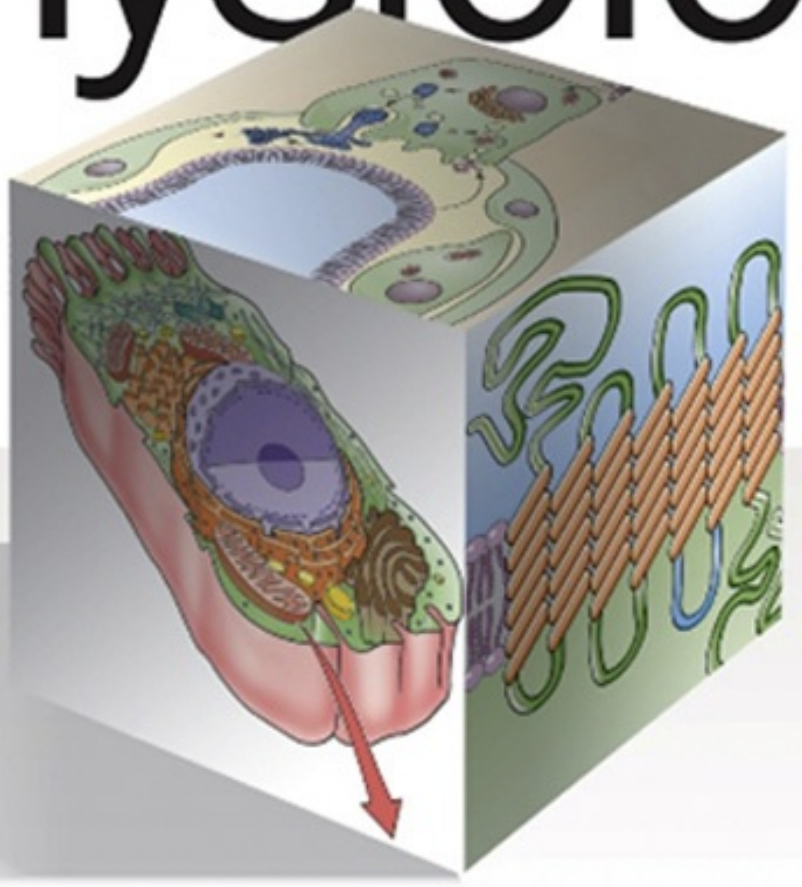
WALTER F. BORON
EMILE L. BOULPAEP

Medical Physiology



THIRD 3 EDITION

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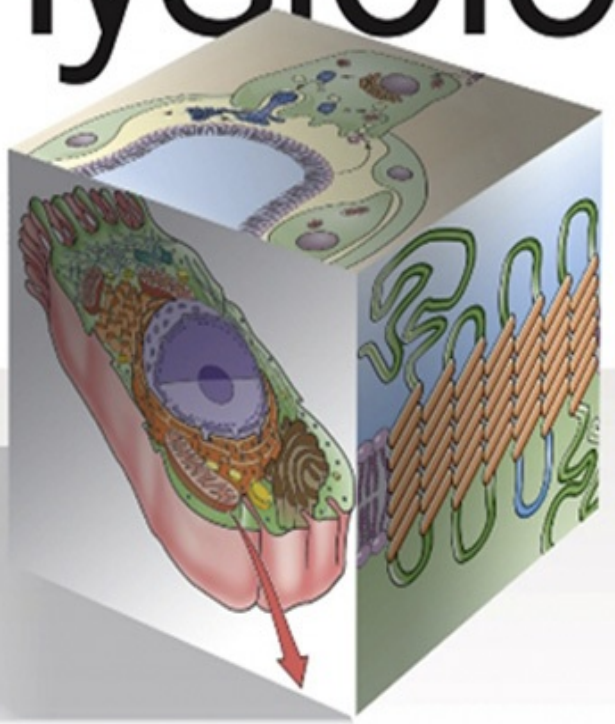

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Preface to the Third Edition

We are delighted that the physiological community so eagerly welcomed the Second Edition of our book. The 3-fold philosophy that has guided us in the previous editions has endured as we prepared the Third Edition.

First, we combine the expertise of several authors with the consistency of a single pen. In the First Edition, we achieved this singleness of pen by sitting—shoulder to shoulder—at a computer as we rewrote the primary copy of our authors, line by line. By the time we began editing the Third Edition, one of us had moved from New Haven to Cleveland. Even so, we continued to edit jointly and in real time—monitor to monitor—using desktop-sharing software. After more than two decades, we have become so accustomed to each other's writing styles that we can literally finish each other's sentences.

Second, we still integrate physiological concepts from the level of DNA and epigenetics to the human body, and everything in between.

Third, we complete the presentation of important physiological principles by pairing them with illustrations from pathophysiology, thereby putting physiology in a clinical context.

In this Third Edition, we have updated the entire book to reflect new molecular insights. In the process, we have shortened the printed version of the book by 40 pages. The Third Edition contains 20 new or redrawn figures as well as enhancements to 125 others. Similarly, we included over 190 tables. In the First Edition, we launched the concept of online-only Notes—electronic footnotes that were available on the Student Consult website. These Notes (indicated by icons in the print version of the book) amplify concepts in the text, provide details and derivations of equations, add clinical illustrations, and include interesting facts (e.g., biographies of famous physiologists). With the increased use of online materials and eBooks, our readers may welcome our updating of the previous Notes as well as a 13% increase in the total number of Notes for the Third Edition, for a total of about 750. In the Second Edition, we

provided the reader with numerous crosslinks to explanatory materials within the book by providing chapter numbers. In the Third Edition, we greatly expand the number of such crosslinks—but now refer the reader to specific pages in the print, and link the reader to specific paragraphs in the eBook. The eBook provides references to scientific literature.

In Section II (Physiology of Cells and Molecules), fresh insights led to substantial revisions in Chapter 4 *(Regulation of Gene Expression)*, including the subchapter on epigenetics, and another on posttranslational modifications. Moreover, advances in physiological genomics and the understanding of genetic diseases led to major expansions of two tables—one on the SLC family of transporters (Table 5-4 in the chapter on *Transport of Solutes and Water*) and the other on ion channels (Table 6-2 in the chapter on *Electrophysiology of the Cell Membrane*). In both tables, our updates help the reader navigate through what sometimes are multiple systems of terminology.

In Section III (The Nervous System), new molecular developments led to major changes in Chapter 15 *(Sensory Transduction),* including the transduction of taste. In Section IV (The Cardiovascular System), we have improved the molecular underpinning of the ionic currents in Chapter 21 *(Cardiac Electrophysiology and the Electrocardiogram).* In Section VI (The Urinary System), we welcome Peter Aronson as a new co-author. Improved molecular insights led to major improvements in Chapter 36, including the subchapters on urea, urate, phosphate, and calcium. In Section VII (The Gastrointestinal System), Chapter 43 *(Pancreatic and Salivary Glands)* underwent significant modernization, including an expansion of the treatment of salivary glands. In Chapter 45 *(Nutrient Digestion and Absorption),* we welcome Charles Mansbach as a new co-author. Section VIII (The Endocrine System) underwent significant updating, including the treatment of phosphate handling in Chapter 52 *(The Parathyroid Glands and Vitamin D).* In Section IX (The Reproductive System), we welcome two new authors. Sam Mesiano extensively reworked Chapters 53 *(Sexual Differentiation)* through Chapter 56 *(Fertilization, Pregnancy, and Lactation),* and George Lister has similarly rewritten Chapter 57 *(Fetal and Neonatal Physiology).* Finally, in Section X (Physiology of Everyday Life), we welcome Shaun Morrison, who extensively rewrote Chapter 59 *(Regulation of Body Temperature).* Chapter

62 *(The Physiology of Aging)* underwent extensive changes, including new treatments of necroptosis and frailty.

The eBook

Although you can still enjoy our book while reading the print version, you can also access the extended content at your computer via the website www.StudentConsult.com. The eBook is also available through the Inkling app on tablets and smart phones. Regardless of the platform for accessing the eBook, the student may access Notes, crosslinks, and references as noted above, and also can "follow" professors and see their highlights and annotations within the text.

Acknowledgments

A textbook is the culmination of successful collaborations among many individuals. First, we thank our chapter authors, who are listed under Contributors on pages *v* and *vi*. We also thank other colleagues who wrote WebNotes, or provided other valuable materials or input. Roberto Dominguez provided Figure 9-5A, and Slavek Filipek and Kris Palczewski provided Figure 15-12. Philine Wangemann made invaluable suggestions for the *Vestibular and Auditory Transduction* subchapter in Chapter 15. George Dubyak responded to numerous queries. We thank all our readers who sent us their suggestions or corrections; we list them in the accompanying **NP-1.**

NP-1

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At the art studio DNA Illustrations, Inc, we thank David and Alex Baker for developing new figures and updating others, while maintaining the textbook's aesthetic appeal, originally established by JB Woolsey and Associates.

At Elsevier, we are most grateful to Elyse O'Grady—Executive Content Strategist—for her trust and endurance. Marybeth Thiel—Senior Content Development Specialist—was the project's communications hub, responsible for coordinating all parties working on the textbook, and for assembling the many elements that comprised the final product. Her meticulous care was indispensible. We thank David Stein—Senior Project Manager—for overseeing production of the textbook. Striving for consistency, Elsevier did us the favor of assigning a single copyeditor—Janet E. Lincoln—to the entire project. We were especially impressed with her meticulous copyediting. Moreover, because she read the manuscript as a dedicated student, she identified several logical or scientific errors, including inconsistencies between chapters.

Finally, we thank four editorial assistants. Charleen Bertolini used every ounce of her friendly, good-humored, and tenacious personality to keep our authors—and us—on track during the first few years as we prepared the Third Edition. Later, three students in the MS in Medical Physiology Program at Case Western Reserve University took the reins from Charleen—Evan Rotar, Alisha Bouzaher, and Anne Jessica Roe.

As we did for the first two editions, we again invite the reader to enjoy learning physiology. If you are pleased with our effort, tell others. If not, tell us.

Preface to the First Edition

We were intrigued by an idea suggested to us by W.B. Saunders: write a modern textbook of physiology that combines the expertise of a multi-author book with the consistency of a single pen. Our approach has been, first, to recruit as writers mainly professors who teach medical physiology at the Yale University School of Medicine, and then to recast the professors' manuscripts in a uniform style. After much effort, we now present our book, which we hope will bring physiology to life and at the same time be a reliable resource for students.

Target Audience

We wrote *Medical Physiology* primarily as an introductory text for medical students, although it should also be valuable for students in the allied health professions and for graduate students in the physiological sciences. The book should continue to be useful for the advanced medical student who is learning pathophysiology and clinical medicine. Finally, we hope that physicians in training, clinical fellows, and clinical faculty will find the book worthwhile for reviewing principles and becoming updated on new information pertinent for understanding the physiological basis of human disease.

Content of the Textbook

Aside from Part I, which is a brief introduction to the discipline of physiology, the book consists of nine major Parts. Part II (Physiology of Cells and Molecules) reflects that, increasingly, the underpinnings of modern physiology have become cellular and molecular. Chapters 2, 4, and 5 would not be present in a traditional physiology text. Chapter 2 (Functional Organization of the Cell), Chapter 4 (Signal Transduction), and Chapter 5 (Regulation of Gene Expression) provide the essentials of cell biology and molecular biology necessary for understanding cell and organ function. The other chapters in Part II cover the *cellular* physiology of transport, excitability, and muscle—all of which are classic topics for traditional physiology texts. In this book we have extended each of these subjects to the *molecular* level. The remainder of the book will frequently send the reader back to the principles introduced in Part II.

Parts III to IX address individual organ systems. In each case, the first chapter provides a general introduction to the system. Part III (Cellular Physiology of the Nervous System) is untraditional in that it deliberately omits those aspects of the physiology of the central nervous system that neuroscience courses generally treat and that require extensive knowledge of neuroanatomical pathways. Rather, Part III focuses on cellular neurophysiology, including synaptic transmission in the nervous system, sensory transduction, and neural circuits. In addition, Part III also treats two subjects—the autonomic nervous system and the neuronal microenvironment—that are important for understanding other physiological systems. Finally, Part X (The Physiology of Everyday Life) is an integrated, multisystem approach to metabolism, temperature regulation, exercise, and adaptations to special environments.

Emphasis of the Textbook

Some important aspects of physiology remain as fundamentally important today as when the pioneers of physiology discovered them a century or more ago. These early observations were generally phenomenological descriptions that physiologists have since been trying to understand at a mechanistic level. Where possible, a goal of this textbook is to extend this understanding all the way to the cell and molecule. Moreover, although some areas are evolving rapidly, we have tried to be as up to date as practical. To make room for the cellular and molecular bricks, we have omitted some classic experimental observations, especially when they were of a "black-box" nature.

Just as each major Part of the textbook begins with an introductory chapter, each chapter generally first describes—at the level of the whole body or organ system (e.g., the kidney)—how the body performs a certain task and/or controls a certain parameter (e.g., plasma K^+ concentration). As appropriate, our discussion then progresses in a reductionistic fashion from organ to tissue to cell and organelles, and ultimately to the molecules that underlie the physiology. Finally, most chapters include a discussion of how the body regulates the parameter of interest at all levels of integration, from molecules to the whole body.

Creating the Textbook

The first draft of each chapter was written by authors with extensive research and/or teaching experience in that field. The editors, sitting shoulder to shoulder at a computer, then largely rewrote all chapters line by line. The goal of this exercise was for the reader to recognize, throughout the entire book, a single voice—a unity provided by consistency in style, in organization, in the sequence for presenting concepts, and in terminology and notation, as well as in consistency in the expression of standard values (e.g., a cardiac output of 5 liters/min). The editors also attempted to minimize overlap among chapters by making extensive use of cross references (by page, figure, or table number) to principles introduced elsewhere in the book.

After the first round of editing, Dr. Malcolm Thaler—a practicing physician and accomplished author in his own right—improved the readability of the text and sometimes added clinical examples. Afterwards, the editors again went through the entire text line by line to decide on the material to be included in specific illustrations, and to match the main text of the book with the content of each figure. The editors then traveled to Philadelphia to visit the art studio of JB Woolsey and Associates. Over many visits, John Woolsey and the editors together developed the content and format for each of the approximately 760 full-color illustrations used in the textbook. These meetings were unique intellectual and pedagogical dialogues concerning the design of the figures. To a large extent, the figures owe their pedagogical style to the creativity of John Woolsey.

The illustrations evolved through several iterations of figure editing, based on suggestions from both the editors and authors. This evolution, as well as text changes requested by authors, led to yet a third round of editing of the entire book, often line by line. Throughout this seemingly endless process, our goal has been to achieve the proper balance among reader friendliness, depth, and accuracy.

Special Features

Compared with other major textbooks of physiology, a much larger fraction of the space in this book is devoted to illustrations. Thus, although our textbook may appear thick, it actually has fewer text words than most other leading medical physiology books. Virtually all illustrations in our book are in full color, conceived de novo, with consistent style and pedagogy. Many of the figures feature "dialogue balloons" that tell a story. The illustrations are also available in digital format on the Evolve Web site (http://evolve.elsevier.com/productPages/s_417.html) for use in the classroom.

The textbook makes considerable use of clinical boxes—highlighted on a color background—that present examples of diseases illustrating important physiological principles. The text includes over 2000 cross references that send the reader from the current page to specific pages, figures, or tables elsewhere in the book for relevant concepts or data. The text also includes hundreds of web icons, which direct the reader to our website at **http://www.wbsaunders.com/MERLIN/BandB/**. These web links provide derivations of mathematical equations, amplification of concepts, material that was deleted for the sake of brevity from earlier drafts of the textbook, and clinical illustrations not included in the clinical boxes.

The website will also contain several other features, including summaries for each subchapter, an expanded list of references (sometimes with direct links to the primary literature), other links that may be of interest to the physiology student (e.g., biographies of famous physiologists), late-breaking scientific developments that occur after publication of the book, and—alas—the correction of errors. Finally, we invite the reader to visit our website to comment on our book, to point out errors, and to make other helpful suggestions.

Acknowledgments

A textbook is the culmination of successful collaborations among many individuals. First, we would like to thank our authors. Second, we acknowledge the expert input of Dr. Malcolm Thaler, both in terms of style and clinical insight. We also thank Dr. Thaler for emphasizing the importance of telling a “good story.” The textbook's aesthetic appeal is largely attributable to JB Woolsey and Associates, particularly John Woolsey and Joel Dubin.

At W.B. Saunders, we are especially thankful to William R. Schmitt—Acquisitions Editor—for his trust and patience over the years that this book has been in gestation. At the times when the seas were rough, he steered a safe course. Melissa Dudlick—Developmental Editor at W.B. Saunders—was the project's nerve center, responsible for day-to-day communication among all parties working on the textbook, and for assembling all of the many components that went into making the final product. Her good humor and careful attention to detail greatly facilitated the creation of the textbook. We thank Frank Polizzano—Publishing Services Manager at W.B. Saunders—for overseeing production of the textbook.

Before this textbook was completed, the author of Part X (The Physiology of Everyday Life), Ethan Nadel, passed away. We are indebted to those who generously stepped up to carefully check the nearly finished manuscripts for the final four chapters: Dr. Gerald Shulman for Chapter 57, Dr. John Stitt for Chapter 58, the late Dr. Carl Gisolfi for Chapter 59, and Dr. Arthur DuBois for Chapter 60. In addition, Dr. George Lister provided expert advice for Chapter 56. We are also grateful to Dr. Bruce Davis for researching the sequences of the polypeptide hormones, to Mr. Duncan Wong for expert information-technology services, and to Mrs. Leisa Strohmaier for administrative assistance.

We now invite the reader to enjoy the experience of learning physiology. If you are pleased with our effort, tell others. If not, tell us.

SECTION I
Introduction

OUTLINE

CHAPTER 1

Foundations of Physiology

Emile L. Boulpaep, Walter F. Boron

What is physiology?

Physiology is the dynamic study of life. Physiology describes the "vital" functions of living organisms and their organs, cells, and molecules. For centuries, the discipline of physiology has been closely intertwined with medicine. Although physiology is not primarily concerned with structure—as is the case for anatomy, histology, and structural biology—structure and function are inextricably linked because the living structures perform the functions.

For some, physiology is the function of the whole person (e.g., exercise physiology). For many practicing clinicians, physiology may be the function of an individual organ system, such as the cardiovascular, respiratory, or gastrointestinal system. For still others, physiology may focus on the cellular principles that are common to the function of all organs and tissues. This last field has traditionally been called *general physiology,* a term that is now supplanted by *cellular and molecular physiology*. Although one can divide physiology according to varying degrees of reductionism, it is also possible to define a branch of physiology—for example, *comparative physiology*—that focuses on differences and similarities among different species. Indeed, comparative physiology may deal with all degrees of reductionism, from molecule to whole organism. In a similar way, *medical physiology* deals with how the human body functions, which depends on how the individual organ systems function, which depends on how the component cells function, which in turn depends on the interactions among subcellular organelles and countless molecules. Thus, medical physiology takes a global view of the human body; but in doing so, it requires an integrated understanding of events at the level of molecules, cells, and organs.

Physiology is the mother of several biological sciences, having given

birth to the disciplines of biochemistry, biophysics, and neuroscience, as well as their corresponding scientific societies and journals. Thus, it should come as no surprise that the boundaries of physiology are not sharply delineated. Conversely, physiology has its unique attributes. For example, physiology has evolved over the centuries from a more qualitative to a more quantitative science. Indeed, many of the leading physiologists were—and still are—trained as chemists, physicists, mathematicians, or engineers.

Physiological genomics is the link between the organ and the gene

The life of the human body requires not only that individual organ systems do their jobs but also that these organ systems work "hand in hand" with each other. They must share information. Their actions must be interdependent. The cells within an organ or a tissue often share information, and certainly the individual cells must act in concert to perform the proper function of the organ or tissue. In fact, cells in one organ must often share information with cells in another organ and make decisions that are appropriate for the health of the individual cell as well as for the health of the whole person.

In most cases, the sharing of information between organs and between cells takes place at the level of atoms or molecules. Cell-to-cell messengers or intracellular messengers may be as simple as H^+ or K^+ or Ca^{2+}. The messengers may also be more complex chemicals. A cell may release a molecule that acts on a neighboring cell or that enters the bloodstream and acts on other cells a great distance away. In other cases, a neuron may send an axon a centimeter or even a meter away and rapidly modulate, through a neurotransmitter molecule, the activity of another cell or another organ. Cells and organs must interact with one another, and the method of communication is almost always molecular.

The grand organizer—the master that controls the molecules, the cells, and the organs and the way they interact—is the genome with its epigenetic modifications. Traditionally, the discipline of physiology has, in its reductionistic journey, always stopped at about the level of cells and certain subcellular organelles as well as their component and controlling molecules. The discipline of physiology left to molecular

biology and molecular genetics the business of how the cell controls itself through its DNA. The modern discipline of physiology has become closely intertwined with molecular biology, however, because DNA encodes the proteins in which physiologists are most interested. Very often, physiologists painstakingly develop elegant strategies for cloning the genes relevant to physiology. Sometimes brute-force approaches, such as the Human Genome Project in the United States, hand the physiologist a candidate gene, homologous to one of known function, on a silver platter. In still other cases, molecular biologists may clone a gene with no known function. In this case, it may be up to the physiologist to determine the *function* of the gene product; that is, to determine its *physiology*.

Physiological genomics (or functional genomics) is a new branch of physiology devoted to the understanding of the roles that genes play in physiology. Traditionally, physiologists have moved in a reductionistic direction from organ to cell to molecule to gene. One of the most fascinating aspects of physiological genomics is that it has closed the circle and linked organ physiology directly with molecular biology. Perhaps one of the most striking examples is the knockout mouse. Knocking out the gene encoding a protein that, according to conventional wisdom, is very important will sometimes have no obvious effect or sometimes unexpected effects. It is up to the physiologist, at least in part, to figure out why. It is perhaps rather sobering to consider that to truly understand the impact of a transgene or a knockout on the physiology of a mouse, one would have to carefully re-evaluate the totality of mouse physiology. To grasp the function of a gene product, the physiologist must retrace the steps up the reductionistic road and achieve an integrated understanding of that gene's function at the level of the cells, organs, and whole body. Physiology is unique among the basic medical sciences in that it is both broad in its scope (i.e., it deals with multiple systems) and integrative in its outlook.

In some cases, important physiological parameters, such as blood pressure, may be under the control of many genes. Certain polymorphisms in several of these many genes could have a cumulative effect that produces high blood pressure. How would one identify which polymorphisms of which genes may underlie high blood pressure? This sort of complex problem does not easily lend itself to a physiologist's

controlled studies. One approach would be to study a population of people, or strains of experimental animals, and use statistical tools to determine which polymorphisms correlate with high blood pressure in a population. Indeed, epidemiologists use statistical tools to study group effects in populations. However, even after the identification of variants in various genes, each of which may make a small contribution to high blood pressure, the physiologist has an important role. First, the physiologist, performing controlled experiments, must determine whether a particular genetic variant does indeed have at least the potential to modulate blood pressure. Second, the physiologist must determine the mechanism of the effect.

Cells live in a highly protected milieu intérieur

In his lectures on the phenomena of life, Claude Bernard noted in 1878 on the conditions of the constancy of life, which he considered a property of higher forms of life. According to Bernard, animals have two environments: the "milieu extérieur" that physically surrounds the whole organism; and the "milieu intérieur," in which the tissues and cells of the organism live. This internal environment is neither the air nor the water in which an organism lives but rather—in the case of the human body—the well-controlled liquid environment that Bernard called "the organic liquid that circulates and bathes all the anatomic elements of the tissues, the lymph or the plasma." In short, this internal environment is what we today call the extracellular fluid. He argued that physiological functions continue in a manner indifferent to the changing environment because the milieu intérieur isolates the organs and tissues of the body from the vagaries of the physical conditions of the environment. Indeed, Bernard described the milieu intérieur as if an organism had placed itself in a greenhouse.

According to Bernard's concept of milieu intérieur, some fluids contained within the body are not really inside the body at all. For example, the *contents* of the gastrointestinal tract, sweat ducts, and renal tubules are all outside the body. They are all continuous with the milieu extérieur.

Bernard compares a complex organism to an ensemble of anatomical elements that live together inside the milieu intérieur. Therefore, in

Section II of this textbook, we examine the physiology of these cells and molecules. In Chapter 2 ("Functional Organization of the Cell"), we begin our journey through physiology with a discussion of the biology of the cells that are the individual elements of the body. Chapter 3 ("Signal Transduction") discusses how cells communicate *directly* through gap junctions or *indirectly* by molecules released into the extracellular fluid. These released molecules can bind to receptors on the cell membrane and initiate signal-transduction cascades that can modify gene transcription (a genomic response) and a wide range of other cell functions (nongenomic responses). Alternatively, these released molecules can bind to receptors in the cytoplasm or nucleus and alter the transcription of genes. In Chapter 4 ("Regulation of Gene Expression"), we examine the response of the nucleus. Chapter 5 ("Transport of Solutes and Water") addresses how the plasma membrane separates the cell interior from Bernard's milieu intérieur and establishes the composition of the cell interior. In the process of establishing the composition of the intracellular fluid, the plasma membrane also sets up ion and voltage gradients across itself. Excitable cells—mainly nerve and muscle cells—can exploit these gradients for the long-distance "electrical" transmission of information. The property of "excitability," which requires both the perception of a change (a signal) and the reaction to it, is the topic of Chapters 6 to 9. In Section III, we examine how the nervous system exploits excitability to process information.

Another theme developed by Bernard was that the "fixité du milieu intérieur" (the constancy of the extracellular fluid) is the condition of "free, independent life." He explains that organ differentiation is the exclusive property of higher organisms and that each organ contributes to "compensate and equilibrate" against changes in the external environment. In that sense, each of the systems discussed in Sections IV to VIII permits the body to live within an adverse external environment because the cardiovascular system, the respiratory system, the urinary system, the gastrointestinal system, and the endocrine system create and maintain a constant internal environment. Individual cell types in various organ systems act in concert to support the constancy of the internal milieu, and the internal milieu in turn provides these cells with a culture medium in which they can thrive.

The discipline of physiology also deals with those characteristics that

are the property of a living organism as opposed to a nonliving organism. Four fundamental properties distinguish the living body. First, only living organisms exchange matter and energy with the environment to continue their existence. Several organ systems of the body participate in these exchanges. Second, only living organisms can receive signals from their environment and react accordingly. The principles of sensory perception, processing by the nervous system, and reaction are discussed in the chapters on excitability and the nervous system. Third, what distinguishes a living organism is the life cycle of growth and reproduction, as discussed in the chapters on reproduction (Section IX). Finally, the living organism is able to adapt to changing circumstances. This is a theme that is developed throughout this textbook but especially in the chapters on everyday life (Section X).

Homeostatic mechanisms—operating through sophisticated feedback control mechanisms—are responsible for maintaining the constancy of the milieu intérieur

Homeostasis is the control of a vital parameter. The body carefully controls a seemingly endless list of vital parameters. Examples of tightly controlled parameters that affect nearly the whole body are arterial pressure and blood volume. At the level of the milieu intérieur, tightly regulated parameters include body core temperature and plasma levels of oxygen, glucose, potassium ions (K^+), calcium ions (Ca^{2+}), and hydrogen ions (H^+). Homeostasis also occurs at the level of the single cell. Thus, cells regulate many of the same parameters that the body as a whole regulates: volume, the concentrations of many small inorganic ions (e.g., Na^+, Ca^{2+}, H^+), and energy levels (e.g., ATP).

One of the most common themes in physiology is the **negative-feedback mechanism** responsible for homeostasis. Negative feedback requires at least four elements. First, the system must be able to sense the vital parameter (e.g., glucose level) or something related to it. Second, the system must be able to compare the input signal with some internal reference value called a *set-point,* thereby forming a difference signal. Third, the system must multiply the error signal by some proportionality

factor (i.e., the gain) to produce some sort of output signal (e.g., release of insulin). Fourth, the output signal must be able to activate an effector mechanism (e.g., glucose uptake and metabolism) that opposes the source of the input signal and thereby brings the vital parameter closer to the set-point (e.g., decrease of blood glucose levels back to normal). **N1-1** Sometimes the body controls a parameter, in part, by cleverly employing positive-feedback loops.

N1-1

Feedback Control

Contributed by Arthur DuBois

In proportional control, the set-point is not reached because the difference signal would disappear, and control would come to an end. Engineers devised a way around this. They took the time integral of the difference signal and used that to activate the effector mechanism to achieve integral control that would allow return to the set-point. There was another problem. Since there is a time delay in processing the input signal, there is a delay in returning to the set-point. Engineers also had a way around that. They took the time-derivative of the difference signal and added that to the corrective signal, speeding up the return toward the set-point.

Another problem turned up. If you have a heater and a cooler, each with its own thermostat, and you want the room to be 23°C to 25°C, you must set one thermostat to turn on the heater at temperatures <23°C but to shut it off at ≥23°C. The thermostat for the cooler has to turn it on >25°C but shut it off at ≤25°C to avoid running the heater and cooler both at once. If the room is cold, the heater will warm it up to 23°C, then shut off. If the room is warm, the cooler will cool it down to 25°C, then shut off. By analogy, the body has separate systems for shivering and sweating, so both do not occur at once. One can picture that anabolic and catabolic pathways should cycle separately and not simultaneously. Many body systems such as respiratory and circulatory controls oscillate between slightly above and slightly below the desired average, hunting for it rather than sitting on a single ideal value. In a case in which the control system is less precise, the swings become wider, as they do when

a drunk driver wanders back and forth across the road proceeding home.

A single feedback loop often does not operate in isolation but rather as part of a larger network of controls. Thus, a complex interplay may exist among feedback loops within single cells, within a tissue, within an organ or organ system, or at the level of the whole body. After studying these individual feedback loops in isolation, the physiologist may find that two feedback loops act either synergistically or antagonistically. For example, insulin lowers blood glucose levels, whereas epinephrine and cortisol have the opposite effect. Thus, the physiologist must determine the relative weights of feedback loops in **competition** with one another. Finally, the physiologist must also establish **hierarchy** among various feedback loops. For example, the hypothalamus controls the anterior pituitary, which controls the adrenal cortex, which releases cortisol, which helps control blood glucose levels.

Another theme of homeostasis is **redundancy.** The more vital a parameter is, the more systems the body mobilizes to regulate it. If one system should fail, others are there to help maintain homeostasis. It is probably for this reason that genetic knockouts sometimes fail to have their expected deleterious effects. The result of many homeostatic systems controlling many vital parameters is a milieu intérieur with a stable composition.

Whether at the level of the milieu intérieur or the cytoplasm of a single cell, homeostasis occurs at a price: energy. When a vital parameter (e.g., the blood glucose level) is well regulated, that parameter is not in equilibrium. **Equilibrium** is a state that does not involve energy consumption. Instead, a well-regulated parameter is generally in a **steady state.** That is, its value is constant because the body or the cell carefully matches actions that lower the parameter value with other actions that raise it. The net effect is that the vital parameter is held at a constant value.

An important principle in physiology, to which we have already alluded, is that each cell plays a specialized role in the overall function of the body. In return, the body—which is the sum of all these cells—provides the milieu intérieur appropriate for the life of each cell. As part of the bargain, each cell or organ must respect the needs of the body as a

whole and not run amok for its own greedy interests. For example, during exercise, the system that controls body core temperature sheds heat by elaborating sweat for evaporation. However, the production of sweat ultimately reduces blood volume. Because the body as a whole places a higher **priority** on the control of blood volume than on the control of body core temperature, at some point the system that controls blood volume will instruct the system that controls body core temperature to reduce the production of sweat. Unfortunately, this juggling of priorities works only if the individual stops exercising; if not, the result may be heat stroke.

The **adaptability** of an organism depends on its ability to alter its response. Indeed, flexible feedback loops are at the root of many forms of physiological adaptation. For instance, at sea level, experimentally lowering the level of oxygen (the sensory stimulus) in the inspired air causes an increase in breathing (the response). However, after **acclimatization** at high altitude to low oxygen levels, the same low level of oxygen (the same sensory stimulus) causes one to breathe much faster (a greater response). Thus, the response may depend on the previous history and therefore the "state" of the system. In addition to acclimatization, genetic factors can also contribute to the ability to respond to an environmental stress. For example, certain populations of humans who have lived for generations at high altitude withstand hypoxia better than lowlanders do, even after the lowlanders have fully acclimatized.

Medicine is the study of "physiology gone awry"

Medicine borrows its physicochemical principles from physiology. Medicine also uses physiology as a reference state: it is essential to know how organs and systems function in the healthy person to grasp which components may be malfunctioning in a patient. A large part of clinical medicine is simply dealing with the abnormal physiology brought about by a disease process. One malfunction (e.g., heart failure) can lead to a *primary* pathological effect (e.g., a decrease in cardiac output) that—in chain-reaction style—leads to a series of *secondary* effects (e.g., fluid overload) that are the appropriate responses of physiological feedback loops. Indeed, as clinician-physiologists have explored the basis of

disease, they have discovered a great deal about physiology. For this reason, we have tried to illustrate physiological principles with clinical examples, some of which are displayed in clinical boxes in this text.

Physiologists have developed many tools and tests to examine normal function. A large number of functional tests—used in diagnosis of a disease, monitoring of the evolution of an illness, and evaluation of the progress of therapy—are direct transfers of technology developed in the physiology laboratory. Typical examples are cardiac monitoring, pulmonary function tests, and renal clearance tests as well as the assays used to measure plasma levels of various ions, gases, and hormones. Refinements of such technology in the hospital environment, in turn, benefit the study of physiology. Thus, the exchange of information between medicine and physiology is a two-way street. The understanding of physiology summarized in this book comes from some experiments on humans but mostly from research on other mammals and even on squids and slime molds. However, our ultimate focus is on the human body.

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SECTION II
Physiology of Cells and Molecules

OUTLINE

CHAPTER 2

Functional Organization of the Cell

Michael J. Caplan

In the minds of many students, the discipline of physiology is linked inextricably to images from its past. This prejudice is not surprising because many experiments from physiology's proud history, such as those of Pavlov on his dogs, have transcended mere scientific renown and entered the realm of popular culture. Some might believe that the science of physiology devotes itself exclusively to the study of whole animals and is therefore an antique relic in this era of molecular reductionism. Nothing could be further from the truth. Physiology is and always has been the study of the homeostatic mechanisms that allow an organism to persist despite the ever-changing pressures imposed by a hostile environment. These mechanisms can be appreciated at many different levels of resolution.

Certainly it would be difficult to understand how the body operates unless one appreciates the functions of its organs and the communication between these organs that allows them to influence one another's behaviors. It would also be difficult to understand how an organ performs its particular tasks unless one is familiar with the properties of its constituent cells and molecules.

The modern treatment of physiology that is presented in this textbook is as much about the interactions of molecules in cells as it is about the interactions of organs in organisms. It is necessary, therefore, at the outset to discuss the structure and characteristics of the cell. Our discussion focuses first on the architectural and dynamic features of a generic cell. We then examine how this generic cell can be adapted to serve in diverse physiological capacities. Through adaptations at the cellular level, organs acquire the machinery necessary to perform their individual metabolic tasks.

Structure of Biological Membranes

The surface of the cell is defined by a membrane

The chemical composition of the cell interior is very different from that of its surroundings. This observation applies equally to unicellular paramecia that swim freely in a freshwater pond and to neurons that are densely packed in the cerebral cortex of the human brain. The biochemical processes involved in cell function require the maintenance of a precisely regulated intracellular environment. The cytoplasm is an extraordinarily complex solution, the constituents of which include myriad proteins, nucleic acids, nucleotides, and sugars that the cell synthesizes or accumulates at great metabolic cost. The cell also expends tremendous energy to regulate the intracellular concentrations of numerous ions. If there were no barrier surrounding the cell to prevent exchange between the intracellular and extracellular spaces, all of the cytoplasm's hard-won compositional uniqueness would be lost by diffusion in a few seconds.

The requisite barrier is provided by the **plasma membrane,** which forms the cell's outer skin. The plasma membrane is *impermeable* to large molecules such as proteins and nucleic acids, thus ensuring their retention within the cytosol. It is *selectively permeable* to small molecules such as ions and metabolites. However, the metabolic requirements of the cell demand a plasma membrane that is much more sophisticated than a simple passive barrier that allows various substances to leak through at different rates. Frequently, the concentration of a nutrient in the extracellular fluid (ECF) is several orders of magnitude lower than that required inside the cell. If the cell wishes to use such a substance, therefore, it must be able to *accumulate* it against a concentration gradient. A simple pore in the membrane cannot concentrate anything; it can only modulate the rate at which a gradient dissipates. To accomplish the more sophisticated feat of creating a concentration gradient, the membrane must be endowed with special machinery that uses metabolic energy to drive the *uphill* movements of substances—**active transport**—into or out of the cell. In addition, it would be useful to rapidly modulate the

permeability properties of the plasma membrane in response to various metabolic stimuli. Active transport and the ability to control passive permeabilities underlie a wide range of physiological processes, from the electrical excitability of neurons to the resorptive and secretory functions of the kidney. In Chapter 5, we will explore how cells actively transport solutes across the plasma membrane. The mechanisms through which the plasma membrane's dynamic selectivity is achieved, modified, and regulated are discussed briefly below in this chapter and in greater detail in Chapter 7.

The cell membrane is composed primarily of phospholipids

Our understanding of biological membrane structure is based on studies of red blood cells, or erythrocytes, that were conducted in the early part of the 20th century. The erythrocyte lacks the nucleus and other complicated intracellular structures that are characteristic of most animal cells. It consists of a plasma membrane surrounding a cytoplasm that is rich in hemoglobin. It is possible to break open erythrocytes and release their cytoplasmic contents. The plasma membranes can then be recovered by centrifugation to provide a remarkably pure preparation of cell surface membrane. Biochemical analysis reveals that this membrane is composed of two principal constituents: lipid and protein.

Most of the lipid associated with erythrocyte plasma membranes belongs to the molecular family of **phospholipids.** In general, phospholipids share a **glycerol** backbone, two hydroxyl groups of which are esterified to various **fatty-acid** or **acyl groups** (Fig. 2-1*A*). These acyl groups may have different numbers of carbon atoms and also may have double bonds between carbons. For glycerol-based phospholipids, the third glycerolic hydroxyl group is esterified to a **phosphate** group, which is in turn esterified to a small molecule referred to as a **head group.** The identity of the head group determines the name as well as many of the properties of the individual phospholipids. For instance, glycerol-based phospholipids that bear an ethanolamine molecule in the head group position are categorized as **phosphatidylethanolamines** (see Fig. 2-1*A*).

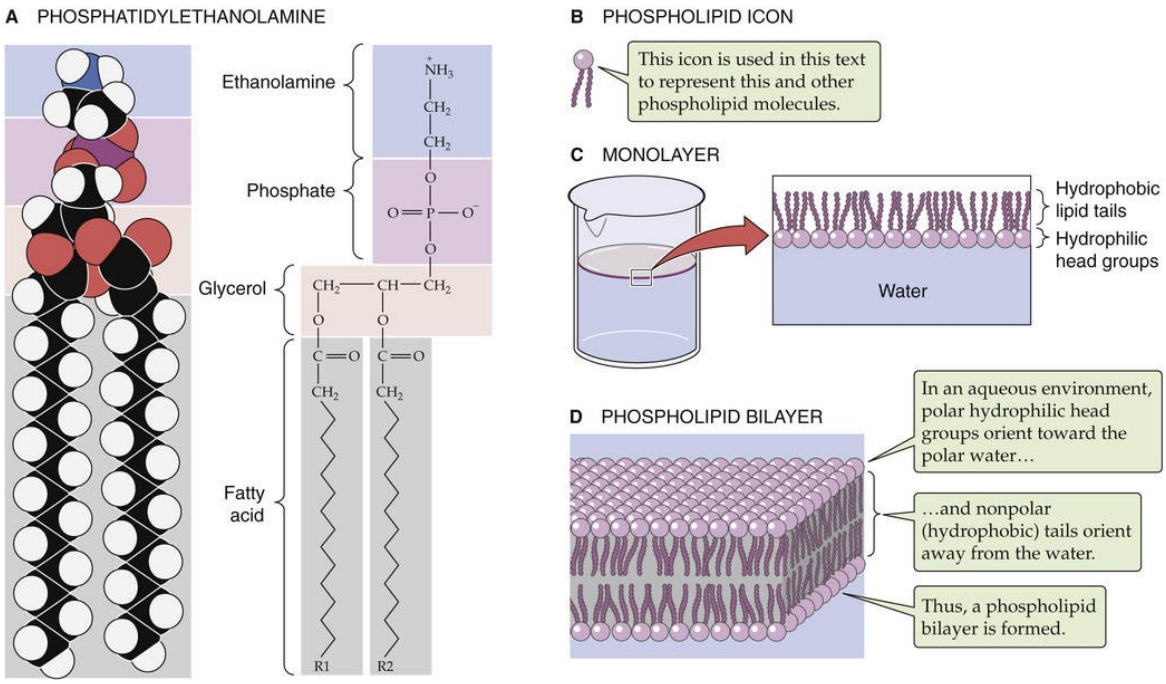


FIGURE 2-1 Phospholipids.

Phospholipids form complex structures in aqueous solution

The unique structure and physical chemistry of each phospholipid (see Fig. 2-1*B*) underlie the formation of biological membranes and explain many of their most important properties. Fatty acids are nonpolar molecules. Their long carbon chains lack the charged groups that would facilitate interactions with water, which is polar. Consequently, fatty acids dissolve poorly in water but readily in organic solvents; thus, fatty acids are **hydrophobic.** On the other hand, the head groups of most phospholipids are charged or polar. These head groups interact well with water and consequently are very water soluble. Thus, the head groups are **hydrophilic.** Because phospholipids combine hydrophilic heads with hydrophobic tails, their interaction with water is referred to as **amphipathic.**

When mixed with water, phospholipids organize themselves into structures that prevent their hydrophobic tails from making contact with water while simultaneously permitting their hydrophilic head groups to be fully dissolved. When added to water at fairly low concentrations,

phospholipids form a **monolayer** (see Fig. 2-1*C*) on the water's surface at the air-water interface. It is energetically less costly to the system for the hydrophobic tails to stick up in the air than to interact with the solvent.

At higher concentrations, phospholipids assemble into **micelles.** The hydrophilic head groups form the surfaces of these small spheres, whereas the hydrophobic tails point toward their centers. In this geometry, the tails are protected from any contact with water and instead are able to participate in energetically favorable interactions among themselves. At still higher concentrations, phospholipids spontaneously form **bilayers** (see Fig. 2-1*D*). In these structures, the phospholipid molecules arrange themselves into two parallel sheets or **leaflets** that face each other tail to tail. The hydrophilic head groups form the surfaces of the bilayer; the hydrophobic tails form the center of the sandwich. The hydrophilic surfaces insulate the hydrophobic tails from contact with the solvent, leaving the tails free to associate exclusively with one another.

The physical characteristics of a lipid bilayer largely depend on the chemical composition of its constituent phospholipid molecules. For example, the width of the bilayer is determined by the length of the fatty-acid side chains. Dihexadecanoic phospholipids (whose two fatty-acid chains are each 16 carbons long) produce bilayers that are 2.47 nm wide; ditetradecanoic phospholipids (bearing 14-carbon fatty acids) generate 2.3-nm bilayers. Similarly, the nature of the head groups determines how densely packed adjacent phospholipid molecules are in each leaflet of the membrane.

Detergents can dissolve phospholipid membranes because, like the phospholipids themselves, they are amphipathic. They possess very hydrophilic head groups and hydrophobic tails and are water soluble at much higher concentrations than are the phospholipids. When mixed together in aqueous solutions, detergent and phospholipid molecules interact through their hydrophobic tails, and the resulting complexes are water soluble, either as individual dimers or in mixed micelles. Therefore, adding sufficient concentrations of detergent to phospholipid bilayer membranes disrupts the membranes and dissolves the lipids. Detergents are extremely useful tools in research into the structure and composition of lipid membranes.

The diffusion of individual lipids within a leaflet of a bilayer is determined by the chemical makeup of its constituents

Despite its highly organized appearance, a phospholipid bilayer is a fluid structure. An individual phospholipid molecule is free to diffuse within the entire leaflet in which it resides. The rate at which this two-dimensional diffusion occurs is extremely temperature dependent. At high temperatures, the thermal energy of any given lipid molecule is greater than the interaction energy that would tend to hold adjacent lipid molecules together. Under these conditions, lateral diffusion can proceed rapidly, and the lipid is said to be in the **sol state.** At lower temperatures, interaction energies exceed the thermal energies of most individual molecules. Thus, phospholipids diffuse slowly because they lack the energy to free themselves from the embraces of their neighbors. This behavior is characteristic of the **gel state.**

The temperature at which the bilayer membrane converts from the gel to the sol phase (and vice versa) is referred to as the **transition temperature.** The transition temperature is another characteristic that depends on the chemical makeup of the phospholipids in the bilayer. Phospholipids with long, saturated fatty-acid chains can extensively interact with one another. Consequently, a fair amount of thermal energy is required to overcome these interactions and permit diffusion. Not surprisingly, such bilayers have relatively high transition temperatures. For example, the transition temperature for dioctadecanoic phosphatidylcholine (which has two 18-carbon fatty-acid chains, fully saturated) is 55.5°C. In contrast, phospholipids that have shorter fatty-acid chains or double bonds (which introduce kinks) cannot line up next to each other as well and hence do not interact as well. Considerably less energy is required to induce them to participate in diffusion. For example, if we reduce the length of the carbon chain from 18 to 14, the transition temperature falls to 23°C. If we retain 18 carbons but introduce one double bond (making the fatty-acid chains monounsaturated), the transition temperature also falls dramatically.

By mixing other types of lipid molecules into phospholipid bilayers, we can markedly alter the membrane's fluidity properties. The *glycerol-based* phospholipids, the most common membrane lipids, include the

phosphatidylethanolamines described above (see Fig. 2-1*A*), as well as the **phosphatidylinositols** (Fig. 2-2*A*), **phosphatidylserines** (see Fig. 2-2*B*), and **phosphatidylcholines** (see Fig. 2-2*C*). The second major class of membrane lipids, the **sphingolipids** (derivatives of *sphingosine*), is made up of three subgroups: **sphingomyelins** (see Fig. 2-2*D*), **N2-1** **glycosphingolipids** such as the galactocerebrosides (see Fig. 2-2*E*), and **gangliosides** (not shown in figure). Cholesterol (see Fig. 2-2*F*) is another important membrane lipid. Because these other molecules are not shaped exactly like the glycerol-based phospholipids, they participate to different degrees in intermolecular interactions with phospholipid side chains. **N2-2** The presence of these alternative lipids changes the strength of the interactions that prevents lipid molecules from diffusing. Consequently, the membrane has a different fluidity and a different transition temperature. This behavior is especially characteristic of the cholesterol molecule, whose rigid steroid ring binds to and partially immobilizes fatty-acid side chains. Therefore, at modest concentrations, cholesterol decreases fluidity. However, when it is present in high concentrations, cholesterol can substantially disrupt the ability of the phospholipids to interact among themselves, which increases fluidity and lowers the gel-sol transition temperature. This issue is significant because animal cell plasma membranes can contain substantial quantities of cholesterol.

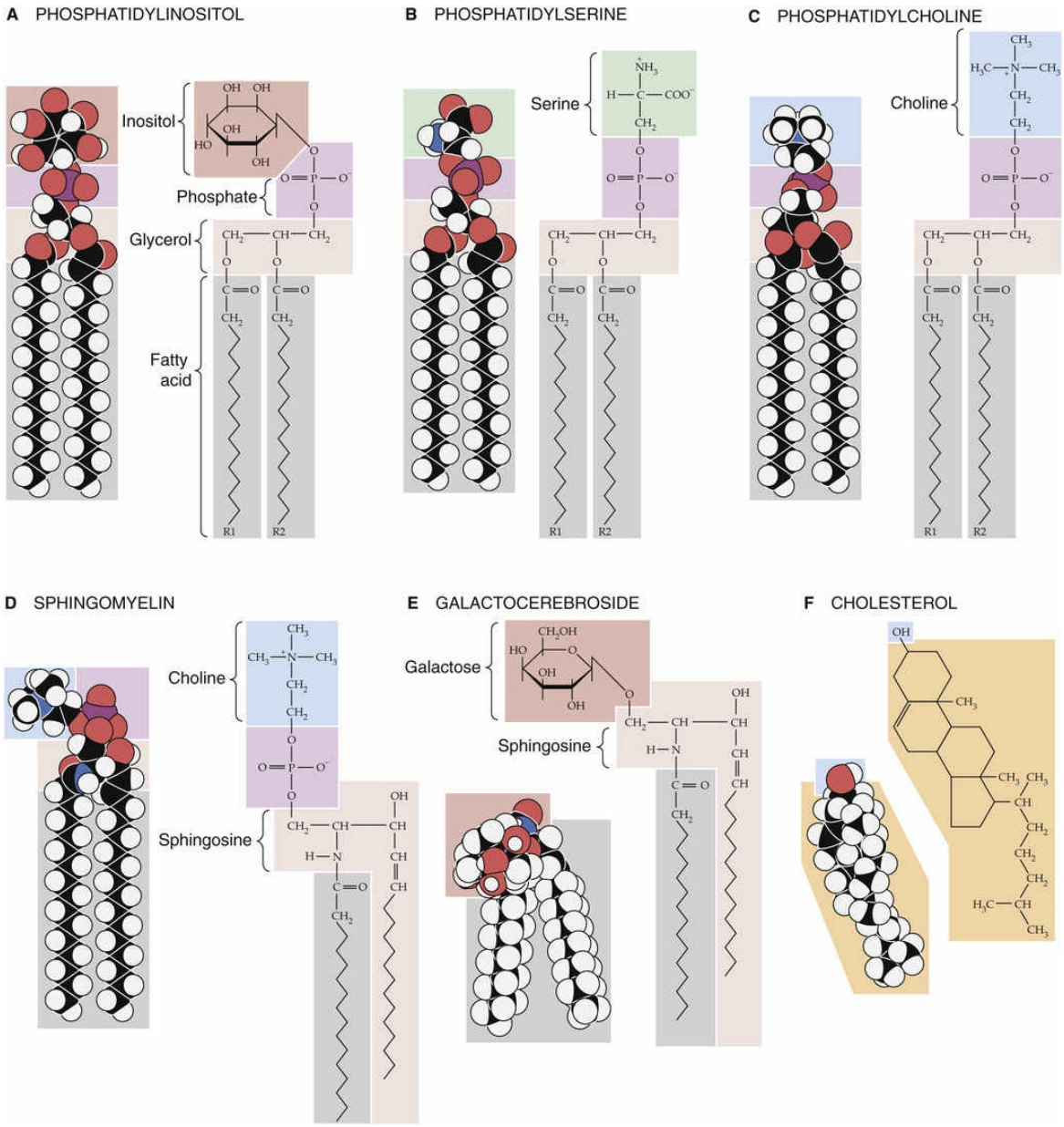


FIGURE 2-2 Structures of some common membrane lipids.

N2-1

Sphingomyelins

Contributed by Emile Boulpaep, Walter Boron

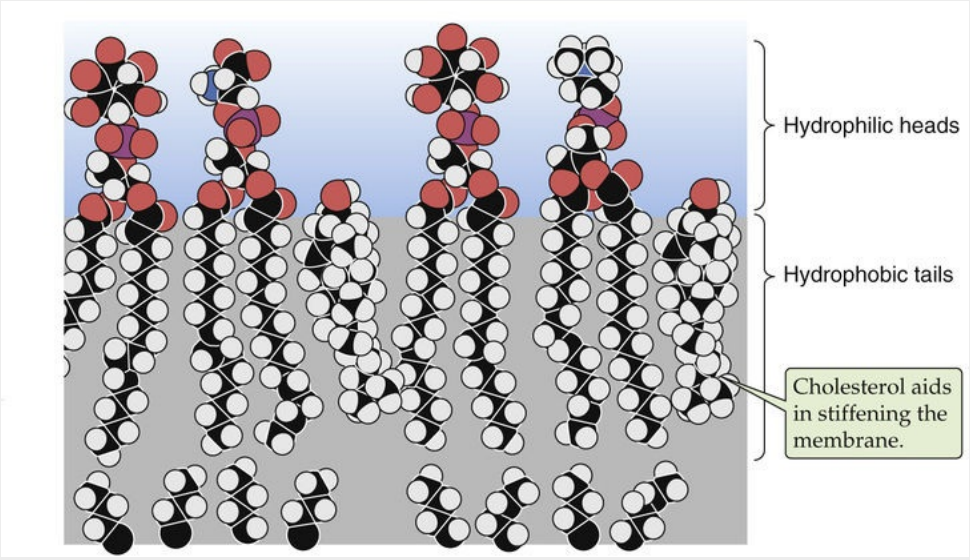
The polar head group of sphingomyelins can be either phosphocholine, as shown in Figure 2-2*D*, or phosphoethanolamine (analogous to the phosphoethanolamine moiety in Fig. 2-1*A*). Note that sphingomyelins

are both (1) sphingolipids because they contain sphingosine, and (2) phospholipids because they contain a phosphate group as do the glycerol-based phospholipids shown in Figures 2-1*A* and 2-2*A–C*.

N2-2

Diversity of Lipids in a Bilayer

Contributed by Michael Caplan



EFIGURE 2-1 The upper leaflet of this lipid bilayer contains, from left to right, phosphatidylinositol, phosphatidylserine, cholesterol, phosphatidylinositol, phosphatidylcholine, and cholesterol.

Bilayers composed of several different lipids do not undergo the transition from gel to sol at a single, well-defined temperature. Instead, they interconvert more gradually over a temperature range that is defined by the composition of the mixture. Within this transition range in such multicomponent bilayers, the membrane can become divided into compositionally distinct zones. The phospholipids with long-chain, saturated fatty acids will adhere to one another relatively tightly, which results in the formation of regions with gel-like properties. Phospholipids bearing short-chain, unsaturated fatty acids will be excluded from these

regions and migrate to sol-like regions. Hence, "lakes" of lipids with markedly different physical properties can exist side by side in the plane of a phospholipid membrane. Thus, the same thermodynamic forces that form the elegant bilayer structure can partition distinct lipid domains within the bilayer. As discussed below, the segregation of lipid lakes in the plane of the membrane may be important for sorting membrane proteins to different parts of the cell.

Although phospholipids can diffuse in the plane of a lipid bilayer membrane, they do not diffuse between adjacent leaflets (Fig. 2-3). The rate at which phospholipids spontaneously "flip-flop" from one leaflet of a bilayer to the other is extremely low. As mentioned above, the center of a bilayer membrane consists of the fatty-acid tails of the phospholipid molecules and is an extremely hydrophobic environment. For a phospholipid molecule to jump from one leaflet to the other, its highly hydrophilic head group would have to transit this central hydrophobic core, which would have an extremely high energy cost. This caveat does not apply to cholesterol (see Fig. 2-3), whose polar head is a single hydroxyl group. The energy cost of dragging this small polar hydroxyl group through the bilayer is relatively low, which permits relatively rapid cholesterol flip-flop.

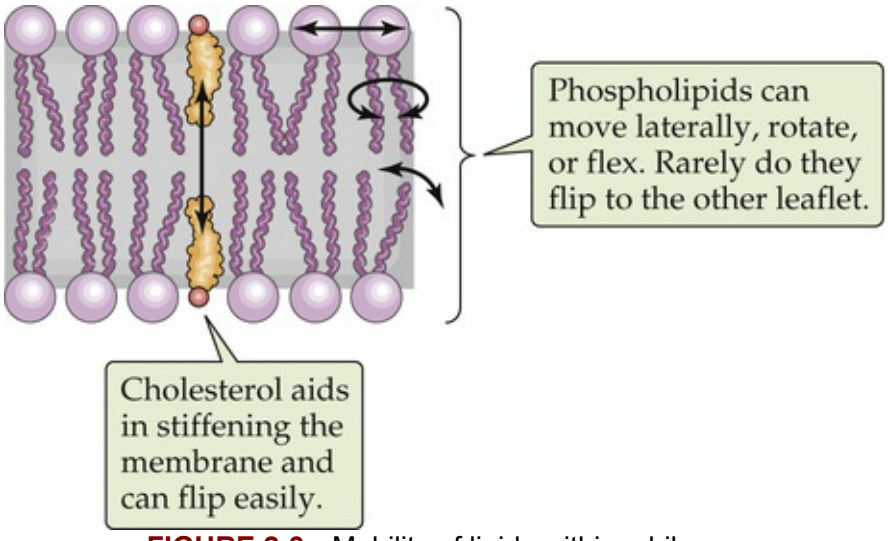


FIGURE 2-3 Mobility of lipids within a bilayer.

Phospholipid bilayer membranes are impermeable to charged molecules

The lipid bilayer is ideally suited to separate two aqueous compartments. Its hydrophilic head groups interact well with water at both membrane surfaces, whereas the hydrophobic center ensures that the energetic cost of crossing the membrane is prohibitive for charged atoms or molecules. Pure phospholipid bilayer membranes are extremely impermeable to almost any charged water-soluble substance. Ions such as Na^+, K^+, Cl^-, and Ca^{2+} are insoluble in the hydrophobic membrane core and consequently cannot travel from the aqueous environment on one side of the membrane to the aqueous environment on the opposite side. The same is true of large water-soluble molecules, such as proteins, nucleic acids, sugars, and nucleotides.

Whereas phospholipid membranes are impermeable to water-soluble molecules, small *uncharged* polar molecules can cross fairly freely. This is often true for O_2, CO_2, NH_3, and, remarkably, water itself. Water molecules may, at least in part, traverse the membrane through transient cracks between the hydrophobic tails of the phospholipids without having to surmount an enormous energetic barrier. The degree of permeability of water (and perhaps that of CO_2 and NH_3 as well) varies extensively with lipid composition; some phospholipids (especially those with short or kinked fatty-acid chains) permit a much greater rate of transbilayer water diffusion than others do.

The plasma membrane is a bilayer

As may be inferred from the preceding discussion, the membrane at the cell surface is, in fact, a phospholipid bilayer. The truth of this statement was established by a remarkably straightforward experiment. In 1925, Gorter and Grendel measured the surface area of the lipids they extracted from erythrocyte plasma membranes. They used a device called a Langmuir trough in which the lipids are allowed to line up at an air-water interface (see Fig. 2-1C) and are then packed together into a continuous monolayer by a sliding bar that decreases the surface available to them. The area of the monolayer that was created by the erythrocyte lipids was exactly twice the surface area of the erythrocytes

from which they were derived. Therefore, the plasma membrane must be a bilayer.

Confirmation of the bilayer structure of biological membranes has come from x-ray diffraction studies performed on the repetitive whorls of membrane that form the myelin sheaths surrounding neuronal axons (see pp. 292–293).

The membrane's bilayer structure can be visualized directly in the high-magnification electron micrograph depicted in Figure 2-4. The osmium tetraoxide molecule (OsO_4) with which the membrane is stained binds to the head groups of phospholipids. Thus, both surfaces of a phospholipid bilayer appear black in electron micrographs, whereas the membrane's unstained central core appears white.

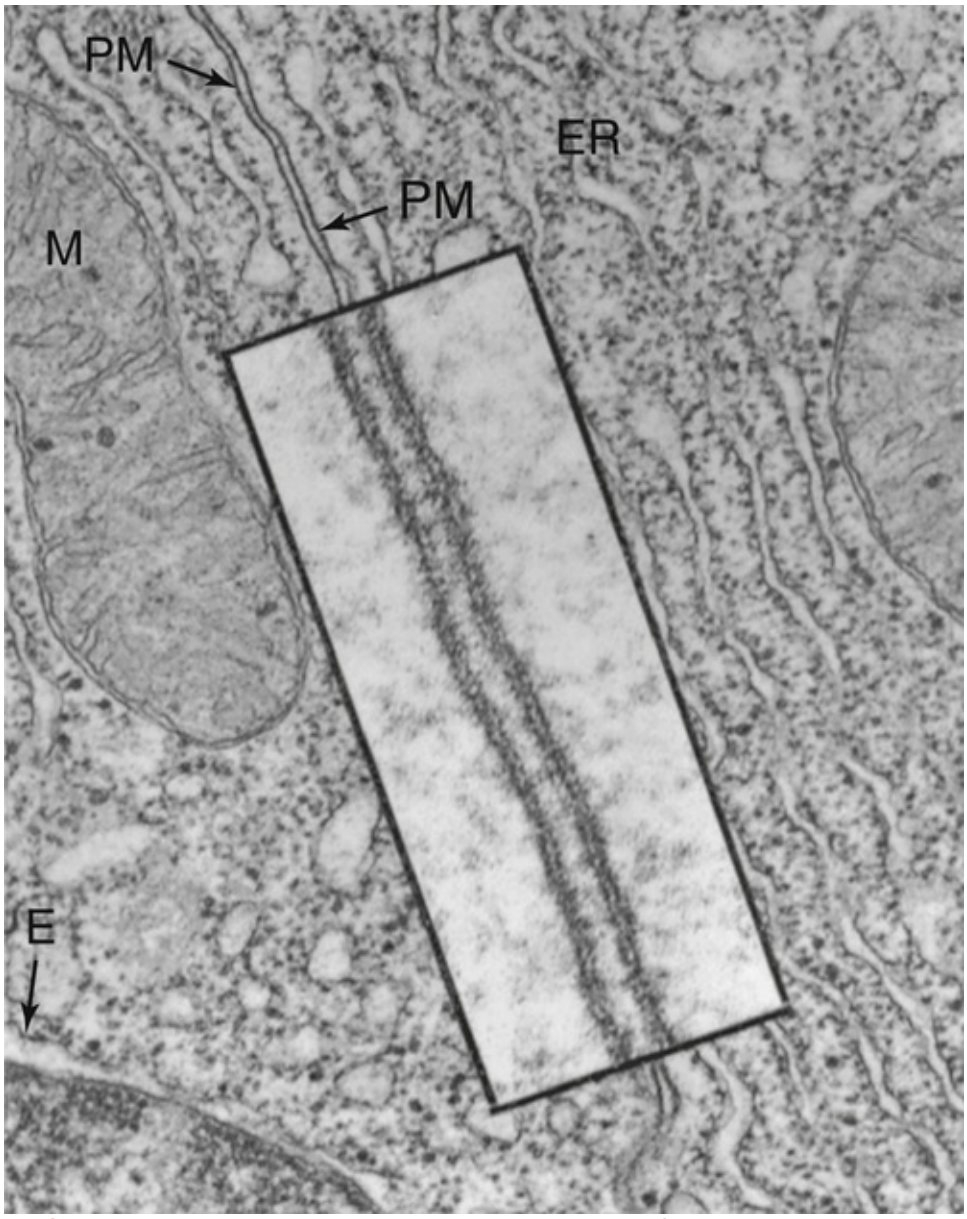


FIGURE 2-4 Transmission electron micrograph of a cell membrane. The photograph shows two adjacent cells of the pancreas of a frog (original magnification ×43,000). The inset is a high-magnification view (original magnification ×216,000) of the plasma membranes (PM) of the cells. Note that each membrane includes two dense layers with an intermediate layer of lower density. The dense layers represent the interaction of the polar head groups of the phospholipids with the OsO_4 used to stain the preparation. E, nuclear envelope; M, mitochondrion. (From Porter KR, Bonneville MR: Fine Structure of Cells and Tissues, 4th ed. Philadelphia, Lea & Febiger, 1973.)

The phospholipid compositions of the two leaflets of the plasma membrane are not identical. Labeling studies performed on erythrocyte plasma membranes reveal that the surface that faces the cytoplasm

contains phosphatidylethanolamine and phosphatidylserine, whereas the outward-facing leaflet is composed almost exclusively of phosphatidylcholine. As is discussed below in this chapter, this asymmetry is created during the biosynthesis of the phospholipid molecules. It is not entirely clear what advantage this distribution provides to the cell. The interactions between certain proteins and the plasma membrane may require this segregation. The lipid asymmetry may be especially important for those phospholipids that are involved in second-messenger cascades. Phosphatidylinositols, for example, give rise to phosphoinositides, which play critical roles in signaling pathways (see pp. 58–61). In addition, the phosphatidylinositol composition of the cytoplasmic face of an organelle helps to define the identity of the organelle and to govern its trafficking and targeting properties. Finally, the phospholipids that are characteristic of animal cell plasma membranes generally have one saturated and one unsaturated fatty-acid residue. Consequently, they are less likely to partition into sol-like or gel-like lipid domains than are phospholipids that bear identical fatty-acid chains. **N2-3**

N2-3

Membrane Microdomains

Contributed by Michael Caplan

According to current models (see Anderson and Jacobson, 2002; Edidin, 2003), lipids and proteins are not uniformly distributed in the plane of the membranes that surround cells and organelles. Instead, certain lipids and associated proteins cluster to form microdomains that differ in composition, structure, and function from the rest of the membrane that surrounds them. These microdomains can be thought of as small islands bordered by the "lake" of lipids and proteins that constitute the bulk of the membrane. These two-dimensional structures are composed of lipids that tend to form close interactions with one another, resulting in the self-assembly of organized domains that include specific types of lipids and exclude others. The lipids that tend to be found in microdomains include sphingomyelin, cholesterol, and glycolipids. Proteins that are able to interact closely with microdomain-forming lipids can also

become selectively incorporated into these microdomains. A number of different names are used to refer to these microdomains, the most common of which are caveolae and rafts.

Caveolae (see pp. 42–43) were originally identified in the electron microscope as flask-shaped invaginations of the plasma membrane. They carry a coat composed of proteins called **caveolins,** and they tend to be at least 50 to 80 nm in diameter. Caveolae have been shown to participate in endocytosis of specific subsets of proteins and are also richly endowed with signaling molecules, such as receptor tyrosine kinases.

Rafts are less well understood structures, which are defined by the biochemical behaviors of their constituents when the surrounding membrane is dissolved in nonionic detergents. Lipid microdomains rich in sphingomyelin, cholesterol, and glycolipids tend to resist solubilization in these detergents under certain conditions and can be recovered intact by density centrifugation. Once again, a number of interesting proteins involved in cell signaling and communication, including kinases, ion channels, and G proteins, tend to be concentrated in rafts, or to become associated with rafts upon the activation of specific *signal-transduction* pathways. Rafts are thought to collect signaling proteins into small, highly concentrated zones, thereby facilitating their interactions and hence their ability to activate particular pathways. Rafts are also involved in membrane trafficking processes. In polarized epithelial cells, the sorting of a number of proteins to the apical plasma membrane is dependent upon their ability to partition into lipid rafts that form in the plane of the membrane of the *trans*-Golgi network. Little is known about what lipid rafts actually look like in cell membranes in situ. It is currently thought that they are fairly small (<100 nm), although it is possible that they can be induced to coalesce into larger structures under certain circumstances. Much remains to be learned about the structures and functions of rafts and caveolae, but it is clear that they are dynamic and important entities that subdivide membrane into specialized regions that cells exploit for a wide variety of tasks.

References

Anderson RG, Jacobson K. A role for lipid shells in targeting

proteins to caveolae, rafts, and other lipid domains. *Science*. 2002;296:1821–1825.
Edidin M. The state of lipid rafts: From model membranes to cells. *Annu Rev Biophys Biomol Struct*. 2003;32:257–283.

Membrane proteins can be integrally or peripherally associated with the plasma membrane

The demonstration that the plasma membrane's lipid components form a bilayer leaves open the question of how the membrane's protein constituents are organized. Membrane proteins can belong to either of two broad classes, peripheral or integral. **Peripherally associated membrane proteins** are neither embedded within the membrane nor attached to it by covalent bonds; instead, they adhere tightly to the cytoplasmic or extracellular surfaces of the plasma membrane (Fig. 2-5*A*). They can be removed from the membrane, however, by mild treatments that disrupt ionic bonds (very high salt concentrations) or hydrogen bonds (very low salt concentrations).

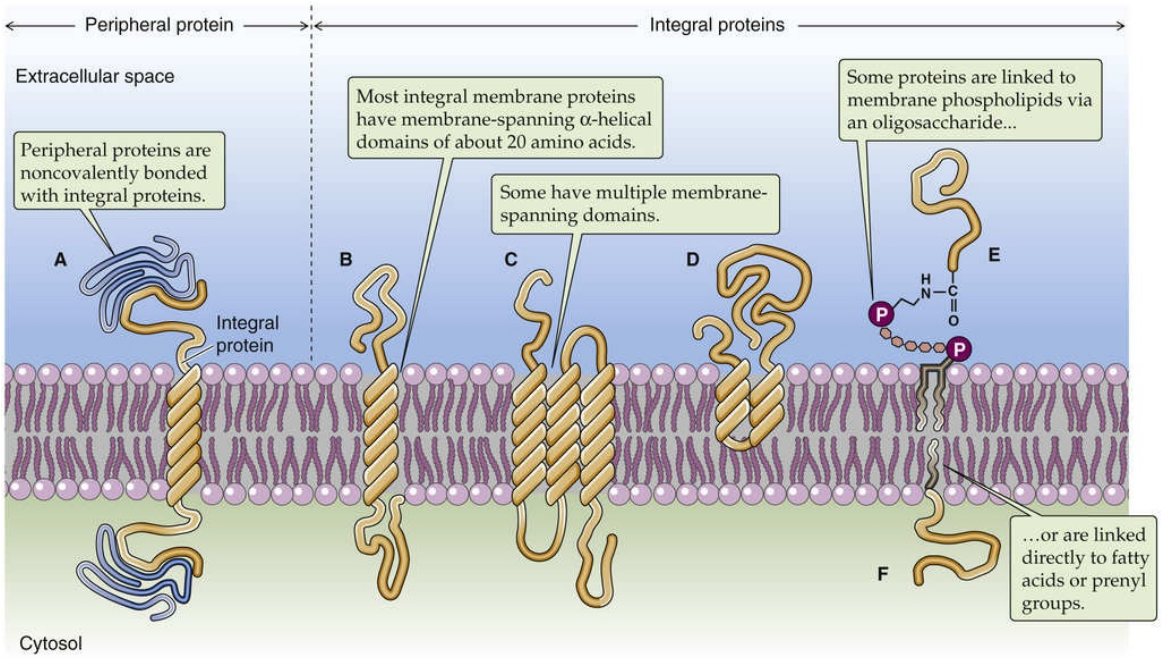


FIGURE 2-5 Classes of membrane proteins. In **E,** protein is coupled via a

GPI linkage.

In contrast, **integral membrane proteins** are intimately associated with the lipid bilayer. They cannot be eluted from the membrane by these high- or low-salt washes. For integral membrane proteins to be dislodged, the membrane itself must be dissolved by adding detergents. Integral membrane proteins can be associated with the lipid bilayer in any of three ways. First, some proteins actually span the lipid bilayer once or several times (see Fig. 2-5*B, C*) and hence are referred to as **transmembrane proteins.** Experiments performed on erythrocyte membranes reveal that these proteins can be labeled with protein-tagging reagents applied to either side of the bilayer.

The second group of integral membrane proteins is embedded in the bilayer without actually crossing it (see Fig. 2-5*D*). A third group of membrane-associated proteins is not actually embedded in the bilayer at all. Instead, these lipid-anchored proteins are attached to the membrane by a covalent bond that links them either to a lipid component of the membrane or to a fatty-acid derivative that intercalates into the membrane. For example, proteins can be linked to a special type of glycosylated phospholipid molecule (see Fig. 2-5*E*), which is most often **glycosylphosphatidylinositol (GPI),** on the outer leaflet of the membrane. This family is referred to collectively as the **glycophospholipid-linked proteins.** Another example is a direct linkage to a fatty acid (e.g., a myristyl group) or a prenyl (e.g., farnesyl) group that intercalates into the inner leaflet of the membrane (see Fig. 2-5*F*).

The membrane-spanning portions of transmembrane proteins are usually hydrophobic α helices

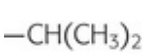
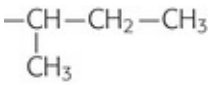
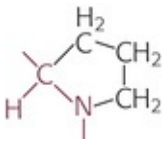
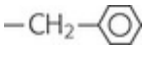
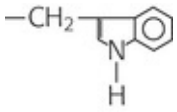
How can membrane-spanning proteins remain stably associated with the bilayer in a conformation that requires at least some portion of their amino-acid sequence to be in continuous contact with the membrane's hydrophobic central core? The answer to this question can be found in the special structures of those protein domains that actually span the membrane.

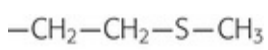
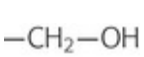
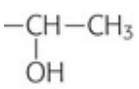
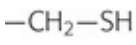
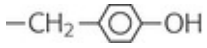
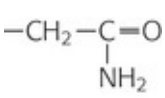
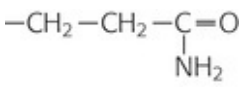
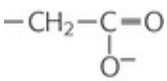
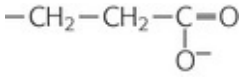
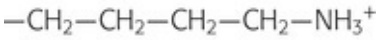
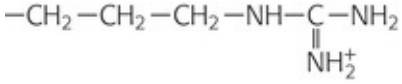
The side chains of the eight amino acids listed in the upper portion of

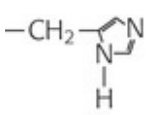
Table 2-1 are hydrophobic. These aromatic or uncharged aliphatic groups are almost as difficult to solvate in water as are the fatty-acid side chains of the membrane phospholipids themselves. Not surprisingly, therefore, these hydrophobic side chains are quite comfortable in the hydrophobic environment of the bilayer core. Most **membrane-spanning segments**—that is, the short stretches of amino acids that pass through the membrane once—are composed mainly of these nonpolar amino acids, in concert with polar, uncharged amino acids.

TABLE 2-1

Classification of the Amino Acids Based on the Chemistry of Their Side Chains

	NAME	THREE-LETTER CODE	SINGLE-LETTER CODE	STRUCTURE OF THE SIDE CHAIN	HYDROPATHY INDEX*
Nonpolar	Alanine	Ala	A		+1.8
	Valine	Val	V		+4.2
	Leucine	Leu	L		+3.8
	Isoleucine	Ile	I		+4.5
	Proline	Pro	P		−1.6
	Phenylalanine	Phe	F		+2.8
	Tryptophan	Trp	W		−0.9

	Methionine	Met	M		+1.9
Polar uncharged	Glycine	Gly	G		−0.4
	Serine	Ser	S		−0.8
	Threonine	Thr	T		−0.7
	Cysteine	Cys	C		+2.5
	Tyrosine	Tyr	Y		−1.3
	Asparagine	Asn	N		−3.5
	Glutamine	Gln	Q		−3.5
Polar, charged, acidic	Aspartate	Asp	D		−3.5
	Glutamate	Glu	E		−3.5
Polar, charged, basic	Lysine	Lys	K		−3.9
	Arginine	Arg	R		−4.5
	Histidine	His	H		−3.2

*Kyte and Doolittle generated these values (arbitrary scale from −4.5 to +4.5) by averaging two kinds of data. The first is an index of the energy that is required to transfer the side chain from the vapor phase into water. The second indicates how likely it is to find the side chain buried in (as opposed to being on the surface of) 12 globular proteins, whose structures were solved by x-ray crystallography. A positive value indicates that the side chain is hydrophobic.

Note: The portion shown in red is part of the peptide backbone.

(From Kyte J, Doolittle RF: A simple method for displaying the hydropathic character of a protein. J Mol Biol 157:105–132, 1982.)

The hydrophobic, membrane-spanning segments of transmembrane proteins are specially adapted to the hydrophobic milieu in which they reside. The phospholipid molecules of the membrane bilayer actually protect these portions of transmembrane proteins from energetically unfavorable interactions with the aqueous environment. Transmembrane proteins tend to be extremely insoluble in water. If we separate the membrane-spanning segments of these proteins from the amphipathic phospholipids that surround them, these hydrophobic sequences tend to interact tightly with one another rather than with water. The resulting large protein aggregates are generally insoluble and precipitate out of solution. If, however, we disrupt the phospholipid membrane by adding detergent, the amphipathic detergent molecules can substitute for the phospholipids. The hydrophobic membrane-spanning sequences remain insulated from interactions with the aqueous solvent, and the proteins remain soluble as components of **detergent micelles.** This ability of detergents to remove transmembrane proteins from the lipid bilayer—while maintaining the solubility and native architectures of these proteins—has proved important for purifying individual membrane proteins.

Transmembrane proteins can have a single membrane-spanning segment (see Fig. 2-5*B*) or several (see Fig. 2-5*C*). Those with a single transmembrane segment can be oriented with either their amino (N) or their carboxyl (C) terminus facing the extracellular space. Multispanning membrane proteins weave through the membrane like a thread through cloth. Again, the N and C termini can be exposed to either the cytoplasmic or extracellular compartments. The pattern with which the transmembrane protein weaves across the lipid bilayer defines its

membrane **topology.**

The amino-acid sequences of membrane-spanning segments tend to form α helices, with ~3.6 amino acids per turn of the helix (see Fig. 2-5*B*). In this conformation, the polar atoms of the peptide backbone are maximally hydrogen bonded to one another—from one turn of the helix to the next—so they do not require the solvent to contribute hydrogen-bond partners. Hence, this structure ensures the solubility of the membrane-spanning sequence in the hydrophobic environment of the membrane. Whereas most transmembrane proteins appear to traverse the membrane with α-helical spans, it is clear that an intriguing subset of membrane polypeptides makes use of a very different structure. For example, the porin protein (see p. 109), which serves as a channel in bacterial membranes, has membrane-spanning portions arranged as a β barrel.

In the case of multispanning membrane proteins, their transmembrane helices probably pack together tightly (see Fig. 2-5*C*). Molecular analysis of a number of known membrane-spanning sequences has helped in the development of algorithms predicting the likelihood that a given amino-acid sequence can span the membrane. These algorithms are widely used to assess the likelihood that newly identified genes encode transmembrane proteins and to predict the number and location of membrane-spanning segments.

Many membrane proteins form tight, noncovalent associations with other membrane proteins in the plane of the bilayer. These **multimeric proteins** can be composed of a single type of polypeptide or of mixtures of two or more different proteins. The side-to-side interactions that hold these complexes together can involve the membrane-spanning segments or regions of the proteins that protrude at either surface of the bilayer. By assembling into multimeric complexes, membrane proteins can increase their stability. They can also increase the variety and complexity of the functions that they are capable of performing.

Some membrane proteins are mobile in the plane of the bilayer

As is true for phospholipid molecules (see Fig. 2-3), some transmembrane proteins can diffuse within the surface of the membrane. In the absence

of any protein-protein attachments, transmembrane proteins are free to diffuse over the entire surface of a membrane. This fact was demonstrated by Frye and Edidin in 1970 (Fig. 2-6). They labeled the surface proteins of a population of *mouse* lymphocytes with a lectin (a plant protein that binds strongly to certain sugar groups attached to proteins) that was linked to the fluorescent dye fluorescein. They also tagged the surface proteins of a second population of *human* lymphocytes with a lectin that was conjugated to a different fluorescent dye, rhodamine. Because fluorescein glows green and rhodamine glows red when excited by the light of the appropriate wavelengths, these labeling molecules can be easily distinguished from one another in a fluorescence microscope. Frye and Edidin mixed the two lymphocyte populations and treated them with a reagent that caused the cells to fuse to each other. Immediately after fusion, the labeled surface proteins of the newly joined cells remained separate; half of the fused cell surface appeared red, whereas the other half appeared green. During a period of ~30 minutes, however, the green and red protein labels intermixed until the entire surface of the fused cell was covered with both labeling molecules. The rate at which this intermingling occurred increased with temperature, which is not surprising given the temperature dependence of membrane fluidity.

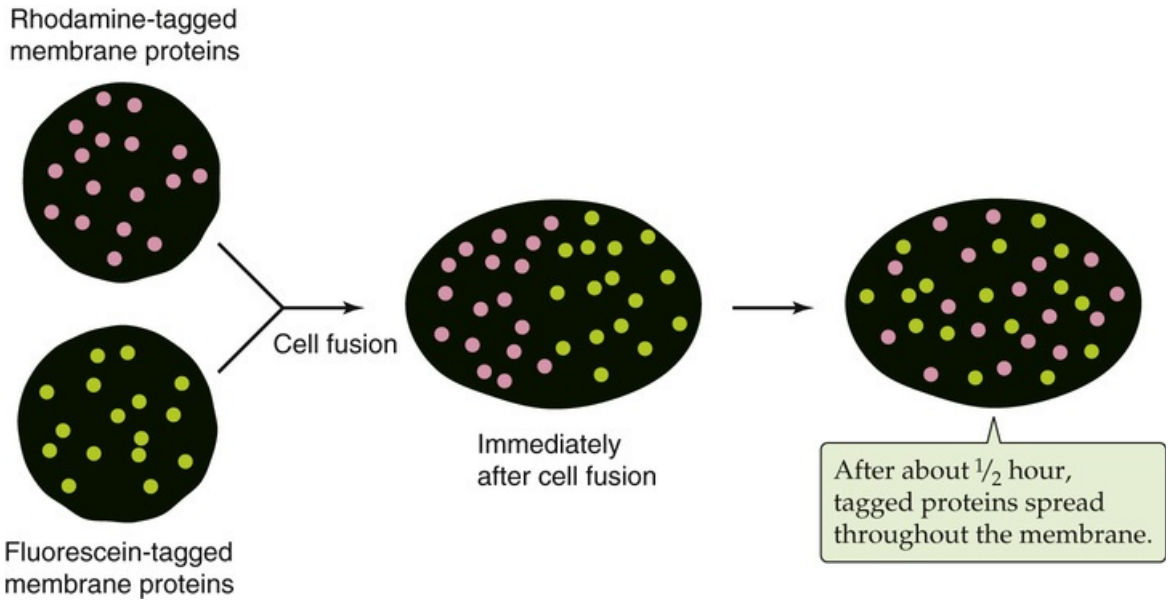


FIGURE 2-6 Diffusion of membrane proteins within the plane of the cell

membrane. The surface proteins of a *human* lymphocyte are tagged with a lectin conjugated to rhodamine, a fluorescent dye; the surface proteins of a *mouse* lymphocyte are tagged with a lectin linked to fluorescein, another fluorescent dye. Immediately after fusion of the two cells, the labeled surface proteins remain segregated. However, the membrane proteins intermingle during a period of ~30 minutes.

Because transmembrane proteins are large molecules, their diffusion in the plane of the membrane is much slower than that of lipids. Even the fastest proteins diffuse ~1000 times more slowly than the average phospholipid. The diffusion of many transmembrane proteins appears to be further impeded by their attachments to the cytoskeleton, just below the surface of the membrane. Tight binding to this meshwork can render proteins essentially immobile. Other transmembrane proteins appear to travel in the plane of the membrane via directed processes that are much faster and less directionally random than diffusion is. Motor proteins that are associated with the cytoplasmic cytoskeleton (discussed below) appear to grab onto certain transmembrane proteins, dragging them in the plane of the membrane like toy boats on strings. Finally, like phospholipids, proteins can diffuse only in the plane of the bilayer. They cannot flip-flop across it. The energetic barrier to dragging a transmembrane protein's hydrophilic cytoplasmic and extracellular domains across the bilayer's hydrophobic core is very difficult to surmount. Thus, a membrane protein's topology does not change over its life span.

Function of Membrane Proteins

Integral membrane proteins can serve as receptors

All communication between a cell and its environment must involve or at least pass through the plasma membrane. For the purposes of this discussion, we define communication rather broadly as the exchange of any signal between the cell and its surroundings. Except for lipid-soluble signaling molecules such as steroid hormones, essentially all communication functions served by the plasma membrane occur via membrane proteins. From an engineering perspective, membrane proteins are perfectly situated to transmit signals because they form a single, continuous link between the two compartments that are separated by the membrane.

Ligand-binding receptors comprise the group of transmembrane proteins that perhaps most clearly illustrate the concept of transmembrane signaling (Fig. 2-7*A*). For water-soluble hormones such as epinephrine to influence cellular behavior, their presence in the ECF compartment must be made known to the various intracellular mechanisms whose behaviors they modulate. The interaction of a hormone with the extracellular portion of the hormone receptor, which forms a high-affinity binding site, produces conformational changes within the receptor protein that extend through the membrane-spanning domain to the intracellular domain of the receptor. As a consequence, the intracellular domain either becomes enzymatically active or can interact with cytoplasmic proteins that are involved in the generation of so-called second messengers. Either mechanism completes the transmission of the hormone signal across the membrane. The transmembrane disposition of a hormone receptor thus creates a single, continuous communication medium that is capable of conveying, through its own structural modifications, information from the environment to the cellular interior. The process of transmembrane signal transduction is discussed in Chapter 3.