining in a $5' \rightarrow 3'$ direction. See text.

Lamella (pl. Lamellae) — In reference to the cornea, flat sheets of collagen fibers that are stacked one upon another, but whose fiber direction varies from lamella to lamella.

Laminin — An extracellular membrane (matrix) protein. This protein is required, for example, in the spreading and attachment of corneal epithelial cells during corneal repair.

Latency — A stage of viral infection when a virus is apparently inactive biochemically (also called the *latency stage*).

Latency associated transcript — Also known as LAT, a mRNA in herpesvirus that is associated with the latency stage.

Lateral geniculate nucleus — A subcortical structure in which ganglion cells from the retina synapse onto neurons sending processes to area 17 of the brain (the primary visual cortex).

Ligase — An enzyme that joins DNA.

Light adaptation — A change in the membrane voltage of photoreceptor and other retinal cells that occurs in response to continued exposure of light of the same intensity. Other definitions are possible.

Light scattering — A phenomenon in which light is reflected from a surface in all directions in a nonuniform manner.

Lipid — A class of nonprotein compounds that are predominately hydrophobic, but which have hydrophilic moieties or parts.

Lipofuscin — Fatty deposit in the retina containing chromogenic (color producing) material.

Lipophilic — Lipid soluble, literally "fat loving."

Lipoxygenase — Enzymes that form HETE and leukotriene eicosanoids.

Liquid crystal — A state in which a substance exists in an intermediate flexible phase between a liquid and a solid.

Lumican — A proteoglycan found in the cornea.

Macrophage — A white blood cell that enters into a tissue in response to an infection as part of the inflammatory response.

Maillard reaction — Chemical reactions involved in the formation of AGEs from glucose and proteins.

Masking — The biochemical alteration of the N-terminal end of a protein in order to prevent its degradation. This is often done in cells by adding acetyl groups.

Messenger RNA (mRNA) — RNA that carries the genetic code for the synthesis of polypeptides (proteins) on a ribosome.

Metabolism — The sum total of chemical reactions that occur in cells. Anabolic reactions are involved with synthesis while catabolic reactions are concerned with degradation and the generation of energy.

Metal chelation — Binding of metals (e.g., iron, copper, cobalt) by surrounding the metal with a molecular cagelike structure.

Metaphase — Early part of the mitotic phase of the cell cycle in which chromatids become aligned along the center of a cell.

Michaelis-Menten enzyme — An enzyme whose activity is governed by Michaelis-Menten kinetics (see text and "velocity").

Micrococcus Agar Diffusion Assay — An assay for lysozyme activity (and tear disfunction) in which lysozyme in sample tears clear an agar plate containing the organism *Micrococcus lysodeiticus* over a set time period. The area of clearing is propotional to the amount (i.e., activity) of enzyme present. The assay is also known as the *Schirmer Lysoplate Assay*. [See description in van Bijsterveld (1974).]

Mineralocorticoid activity — Ability of steroid hormones to affect the concentration of cations in the bloodstream by alteration of transport of such ions in the kidney tubules.

Mitochondrion (pl. Mitochondria) — A subcellular oganelle in which certain metabolic processes are isolated from the remainder of the cell, prominently oxidative phosphorylation. This organelle is composed of an outer membrane, an intermembrane space, an inner membrane containing infoldings (*cristae*), and an inner space (*matrix*).

Mitogen-activated protein kinase (MAPK) — A phosphorylating enzyme that acts at the cell nucleus. In the diabetic state, this enzyme probably acts in a manner similar to *protein kinase C*.

Monocyte — A mononuclear white blood cell. They have a number of roles in the cellular immune system, one of which is to engulf and consume tagged antigenic cells (phagocytosis).

Monosaccharide — A carbohydrate consisting of a single sugar unit. Glucose is an example.

Mooren's ulcer — A corneal disease beginning in the stroma presumably of autoimmune origin. The cornea eventually becomes scarred.

Motif — A subset of a domain. Two or more motifs may form a domain, but not a complete polypeptide. Sometimes the distinction between motifs and domains is not apparent.

Mucins — Mucus glycoproteins found principally in a mucoid layer of the precorneal tear film.

Mucopolysaccharide (MPS); Mucopolysaccharidosis — Mucopolysaccharide is the discontinued name for a glycosaminoglycan (GAG). A mucopolysaccharidosis is a lysosomal storage disease involving the incomplete metabolism of GAGs. The term, based on the discontinued name for GAGs, is still used.

Myelin (layer) — A spiral wound coating of lipid around a nerve that insulates it and makes saltatory conduction possible.

MYOC — A gene on chromosome 1 whose mutations are associated with the formation of primary open angle glaucoma. The gene,

variously known as *GLC1A* and *TIGR*, has an unknown normal fuction.

Necrosis — Nonprogrammed cell death in which cell membranes become permeable and cell contents are destroyed by lysosomal enzymes.

Neuromodulator — Any chemical substance that alters normal nervous transmission without acting as a transmitter.

Neurotransmitter — Any chemical substance capable of transmitting a chemical signal across a nerve synapse.

Nitrous oxide — Second messenger hormone in gaseous form. Penetrates its target cell to activate a cGMP mechanism.

Node — An area around a myelinated nerve where myelin is not present. Transport of ions across the membrane occurs there.

Non-steroidal, anti-inflammatory drug (NSAID) — A drug (e.g., aspirin) that inhibits prostaglandin synthase. These drugs differ from steroidal anti-inflammatory drugs that inhibit phospholipase A. Both drugs inhibit formation of prostaglandins that may be inflammatory.

Nucleic acid bases — Nitrogen containing, hydrophobic compounds that form the inner, hydrophobic components of nucleic acids. A sequence of three bases may form a genetic code for an amino acid. See text.

Nucleoside; nucleotide — A nucleoside is a base bound to a pentose. A nucleotide is a base bound to a pentose plus one or more bound phosphates.

Ocular fluids — These include the aqueous humor, vitreous and precorneal tears as well as blood, interstitual fluid, and intracellular cytoplasm (when in the eye).

Okazaki fragments — Small segments of newly synthesized DNA in the lagging strand of DNA replication. They consist of both an RNA primer and new DNA.

Oligosaccharide — A carbohydrate generally consisting of between three and nine sugar units. The units may be branched (see Figure 4–40).

Opsin — The apoprotein of rhodopsin to which no vitamin A is attached.

Opsonization — The process of tagging an antigen with Ig and other protein markers prior to phagocytosis.

Opticin — A protein that binds to vitreal collagen and prevents the aggreagation of collagen fibers.

Oxidation-reduction reaction — A chemical transformation in which electrons are transferred from one substance (oxidation) to another (reduction).

Oxidative phosphorylation — The formation of ATP by a complex metabolic process requiring oxygen, electron transport, and proton flow inside of mitochondria. (See *Adenosine triphosphate* and *Substrate-level phosphorylation*.)

Oxidative stress — A biochemical process in which the compound glyoxal is formed from glucose and oxygen in the diabetic state

during the Maillard reaction. Glyoxal is an intermediate that occurs prior to the formation of advanced glycation end products (AGEs).

Paracrine cells — Cells that secrete chemical substances only to the immediately surrounding (i.e., local) tissue via the interstitual fluid.

Partial charge — A positive or negative charge less than that exhibited by a single electron or proton. These weak charges are formed (induced) when molecules (e.g., water) possess one or more atoms that attract electrons more strongly than the other atoms in the molecule and slightly move (displace) electrons within the molecule.

Partial pressure — Usually indicated by a small *p* in front of a gas, represents the pressure exerted by that gas (in a mixture of gases) as if it were present alone in a container or a given tissue.

PAX-6 genes — The name PAX stands for *pa*ired bo*x*-containing genes. Such genes produce proteins with a very high degree of protein similarity (i.e., they are nearly identical as if a box [area] of their sequences were compared). Pax genes are a family of genes whose protein products are involved in organizational development of an embryo where the genes are expressed in the anteroposterior axis of the neural tube. See Walther et al., 1991. The PAX-6 gene is involved in ocular development.

Pedicle — The end or terminal structure of a cone photoreceptor. Each pedicle contains several cone triad synaptic complexes into which are inserted one bipolar and at least two horizontal cell processes.

Pedigree — Lineage, family history, or family traits.

Peptide — Generally, two or more amino acids joined by a peptide bond. Polypeptides are high molecular weight peptides and may fall under the definition of a protein.

Peptidoglycan — A polymer composed of both peptides and sugar derivatives.

Peptidyl transferase activity — Catalytic activity of the 23S rRNA that forms a peptide bond. No enzyme is involved.

Peripheral nervous system — In the strict sense includes both the *somatic* and *autonomic nervous systems*. As used in this text, it only refers to the somatic system that includes innervation of the skin, muscles, and joints.

Peripheral protein — A protein that is attached to a bilipid membrane but does not enter it. Also known as an *extrinsic protein*.

Phagocytosis — The engulfment and lytic digestion of foreign substances by white blood cells associated with immune reactions. This process also occurs under other circumstances (e.g., the phagocytosis of rod outer segments by pigment epithelial cells).

Phenotype — The physical and functional characteristics of genes.

Phospholipase C — Enzyme that hydrolyzes membrane bound phosphatidyl 4,5-bisphosphate to 1,2-diacylglycerol and 1,4,5-trisphosphate (IP_3).

Phospholipid — A lipid in which two fatty acids and a polar head group are esterified to glycerol. Phospholipids are important membrane components.

Phosphorylation — The process of adding a phosphate group (PO_4^{-3}) to a compound or metabolic intermediate. In the generation of high energy ATP (see text) phosphorylation can be either simple (substrate level) or complex (oxidative). In the latter case, electron and proton transports are involved.

PITX genes — *pit*uitary homeobo*x* genes. These genes make transcription factors for RNA associated with eye and other organ development.

PI — Isoelectric point. The pH at which all of the positive and negative charges on a protein or other compound are equal.

Plasma cells — B cells that are actively producing antibodies.

Platelet activating factor — A phospholipid plasmologen having a vinyl ether linkage on one fatty acid (glycerol–CH_2–O–CH=CH–R_1). This compound causes blood pressure lowering, blood clotting, and other effects.

Polar interactions — See *Hydrogen bonding*.

Polar nature — The property or ability of a molecule to possess displaced electrons causing partial charges to exist in the molecule.

Polymerase — An enzyme that synthesizes nucleic acids. There are several types, a DNA directed DNA polymerase makes DNA from a DNA template. DNA-directed RNA polymerases make RNA from a DNA template. Viruses use other types as well.

Polymorphonuclear lymphocytes — PMNs. White blood cells having polylobulated nuclei.

Polyols — Polyhydroxy intermediates formed in the polyol pathway that are a source of osmotic stress for cells, especially those of the lens.

Polysaccharide — A polysaccharide consisting of 10 or more sugar units branched or unbranched. Glycogen is an example.

Postsynaptic inhibition — The process in which nervous transmission is prevented in the postsynaptic nerve or muscle cell. This may take the form of an increased hyperpolarization of that cell. The term *inhibitory postsynaptic potential* (IPSP) is also used. An IPSP may be caused by an inhibitory neurotransmitter or by a decrease in the amount of neurotransmitters released across the cleft (as occurs with cone photoreceptors).

Postsynaptic stimulation — The process in which nervous transmission or depolarization is continued in the postsynaptic nerve or muscle cell. The term *excitatory postsynaptic potential* (EPSP) is also use. However, note that in photoreceptor cells that an EPSP is caused by a presynaptic hyperpolarization that stops the release of neurotransmitters.

Post-translational modification — The biochemical alteration of a protein after the synthesis of its polypeptide chain.

Potential difference — A charged force, usually given in mV, that exists across a cell membrane. Strictly defined, it is the work that must be accomplished to move a charge over a defined distance.

Promoter site (region) — Location on a DNA strand where the synthesis of new RNA is controlled. It is also called a *promoter element*.

Prostaglandin synthase — Also known as *cyclooxygenase* or *COX*, is a multifunctional enzyme that converts arachidonic and other long-chain fatty acids into an initial prostaglandin product. The synthase has both cyclooxygenase and peroxidase activities.

Prosthetic group — A nonprotein portion of a complete or holo-protein.

Protein — Polymer of amino acids linked by peptide bonds usually with a molecular weight of 10,000 daltons or greater.

Protein kinase C (PKC) — An enzyme that is involved in hormonal signal transduction by catalytic phosphorylation. It is calcium dependent. In the diabetic state, PKC induces the synthesis of *endothelin-1*, a protein responsible for pathological changes to blood vessels. PKC itself is stimulated by a metabolite of glucose (i.e., diacylglyerol) derived indirectly from the E-M pathway.

Protein structure — There are several divisions of this term. *Primary:* the amino acid sequence; *Secondary:* the unique shapes of part of the sequence (α-helices, β-pleated sheets, β-turns, and random coils); *Tertiary:* conformation of a single polypeptide; *Quaternary:* conformation of two or more polypeptides that comprise one protein. A *domain* is an intermediary form between secondary and tertiary structure.

Proteoglycan — The complex that results from the binding of a GAG to a protein.

Pseudo V genes — Pseudogenes are normally nonfunctional genes present in the cell's genome. It has been proposed that pseudogenes for the V domain of antibodies might become activated as an extension of the antibody diversity hypothesis.

Pulverulent cataract — A congenital cataract of the nucleus consisting of fine, "powder like" diffused opacities. The cataract has been linked to a defect in chromosome 1.

Pyran — A six-membered carbon ring in which one member of the ring is oxygen. Six-membered carbohydrate rings are based on the structure of this compound.

Pyrimidine dimers — Two adjacent pyrimidine bases on the same DNA chain having a common bond.

Pyruvate dehydrogenase complex — A molecular complex inside of the mitochondrial matrix that is composed of three different enzymes and five co-enzymes. The complex catalyzes the formation of acetyl co-enzyme A from pyruvate.

q arm — The longer arm of a chromosome extending out from its centromere. The other arm is referred to as a *p arm*.

Rate limiting enzyme — An enzyme that governs the formation of products in one direction. Its rate of catalysis is usually slower than other enzymes in a pathway.

Rb — Retinoblastoma protein. A cell cycle control protein often bound to the E2F protein to inhibit the cell cycle at G_1. Like E2F, Rb is a transcription factor that promotes the S phase when

phosphorylated to separate it from E2F. The Rb gene (or Rb1) is defective (nonfunctional) in retinoblastoma and does not make Rb protein. This allows uncontrolled cell division to take place.

Recoverin — Protein, also known as *S-modulin*, that binds to Ca^{+2} ions. It has been proposed to inhibit rhodopsin kinase.

Reducing carbon — Carbon number one on an aldose (i.e., aldehyde carbohydrate) that is able to transfer electrons to a number of other compounds (i.e., it reduces them).

Refractory period — The time during which a nerve cannot depolarize again due to an overshoot in hyperpolarization.

Releasing factors — Peptide hormones of the first order that are released by the hypothalamus. (See Figure 6–3 for an example.)

Replication — The process of making new DNA.

Replication fork — DNA location at which replication commences.

Reporter genes — A gene that synthesizes a protein whose presence can be readily detected (e.g., by chemical assay). Reporter genes can be used to determine whether a transfection is successful.

Respiratory burst — The process of generating large quantities of superoxide and other radicals for the purpose of destroying antigens by leucocytes.

Retinal — Vitamin A aldehyde. It is the prosthetic group bound to opsin to form rhodopsin.

Retinitis pigmentosa — A primary degeneration of the neuroepithelium of the retina, its pigment, and its blood vessels. Main symptoms are night blindness with progressive loss of the visual field. Its cause is unknown.

Retinoblastoma — A carcinoma (cancer) of the retina in which neural precursor cells grow as multiple tumors. See text.

Rhodopsin — Rod pigment protein that contains 11-*cis* retinal and is involved in the initial phase of phototransduction.

Ribonucleic acid (RNA) — A genetic molecule that is involved in supplying the code for protein synthesis. It is similar to DNA but substitutes uracil for thymine and ribose for deoxyribose. See text for types.

Ribosome — A complex molecular assembly of proteins and RNA that is used to house the machinery for the formation of polypeptides and proteins.

RNA primase and RNA primer — The primase enzyme catalyzes the formation of RNA primer from RNA. RNA primer is a short strand of RNA required to initiate Okazaki fragment formation.

S chain — A polypeptide that surrounds an immunoglobulin to protect it from enzyme degradation (found in secretory IgA). S stands for "secretory."

Saccharide — Alternate name for a carbohydrate or sugar.

Saponification — Hydrolysis of an ester, such as a membrane ester, in a base, such as sodium hydroxide.

Schiff base — R_1–CH=NH^+–R_2, the bond that exists between retinal and opsin. The bond is protonated in rhodopsin and is comparatively weak for a covalent bond.

Shirmer Lysoplate Assay — See *Micrococcus Agar Diffusion Assay.*

Second messenger — An internal cell hormone: cAMP, cGMP, Ca^{+2}, or a phosphoinositide.

Sickle cell anemia — A hereditary disease in which the red blood cells of the patient form a sickle shape impeding normal blood flow and causing early destruction of the red blood cells. It is due to the substitution of Val for Glu on β-subunits of hemoglobin. The disease is prevalent among native Africans and in some parts of Asia.

SIX genes — *s*ine oculi*s* (Latin: without eyes) opti*x* and similar genes involved in ocular development.

Solubility — The ability of a molecule to be (and remain) equally distributed in a liquid.

Spectral shifting — The process in which the sensitivity of light of a given wavelength is altered (in visual transduction) by changing the amino acid composition of rhodopsin.

Spherule — The terminal structure of a rod photoreceptor that usually is a single synaptic complex. The synaptic complex, termed a *triad*, contains one bipolar and usually two horizontal cell processes.

Sphingomyelin — The compound formed when a phosphocholine is esterified to ceramide (see Figure 4–12).

Sphingosine — A long-chain dihydroxy amino alcohol used to form glycolipids (see Figure 4–11).

Starch — The polymer storage form of sugar in plants. There is less branching of the main chain compared to glycogen.

Stargardt's disease — A degeneration of the macula lutea.

Steroids — Any chemical substance with four fused rings that is derived from cholesterol. Many of these substances are hormones such as cortisol.

Substrate — Substance or metabolite that is converted to a product by an enzyme.

Substrate-level phosphorylation — In the formation of ATP, the substrate is the immediate source of phosphate. (See *Adenosine triphosphate* and *Oxidative phosphorylation.*)

Sulfoxide bond — A bond formed between sulfur and oxygen in the amino acid methionine found in proteins (see Figure 1–13). It accompanies disulfide bond formation in crystallins.

Svedberg unit (S) — A sedimentation coefficient used in high speed or ultracentrifugation. The unit is roughly proportional to particle mass and it is used when the molecular weight of the particle is unknown. S = the specific volume (mL/g) of the particle divided by the product of the angular velocity per sec times the revolutions per min.

Symport — Transport of substances in the same direction.

Synaptic cleft — A closed compartment between the presynaptic and postsynaptic membranes across which a neurotransmitter diffuses.

Synaptic ribbon — A molecular complex found in the presynapse of photoreceptors and a few other specialized cells that cause a high continuous release of neurotransmitters when the cell is not stimulated.

Target tissues (cells) — Tissues whose cells have receptor proteins either at their plasma membranes or in their cytoplasm for specific endocrine or paracrine hormones.

Tay-Sachs disease — A disease in which there is a deficiency of *hexosaminidase A* leading to an accumulation of *Tay-Saches ganglioside*. It is a glycolipid storage disease. (See text.)

Telomeres — Noncoding sequences of DNA located at the ends of chromosomes. One of their functions is to act as a mitotic clock in cell aging.

Thromboxanes — Eicosanoids formed by thromboxane synthase from a prostaglandin precursor. Their role in ocular function has not been well investigated.

Thyroglobulin — Thyroid gland protein at which the precursors to the thyroid hormones T_4 and T_3 are synthesized.

Thyroid peroxidase — Enzyme that incorporate iodinium ions (I^{+1}) into Tyr amino acids in thyroglobulin.

Thyroid-stimulating immunoglobulins (TSI) — Immunoglobulins that are able to bind to the same receptor as that for the thyroid stimulating hormone.

Thyroxine (T_4) — Tetraiodothyronine, along with triiodothyronine (T_3), are hormones released by the thyroid gland.

Transcription — The process of making RNA from DNA. *Reversed transcription* is forming DNA from RNA as occurs in some viruses and with telomere formation.

Transducin (G_t) — The G protein of photoreceptors that is stimulated by rhodopsin. (See Chapter 6.)

Transduction — Process in which a substance or energy is altered from one form to another (e.g., light may be altered initially to a chemical signal during visual transduction).

Transfection — The process of introducing DNA into a cell by making the cell plasma membrane temporarily permeable to the DNA.

Translation — The process of polypeptide (protein) synthesis from RNA.

Triacylglyerol — A lipid storage form composed of three fatty acids esterified to glycerol.

Triphosphoinositide — Component derived from membrane phosphotidyl inositol-4,5-bisphosphate that acts as a second messenger hormone. It causes the release of Ca^{+2} ions that also act as second messengers.

Tropocollagen — The essential triple helical unit of collagen.

Tumor necrosis factor-α (TNF-α) — A protein secreted by fat cells that inhibits insulin receptor function. Increased secretion of TNF-α is associated with type 2 diabetes.

Type 1 diabetes — Diabetes caused by an insufficient amount of circulating insulin.

Type 2 diabetes — Diabetes caused by a defect in the insulin receptors or the insulin receptor mechanism of insulin dependent cells.

Tyrosine kinase receptor — Hormone receptor for insulin and similar hormones. It ultimately causes the uptake of glucose by activation of tyrosine kinase through a cascade mechanism.

Unsaturation (or saturation) — The amount of double bonds present in a compound. A completely saturated compound has no double bonds.

Upstream promoter (element) — A region of DNA on the 3' side of one or more genes that controls transcription of that gene by DNA-directed RNA polymerase.

Variable domain — A domain of an Ig that participates in binding to an antigen. It has both hypervariable and conserved sequences.

Velocity (V) — Velocity of a reaction is usually given as moles of substrate used or product formed per unit time. For Michaelis-Menten kinetics, the velocity equation (2–4) is derived as follows:

Since: $k_1 [E][S] - (k_2[ES] + k_3[ES]) = O$

when $d[ES]/dt = O$

$$k_1 [E][S] = (k_2 + k_3) [ES]$$

then $$\frac{[E][S]}{[ES]} = \frac{k_2 + k_3}{k_1}$$

where $k_2 + k_3/k_1 = K_m$ (Michaelis-Menten constant)

And $[E] = [E_o] - [ES]$

where $[E_o]$ = enzyme concentration at t = O

and $$\frac{([E_o] - [ES])\,[S]}{[ES]} = K_m$$

by rearrangement

$$\frac{[E_o][S]}{K_m + [S]} = [ES]$$

Since the velocity of a reaction is also

$$v = k_3[ES]$$

By substitution

$$v = \frac{k_3\,[E_o]}{K_m + [S]}$$

The maximal velocity of the reaction

$$V_{max} = k_3[E_o]$$

Therefore, the Michaelis-Menten velocity of a reaction is

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

Versican — A chondroitin sulfate proteoglycan found in the vitreous that also binds to hyaluron.

Virus — A genetic entity, minimally composed of nucleic acids and a capsid, which enters a cell to reproduce itself. In the process, it eventually kills the host cell by manipulating its genetic machinery.

Viscoelasticity — The property of a gel (e.g., that of the vitreous) in which the gel can reform its original shape after sudden deformation and yet still be able to flow as a liquid.

Vitamin — A substance that is essential for biological metabolism in highter organisms, but one that the organisms cannot make. There are two classes, lipid-soluble and water-soluble vitamins.

Vitreous — Usually refers to the secondary vitreous, the gel existing in the eye from the back of the lens/ciliary body to the retina.

Vitreous detachment — Usually posterior, it is the collapse of the vitreous away from the remaining vitreous gel.

Vitrosin — Collagen found in the vitreous. It is type II collagen to which are attached types IX and V/XI.

V_{max} — Maximum velocity of an enzyme catalyzed reaction.

Voltage gated ion channel protein — A membrane bound protein that transports a specific ion when induced by a voltage change in the membrane.

Warburg effect — The production of more ATP than can be explained by aerobic metabolism alone. It was first observed by Otto Warburg in cancer cells in 1926 and commonly occurs in highly metabolizing photoreceptor cells.

Wax — Ester made up of a fatty acid and a long-chain alcohol.

Zonules — Suspensory proteinaceous ligaments that support the lens whose tension normally supports far focus. It is also known as the tertiary vitreous.

Answers to Problems

Chapter 1

1. Bicarbonate buffer. The aqueous fluid is very low in protein content so that protein buffers would have little influence on the pH. The aqueous fluid is also devoid of cells such that phosphate buffer would not be found in the aqueous.

2. 7.4 = 6.86 + log $[HPO_4^{-2}]/[H_2PO_4^{-}]$. (This is from the Henderson-Hasselbalch equation.) Solving: 7.4 – 6.86 = 0.54 = log $[A^-]/[HA]$. Antilog of 0.54 = 3.47/1. The total components of the buffer are 3.47 (numerator) + 1 (denominator) = 4.47. The percentage of the total components that is ionized from the dihydrogen form = 3.47/4.47 × 100 = 78%. This is a good buffer system for topical use since the buffer range of this system extends from 5.86 to 7.86 and is well within the physiological pH of 7.4.

3. Since the aqueous fluid is a filtrate of the blood, any effect on the blood should be reflected on the aqueous fluid. Rapid breathing would be expected to cause a shift in the bicarbonate buffering system to raise the pH due to the high rate of expired CO_2. Therefore, cells bathed by the aqueous would probably experience a higher pH from rapid breathing. However, the pH would never be higher than 7.9 and the change would be temporary.

4. Ascorbate or vitamin C. The substance is concentrated by 14.6-fold (19 mg/1.3 mg per 100 mL). Cells that are bathed by the aqueous fluid (corneal endothelial cells and cells of the lens) may be vulnerable to destructive oxidation. Ascorbate acts as an antioxidant.

5. A high gel/liquid ratio would be expected to stabilize the retinal surface by maintaining pressure on it. Therefore, one would expect cattle to have fewer retinal detachments than humans especially older humans.

Chapter 2

1. A = abc (Beer's Law equation).
   Therefore, $0.660 = 40 \times 10^3\ cm^{-1}\ M^{-1} \times 1\ cm \times c$
   $0.660/40 \times 10^3\ cm^{-1}\ M^{-1} \times 1\ cm = c$
   $0.017 \times 10^{-3}\ M = c$
   $17\ \mu M = c$

2. QBA is a bridge compound between crystallin molecules formed by the oxidation of hydroxykynurenine, itself an oxidized form of the amino acid tryptophan. QBA stands for *q*uinilino*b*enzox*a*mine. It has been identified as the complex that bonds between crystallins and forms a yellow to brown color in nuclear cataracts.

3. A deficiency in lysyl hydroxylase would inhibit the formation of hydroxylysine in collagen. This hydroxylated amino acid is important for crosslinking formation in collagen microfibrils. The crosslinking adds marked strength to the fibers. Therefore, the collagen tissues would be considerably weakened without the activity of this enzyme. A deficiency of procollagen peptidase would prevent the formation of tropocollagen units, the building blocks of collagen fibers. Accordingly, fiber formation itself would be impeded in collagen tissues.

4. The protein has a quarternary structure composed of one tertiary polypeptide of 34,000 D and two tertiary polypeptides of 25,000 D. In the second electrophoresis, mercaptoethanol separated the polypeptides by breaking the bonds between the Cys groups. The darker 25,000-D band confirmed that twice as much of that polypeptide was present as the 34,000-D polypeptide.

5. Chaperone activity is the ability of a protein molecule to maintain the normal structure/function of another protein molecule. That is, it prevents the protein from being denatured. Chaperone activity is probably important in lens fiber cells since these cells soon lose the ability to bring about protein synthesis. It would, therefore, be important for these cells to retain as many functioning proteins as possible (i.e., crystallins) in order to retain their shape and ability to refract light.

Chapter 3

1. The K_m is a measure of the affinity of a substrate for an enzyme. However, as mathematically formulated, a K_m actually represents a dissociation constant. So it is opposite in meaning. Therefore, the higher the K_m value, the more an enzyme and substrate will dissociate rather than associate. Good substrates have low K_m values.

2. First, obtain reciprocal values for all of the data in order to construct the plot. Extend the line drawn through the points to a negative intercept on the x-axis. The interception point should be approximately –2.5/mM. Solving for the intercept as a reciprocal value should give a K_m = 0.40 mM. Note that the negative values of –x and –2.5/mM cancel out.

3. The $V_{max}/2$ is by definition one-half of the maximal velocity of the enzyme. However, the concentration of substrate at which V_{max} is one-half is also influenced by the binding of activator and inhibitor substances.

4. The y-intercept (for noncompetitive inhibition) = $(1 + [I]/K_i)$ divided by V_{max}. Using the information given then, 0.02/mmoles/sec = $(1 + 0.5\ mM/K_i)$ divided by 70 mmoles/sec. When solved, this = 1.25 mM.

5. At $V_{max}/2$, the concentration of the inhibitor is equal to the K_i of the inhibitor or 0.6 mM. This may be verified by solving the equation of y at $V_{max}/2$ (or 0.0625/moles/sec) = (1+[I]/0.6 mM) divided by 32 mmoles/sec.

Chapter 4

1. The E–M pathway is driven by both the available glucose and the cell's need for energy. Under relatively anaerobic conditions, the cell may run the anaerobic exit of the E–M pathway at 18 to 19 times the normal rate to obtain 36 to 38 molecules of ATP (2 ATPs × 18 or 19 molecules of glucose). It should be evident that the cost for this is a higher consumption of glucose.

2. Since electron flow is completely blocked, no protons can be transported to the intermembrane space. The resulting lack of protons will inhibit ATP synthase and the production of ATP. Death follows quickly!

3. Type 2 diabetes. One would place the patient on a weight reduction and exercise program. Fat cells produce tumor necrosis factor-α which, in turn, may inhibit insulin receptor autophosphorylation—part of the activation process for the receptor.

4. Several contributing biochemical factors bring about the degeneration of ocular blood vessels and lead to blindness. Both protein kinase C and MAP kinase may cause the synthesis of endothelin-1, a protein that increases blood vessel leakiness and its thickness. PKC is activated by a glucose metabolite when it is high in concentration. Glycation is another factor that produces denatured AGE proteins. These proteins cause similar pathological effects to the blood vessels.

5. These diseases develop due to an inherited enzyme defect that brings about the accumulation of incompletely catabolized GAGs. The accumulation occurs in both ocular and nonocular tissue. The accumulated GAGs hinder vital body functions and can cause eventual death.

Chapter 5

1. Approximately 0°C. Hexadecenoic acid is a 16-carbon fatty acid with one double bond.

2. The concentration of cholesterol in these membranes would be minimal to maintain the high degree of fluidity necessary for visual transduction.

3. Rhodopsin is an intrinsic or integral membrane protein requiring detergents to displace it from its surrounding lipids. This protein would be expected to penetrate the bilipid layer of its membrane.

4. A diester. That is, it is a lipid containing two ester bonds.

5. In Tay-Sachs disease, a tissue and body fluid accumulation of GM_2 ganglioside occurs. This is the result of a deficiency of hexosaminidase A. In the eye, a cherry red spot in the macula indicates a breakdown of the macula due to lipid degeneration there and the patient becomes blind.

Chapter 6

1. Approximately 5 μg. This may be done by determining the amount left after each 4-min period or by determining the slope of decay by the formula $t_{1/2} = 0.693/k$ and graphing time *vs.* the ln $[A_o]$ where k = the slope of the line and A_o = the original amount of the substance. This equation is derived from a 1st order linear rate equation that may be found in texts dealing with chemical kinetics.

2. Chocolate candy contains caffeine and caffeine inhibits cAMP phosphodiesterase. As a result, the available cAMP would increase and, in turn, further amplify the cascade effect to increase the breakdown of glycogen (as seen in Figure 6–7). This would also magnify the available glucose in the Embden-Meyerhoff pathway and increase the supply of cellular ATP.

3. There may be no receptor proteins on the iris sphincter muscle cells for vasopressin. Accordingly, the mechanism is never activated.

4. With a deficiency of both arrestin and rhodopsin kinase, it would not be possible to quickly shut down the process of visual transduction. That is, activated rhodopsin would tend to prolong the visual signal after it either changed or ceased to exist. Under these circumstances, the vision of the patient might be washed out, overly bright, and possibly blurred.

5. Animal experimentation with, for example, a monkey species could help supply the needed information. One would want to investigate both varied concentrations and time of exposure to establish the initiation of cataract formation. (Note: the reader needs to be aware that prednisone also causes other pathological effects such as: osteoporosis, hyperglycemia, and hypertension. So an answer to the cataract formation problem does not establish an answer to the initiation of other pathological effects.)

Chapter 7

1. The sequence would be: Met-Leu-Gly-Phe-Phe-Gln-Gln-Pro-Lys-Pro-Arg. The peptide would have an estimated molecular weight of: 1375 (based on an average amino acid weight of 125). The actual molecular weight = 1347.66. The peptide is *substance P*. Since this peptide promotes gastrointestinal motility, a larger than normal supply of its mRNA would cause an increase in gastrointestinal motility making frequent trips to the bathroom necessary.

2. Ribozymes are not enzymes, but are specialized rRNA having catalytic activity. Ribozymes catalyze the formation of peptide bonds during polypeptide (protein) synthesis on ribosomes.

3. In full-blown retinoblastoma, both genes that produce the retinoblastoma protein (Rb or Rb1) become inactive so that no Rb protein is produced. The Rb protein normally binds to the protein E2F to retard the cell in the G_1 phase of the cell cycle. If the E2F protein is always available in an unbound state it will continuously permit a cell to pass through the G_1-S checkpoint of the cell cycle and, therefore, allow continuous uncontrolled cell division. This condition, in essence, is a cancerous state.

4. If the random sequence between Greek key 2 and Greek key 3 requires a positive charge for γ-crystallin at that amino acid in the sequence, then its removal by the substitution of Gly for Asp would remove that charge. However, the substitution of Asp with Glu by a different point mutation would not remove that charge. It could, therefore, be predicted that cataract formation might not occur. If one produced a point mutation of GAA in the sequence of the Cryga gene of the lens of an experimental animal (as mentioned previously) and obtained no cataract formation, that would confirm one's prediction.

5. Viral latency in herpes infections is frustrating as it holds the ever present potential of re-infecting the cornea. The mechanism by which re-infection occurs from its stored state in nerve ganglia is poorly understood, but is related to at least one mRNA known as LAT that establishes the latency. Re-infection is related to traumatic events, fever, and sunburn as well as menstruation. The short-term solution to re-infection is, therefore, to avoid these events when possible. The long-term solution is to perform research on this reactivation mechanism, perhaps, by studying ganglion cells in culture that are infected with the virus.

Chapter 8

1. These Na^+ ions are found on the inside of the depolarizing neuron at the point where depolarization occurs. As a wave of depolarization occurs, Na^+ ions enter the neuron from the source of depolarization (i.e., the adjacent channel proteins). The positive ions lower the negative polarity and trigger the opening of the S-4 "gate" of the channel.

2. Norepinephrine is released as a neurotransmitter in the autonomic nervous system in response to the physiological need for increasing pupil diameter, for example, in a dimly lit room. Two explanations can be provided for the effects of phenyephrine: (1) the drug can displace norepinephrine and bind more strongly to the receptors of the iridial dilator muscles; and (2) the supply or reserve of drug in the cornea and aqueous fluid, after topical application, remains the iris tissues for a lengthened period of time.

3. Glutamate neurotransmitters could not be efficiently released into the synaptic cleft. Under this condition, one might see constant flashes of light or flickering light.

4. Decreasing the intensity of light (by turning off a light) causes photoreceptors to release (or increase the release) of glutamate to off center,

bipolar cell receptors. In the case of the off center bipolar cells themselves, this temporarily opens a receptor channel protein for Na^+ ions and depolarizes the cell. Upon depolarization, the bipolar cell releases glutamate at its synapse with a ganglion cell. The ganglion cell, in turn, temporarily depolarizes.

5. It has been shown that the level of dopamine is lowered by half in Parkinson's disease. There is a reduction in visual acuity associated with the loss of dopamine. The association with visual acuity is not understood as dopamine may act either as a neurotransmitter or a neuromodulator.

Chapter 9

1. It has been observed in many tissues that IgM is a first responder to an infection. One of the early and important antigenic defenses in the cornea is complement activation. This requires either IgM or IgG for complement activation by the so called "classical pathway" (activation by the "alternate pathway" using IgA does occur, but is much less efficient). Since IgM is already 4.5-fold higher than IgG and it acts as an initial responder for complement activation. IgA tends more to be a stable watchdog for antigens in the mucoid layer on the cornea and does not necessarily respond by increasing its content.

2. No. Although the hypothesis seems to account for virtually all foreign antigenic invasions, it cannot explain how an antibody can be formed for a rare or new antigen that was previously unrecognized by the body's immune system.

3. Complement activation can only occur where all of its protein components can exist. C1q, for example, is only able to partially penetrate the cornea due to its high molecular weight. Complement activation, therefore, would not normally be expected to occur in the deeper layers of the cornea nor in the anterior chamber of the eye.

4. Chemotactic peptides, such as C5a, directionally orient and cause the migration of leucocytes by using a receptor/G protein mechanism. This mechanism causes a second messenger hormone-like cascade that results in the synthesis of actin proteins on the cell side where the leucocyte must migrate.

5. Significant quantities of oxygen are taken up by leucocytes in a process known as a respiratory burst. The oxygen is dismuted into superoxide radicals and then catalyzed into hydrogen peroxide. The hydrogen peroxide reacts with membrane fatty acids and converts them, sequentially, into epoxides then aldehydes. The aldehyde forms represent degenerated membranes.

Chapter 10

1. Necrosis is a process of cell death in which the cell volume swells and the chromatin of the nucleus condenses. Activation and release of lysosomal enzymes hastens the destruction of the cell's components as

the cell's membranes become permeable. Apoptosis is marked by condensation of the cell volume and subdivision of the cell into smaller globules or masses. Cell destruction is brought about by the activation of caspase enzymes that lyse important cell proteins and also bring about the truncation of genomic DNA via endonuclease activation.

2. In the first place, not all corneal cells die in patients with Fuch's dystrophy. Li et al (1997) have studied corneal cell death in keratocytes and endothelial cells involved with this disease. They found that although Bax proteins supported cell death in keratocytes by being present at increased levels, this was not the case for endothelial cells. The lack of Bax mRNA stimulation for endothelial cells is unexplained. Another part of the apoptotic mechanism may be in operation.

3. The following three proteins are considered essential for vitreous gel formation: collagen type II: collagen type IX; and opticin. Hyaluron, ironically a major GAG in the vitreous, is not essential for gel formation. Experiments were conducted in which hyaluron was excluded from vitreal gel and the gel failed to liquefy. Hyaluron is thought to help stabilize the gel.

4. One approach might be to investigate whether any activity of proteolytic enzymes is present in the vitreous. Matrix metalloproteinases can degrade collagen and other extracellular proteins. Hyalocytes, present in small amounts in the vitreous, could synthesize and release such enzymes in low amounts during the lifetime of an individual. A way to begin might be to isolate these cells and determine their ability to make such enzymes. Other experimental approaches are certainly possible.

5. It is a given that acid, as well as alkali, is destructive to cell membranes and, therefore, kills cells by membrane lysis. In the case of collagen, acid may also bring about swelling and distortion of collagen fibers by binding to *negatively*-charged amino acids as well as also forming hydrogen bonds and van der Waals interactions. This would as likely promote osmotic inclusion of water. Acid, as well as alkali, is known for its ability to break peptide bonds. This effect would be aided by collagen strand swelling and distortion allowing easier accessibility of the protons to the bonds. In the case of proteoglycans, mineral acids are able to break glycosidic bonds on the GAGs themselves making the degradation of proteoglycans more complete.

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