MEMBRANE PHYSIOLOGY

RICHARD A. NYSTROM

MEMBRANE PHYSIOLOGY

Richard A. Nystrom

Department of Biological Sciences
University of Delaware

Prentice-Hall, Inc.
Englewood Cliffs, New Jersey

PREFACE

This book is an integrated and relatively compact presentation of cytological, physiological, and molecular data on plasma membranes of animal cells. For a long time the study of the structure and function of biological membranes has been one of the more active endeavors of biological investigators. So many disciplines are involved — biophysics, biochemistry, morphology, pharmacology, physiology, physical chemistry, molecular biology — and the literature is so extensive that it is difficult for workers of diverse training and experience to fully appreciate the level of understanding and the advances within neighboring fields. A young biologist contemplating investigations on membranes and an older worker trained in another field both require a conceptual insight into the nature of membrane problems and a scientific intuition into their solution. The recent scientific conferences and symposiums dealing with general aspects of membranology, a multitude of research reviews and books, and at least one new journal devoted solely to membrane biology have served to unify the field. However, much of this material characteristically contains articles that are restricted in scope and written for scientifically sophisticated readers. None is sufficiently broad to embrace all aspects of the work on biological membranes and at the same time sufficiently integrated to provide a background necessary to relate one finding to another. None is exceptionally noted for its readability. This book may help as an introduction to that literature in several ways.

By discussing the current state of our understanding of the nature of membrane structure, permeation, and excitability of both living and artificial systems, the book covers a broad range of knowledge, but it is clearly not comprehensive. Within the areas covered, only work that I consider important or interesting is included. Investigations on cytoplasmic membranes and on plant and bacterial membranes are barely mentioned; pinocytosis and phagocytosis are only acknowledged; and the role of the cell surface in cellular contact and immunological relationships is ignored completely — to the dismay of virologists, immunologists, pathologists, tissue culturists, and cancer researchers, among others. This book is restricted but not superficial. The intent is not only to present a readable account of the major ideas of the various approaches to membrane study and their broader implications but also to include the complications, limitations, alternative explanations, and important minor points to these investigations. This book is concise and elementary but not simple. The intent is to inform the reader so that he will be able to more effectively glean the sophisticated books, reviews, and research papers on membranes and evaluate their interpretations in his own mind. Sufficient knowledge is extant to permit an uncomplicated summary of only the widely held conclusions and to tell a pretty story, but a beginner would not be well served and a seasoned veteran would not be well satisfied by such an approach. Difficulties with arguments are readily presented, but experimental and language technicalities and ponderous derivations are reduced. Experimental procedures are discussed where they are important to the proper interpretation of the data that they yield. The important quantitative analyses of membrane data are hinted at, but full and, hence, proper treatment is to be found in the publications cited.

The many references cited are intended more as a guide through the literature than as a historical account of authenticity; so a large number refer to reviews and interpretative work. The examples selected for a more detailed analysis represent my own favorites rather than an attempt to tell all or to give the most recent. Experienced investigators will undoubtedly be unhappy about the treatment given some topics as well as the exclusion of others. The success of my approach will be determined by the usefulness of this small volume to you.

Historically, this manuscript is derived in part from a series of discussions held many years ago with members of the Engineering Physics Department of the E. I. du Pont de Nemours Company, Wilmington, Delaware. The time to finish it became more readily available as a result of the freedom from academic duties beyond normal teaching and research provided by a sabbatical leave from the University of Delaware and a Special Fellowship from the National Institutes of Neurological Diseases and Stroke to work on other things at the Massachusetts Institute of Technology and the Marine Biological Laboratory at Woods Hole.

While the responsibility for this manuscript is clearly mine, I am most grateful and thankful for the encouragement, advice, assistance, and especially the criticism of A. Bortoff, Z. de Schauensee, P. B. Dunham, A. Gould, C. M. Lent, R. A. Levy, P. D. Lunger, D. W. Martin, R. A. Meiss, I. Nadelhaft, V. Shashoua, L. Smucker, W. M. Trippeer, J. T. Tupper, D. F. Wilson, R. A. Yates, and the anonymous referees found by J. R. Riina of Prentice-Hall, Inc. I am also pleased to acknowledge K. D. Roeder, C. L. Prosser, R. R. Ronkin, and F. O. Schmitt, all of whom helped to influence my scientific career.

RICHARD A. NYSTROM

Newark, Delaware

CONTENTS

INTRODUCTION

chapter one

Cellular activities are characterized by their occurrence at or near membranes located throughout the cell (Figs. 1–3). For example, much of cellular metabolism is associated with enzymes aligned on cristae of mitochondria; protein synthesis is associated with ribosomes attached to endoplasmic reticulum; lipid transport, with Golgi apparatus; photosynthesis, with grana within chloroplasts; light reception, with retinal rods. The interest here, however, is concerned with the surface of the cell—the cellular membrane or, more properly, the plasma membrane—the barrier or interface between the living inside and the dead outside of the cell.

A variety of physical measurements indicates that the cell surface is about 75Å thick although the published figures vary greatly. This thin structure is made of protein, some carbohydrate, and lipid, mostly sterol and phospholipid. For more than 30 years the molecular arrangement has been described as a double layer of lipid between two protein layers. Studies using X-ray diffraction, polarized light, electron microscopy, and permeability data have in general confirmed this view although newer techniques and approaches have exposed globular substructures and have confused the satisfaction with this description. Pores in membrane structures have been proposed with regularity, but, as the supporting experiments are all indirect ones and as investigators become more familiar with dynamic concepts, the need for the existence of a pore as a fixed morphological structure to understand membrane permeation is becoming less important.

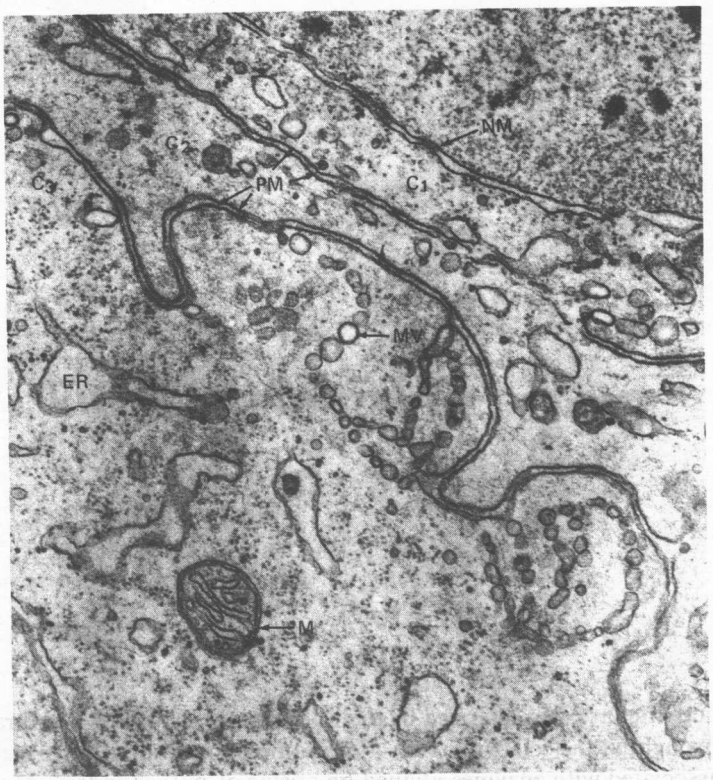

Figure 1. *Examples of membranous structures in cells:* three adjacent frog kidney cells showing at least five types of membrane. C_1, C_2, and C_3 are portions of three cells separated by plasma membranes (PM) and a small amount of extracellular space. The double nuclear membrane (NM) appears at the upper right. A mitochondrion (M) and its cristae are seen at the lower left. Smooth endoplasmic reticulum (ER) and ribosomal particles appear in the cytoplasm of C_3 especially. The rows of membranous vesicles (MV) near the plasma membrane of C_3 are considered by some workers to be plasma membrane that is either forming or degenerating ($\times$ 40,000).

Life exists in a liquid phase. Cells contain and surround themselves with liquid media; water is the solvent. Materials continuously exchange between the inside and outside of cells, dead or alive. Obviously, the cell surface is not an absolute barrier. In living cells, the plasma membrane regulates this exchange with care by processes best described by nonequilibrium thermodynamics; only a dead cell is in complete equilibrium with the solutes in its environment. Larger molecules permeate the membrane more slowly than do smaller ones. Molecules soluble in lipid cross the cell surface barrier faster than others. The transfer of polar molecules is impeded. But water

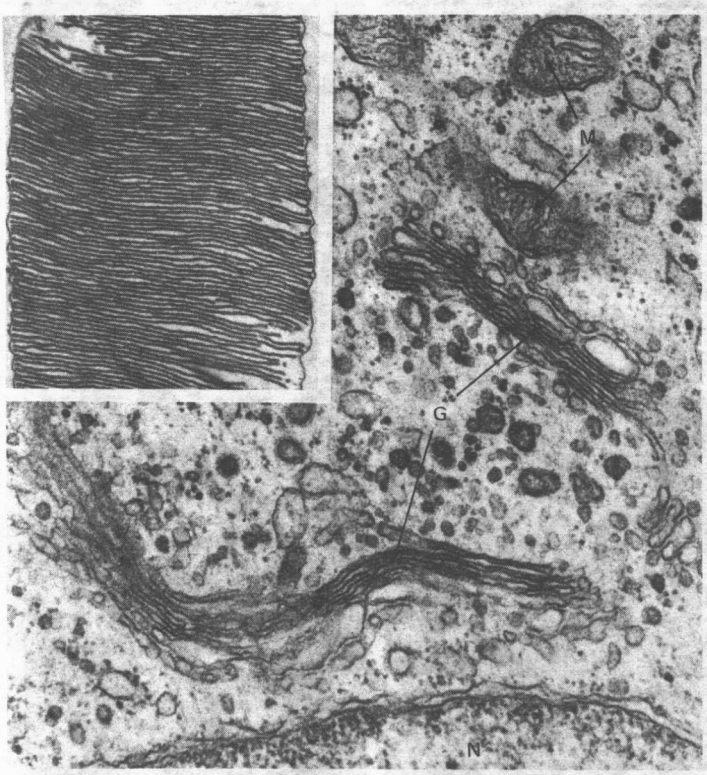

Figure 2. *Examples of membranous structures in cells:* rod membrane in turtle retina and Golgi membrane in frog kidney cells. Portions of three mitochondria (M) can be seen at the upper right and a small part of nucleus (N) and the double nuclear membrane at the bottom as well as Golgi apparatus (G). The endoplasmic reticulum in these cells is highly vesicular (× 40,000). A portion of the outer segment of a retinal rod from turtle eye (insert) illustrates the densely packed membranes characteristic of this light receptor (× 14,500).

molecules are among the fastest to permeate cells. Often the transport of molecules across the plasma membrane cannot be explained solely on the basis of concentration and charge gradients. Metabolic energy appears necessary for some transport processes and a cell will accumulate large concentrations of particular molecules while excluding others. Indeed the pronounced separation of charged molecules across the cell surface is the basis for normal functioning of excitable cells in nerve and muscle. Energy-requiring enzymes, carrier molecules, gates, and special membrane properties become involved in the explanations for the permeation by many substances even in cases where the steady state distribution appears to be adequately explained by the rules of simple diffusion.

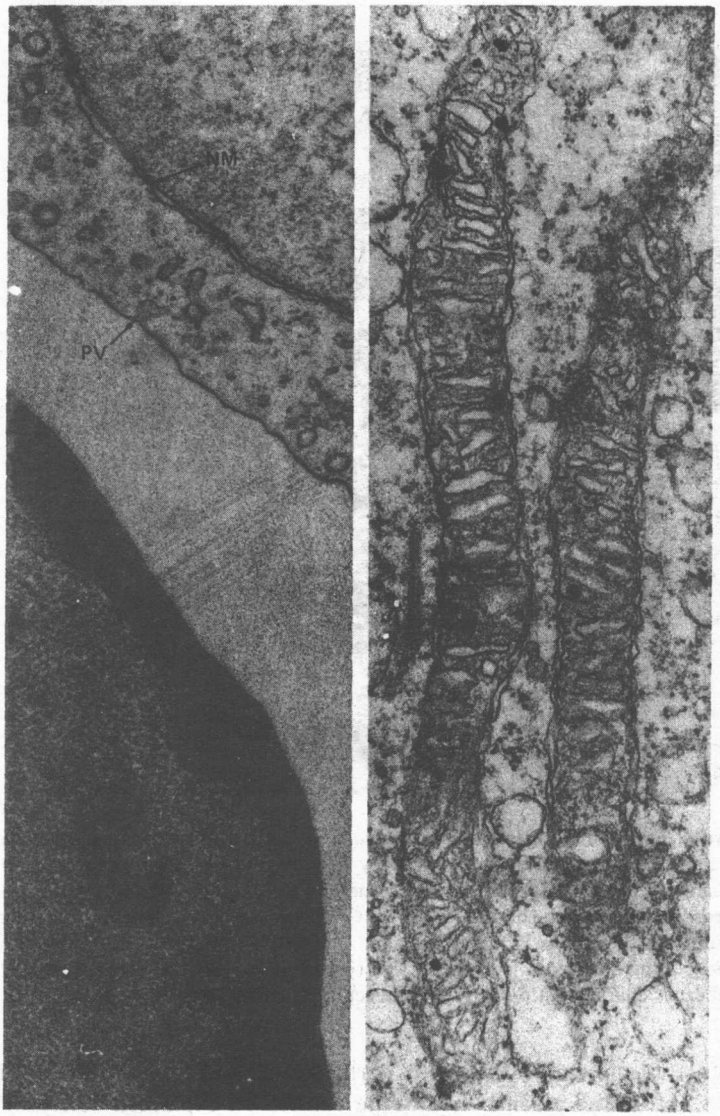

Figure 3. *Examples of membranous structures in cells:* erythrocytes and mitochondria in frog kidney. The left figure shows a nucleated frog red blood cell at the bottom and an endothelial cell with its nucleus at the top. The double nuclear membrane (NM) is clearly discerned in both cells, but the magnification of this electron micrograph (× 27,000) is not sufficient to show the trilaminar structure of the plasma membrane (PM). Note the pinocytotic vesicle (PV) opening into the lumen of the blood vessel. The right figure illustrates two large mitochondria filled with cristae and matrix (× 41,000).

One of the most distinctive properties of the plasma membrane is that of irritability, that is, the capacity to react to changes in the environment. This property is especially prominent in nerve and muscle cells. The excitability of these cells is determined in part by the ability of the membrane to segregate molecules, especially charged ones. The ionic composition inside cells is different from that outside. Sodium is the major cation in the cells' environment, usually tissue fluids; potassium is the major cation inside most cells. An electrical potential difference, commonly thought to result from this unequal distribution of ions, exists across the membrane of resting cells. Upon excitation by any appropriate means, the properties of the membrane change reversibly, often in a sudden and explosive manner, to produce changes in this potential difference. Cells or even parts of a single cell differ in their responses: the propagated impulse of nerve axons, the transducer action of receptor cells, the transmission function at synaptic and neuromuscular junctions. Some cells excite others, some inhibit, and others have more subtle influences. The origin and significance of the electrical phenomena generated by membranes have been vigorously studied but important questions remain.

In addition to the plasma membrane, there are a number of extraneous membranes and coats around cells. These coats are frequently but not always characterized by a high porosity; only large molecules do not pass freely. Some striking examples are the jelly layers, vitelline membranes, and fertilization membranes of some eggs; cellular cement or hyaline plasma layers between cells; the highly sculptured pellicles of protozoans; and plant cell walls. The important functional structure of cells that separates inside from outside, however, is the plasma membrane.

A discussion of the plasma membrane should include these questions: What is it? What does it do? How does it do it? The excitement in this field, which has led to extensive and rigorous study for nearly a century, has produced many excellent ideas, ingenious experiments, and useful models. The voluminous literature that has resulted from this activity, however, is often contradictory, confusing, and incomplete. The basic questions remain.

STRUCTURE OF THE PLASMA MEMBRANE

chapter two

In 1961 Eric Ponder [106] wrote that it is not far wrong to describe him as being "not convinced about the structure, or even the necessary existence, of the cell membrane as it is generally described" and, in asking himself "whether he believes that a cell membrane, lipid, sievelike, or mosaic in structure, perhaps with enzymes incorporated in it, is *solely* responsible for the entrance and egress of substances in the case of the typical cell, he would have to reply that he does not know, and that, on the basis of the existing evidence, he cannot know." He continues, "it is certainly true that many of the conclusions about the cell membrane and its permeability are based on preexisting ideas, on unallowable simplifications, as well as on a disregard of both physical chemistry and of the results of experiments on the cells themselves." As recently as 1966, Loewenstein [79] introduced a conference on biological membranes with the observation that no one has been able to adequately define a biological membrane or even give its space limits.

Most students of cellular surfaces are convinced, however, that such things as membranes exist, that plasma membranes from a number of cells have been isolated, and that their properties have, at least in part, been described. Warnings such as those above are meant to emphasize that (1) it is extremely difficult to distinguish clearly between cell wall, surface ultrastructure, and plasma membrane; (2) it is often possible to describe membrane phenomena as properties of an interface; and (3) it is not possible

at present to relate with each other the different experiments performed by different investigators on different material using different methods. It is easy to envisage the membrane as a discrete package of static molecules arranged in a highly organized and uniform structure. It is more probable that membranes are not uniform but are labile and dynamic structures somewhat disordered and undergoing continuous change in the living state. Variations in the composition of membranes may not alter function or even structure significantly; there may be many ways to build membranes that behave alike. On the other hand, subtle differences, presently unknown, can result in important changes in the functioning of different regions in a single cell, such as a neuron. It is unlikely that membranes are completely distinct from the cellular environment or the cytoplasm and, because of this, membranes cannot be expected to behave in a fully physiological fashion when isolated from their neighboring molecular environment or when this environment is altered in some manner. Yet much can be inferred about membrane structure and function by doing just that.

As long as it is recognized that interpretations of data must be made with caution, it is at least convenient to assume that plasma membranes of cells exist as real structures. This is clearly the view held by the majority of workers in this field.

2.1 Chemical Composition

There are three levels of molecular organization to be considered in describing the architecture of the plasma membrane: (1) thin layers containing a single class of compounds (e.g., lipid, protein, carbohydrate) parallel to the cell surface; (2) specific molecular types (e.g., cholesterol, phosphatidylethanolamine, lecithin, ATPase) that are organized or distributed in particular patterns within each layer; and (3) specific reactive groups (e.g., carboxyl, amino, receptor, antigenic) located at the cell surface and throughout the membrane layers. The earlier work on membrane structure, as well as much of the recent work (e.g., electron microscopy, X-ray diffraction), has dealt mainly with the first level of organization. Only in the past few years has work on isolated membrane preparations been providing useful information in quantity on the other two levels of molecular organization that probably determine permeability properties, specific enzymatic activities, immunochemical specificities, excitation, impulse propagation, and other important membrane phenomena [15, 70, 71, 116].

Chemical analysis of membranes has been accomplished for a number of organelles, cells, and organisms, and the major constituents are lipid, protein, and polysaccharide. One of the earliest and still popular sources of material for all kinds of membrane studies has been mammalian erythrocytes. They

are copiously available in pure cell type at the ends of the investigator's fingertips and can be treated in a variety of simple ways to lose their hemoglobin and to leave only membrane and not much more. Moreover, red cells have no internal membrane structures to contaminate these plasma membrane preparations. Neville [100] opened the way to other membrane preparations by devising a method to isolate plasma membranes from rat liver cells. Neville's techniques have been modified or extended to isolate plasma membrane fractions from cells of liver, intestinal brush border, kidney, muscle, amoebae, Ehrlich ascites carcinomas, mouse fibroblast tissue, HeLa cultures, *Mycoplasm*, and nerve, among many others.

Most of the methods employed to isolate membrane fragments generally treat the cells in some fashion to harden the surface membrane and to block sulfhydryl groups [168]. The membranes are then pulled away from the cytoplasm by, for example, swelling the cells in hypotonic media, homogenizing them, and separating the membrane fragments from other cellular components by physical means, e.g., separation in sucrose density gradients. How these treatments may alter details in the molecular organization of membranes is not clear. Obvious difficulties have not appeared, but few of the analyses can be accomplished on untreated membranes. It is unlikely, however, that these isolation procedures significantly alter the analysis of the individual molecular species that compose membranes.

Membranes are among the most stable of cellular structures. Maddy [88] found no detectable turnover of the mechanical component of membranes, which is consistent with the low turnover of both lipid and protein reported for various plasma membranes [107, 167].

Generally 50% or more of the dry weight of plasma membrane is protein [123]. The low protein content of nerve myelin makes it an unusual membrane preparation. This protein can serve a structural role to confer mechanical stability to membranes or it can possess catalytic functions. A protein molecule itself need not have a specific enzymatic function since it can interact with lipid to provide a matrix for the organization of catalytic factors.

The original suggestion that membranes contained protein was derived from the low surface tensions of biological membranes contrasted to the high surface tensions of pure lipid films on water. Since it is now known that phospholipids also possess low surface tensions in water, other evidence must be presented to demonstrate the value of protein to the mechanical stability of membranes. Proteolytic enzymes, which interfere minimally with the enzymatic and permeability properties of plasma membranes, deform the cellular surface [176] or reduce the force necessary to deform the cell [88]. Divalent cations may also serve to stabilize membranes, however [123].

Evidence has accumulated over the years to show that membranes are asymmetric or that the outer surface is or behaves differently from the inner

surface. The inner membrane of mitochondria possesses an isotropic organization of the electron transport chain [93]; the sodium pump in plasma membranes is outwardly directed in most instances [177]. Robertson's electron micrographs of myelin membranes show structural asymmetry [116] (see Fig. 11, p. 24) and the action of many pharmacological and immunological agents on cells can be demonstrated only by application to one or the other of the two membrane surfaces (e.g., see Chapter 4). Sialic acid is found bound to proteins on the surfaces of many cell types [23] and is located on the outer surface of the membrane as indicated by the action of neuraminidase on red cells [36] and electron micrographs of liver cells [7]. Sialic acid has also been proposed to conver mechanical strength to membranes [175] as it does to mucoproteins [58]. No evidence is currently available to distinguish a difference in the role of protein or lipid in generating and maintaining the anisotropic membrane conditions.

The protein components of human erythrocyte membranes outweigh the lipid components and can be separated into several different fractions. Rosenberg and Guidotti [123] separated eight fractions containing at least twelve different proteins that were present in significant amount. Molecular weights ranged from 10,000 to 150,000 daltons. The fractions differed in their proportions of nonpolar and acidic amino acids. Four of the eight fractions contained large amounts of sialic acid, but Rosenberg and Guidotti did not find a major protein component in red cell membrane that has the characteristics of the structural protein isolated from mitochondrial membranes [77]. Proteins extracted from plasma membranes of rat kidney cells fall into two major fractions [49]. One fraction contained at least five different proteins of molecular weights greater than 1 million daltons. The Na-K-dependent, ouabain-sensitive, ATPase activity that is unique to plasma membranes was found in this fraction. The other fractions contained at least eight different proteins of molecular weight near 45,000 daltons. Some of these smaller proteins were insoluble in butanol and may be structural components. Also, after incorporation into the membranes of living cells, radioactive leucine becomes unevenly distributed among the different protein fractions, suggestive of a heterogeneity of membrane protein constituents.

Roughly 20–30% of the dry weight of plasma membranes is lipid [161], about 50% of the total solids of mammalian brain is lipid, and isolated myelin fractions contain an even higher proportion of lipid [71]. Clearly, lipids are a major component of cells and are specifically concentrated in membranes. Since other membrane components, such as proteins, appear in a variety of cellular structures, lipid can be considered as the characteristic component of membranes [85, 124]. The presence of lipid in membranes was originally postulated to explain the permeability behavior of cells, and much of the behavior of membranes can be mimicked by purely lipid model systems (see Chapter 5). While lipids are an essential component of membranes,