Heidelberg
science library

THE PLASMA MEMBRANE:

DYNAMIC PERSPECTIVES, GENETICS AND PATHOLOGY

By
Donald F. Hoelzl Wallach

The Plasma Membrane: Dynamic Perspectives, Genetics and Pathology

Donald F. Hoelzl Wallach

The English Universities Press Ltd. London
Springer-Verlag New York • Heidelberg • Berlin
1972

Donald F. Hoelzl Wallach, M.D.
Director Radiobiology Division
Professor of Therapeutic Radiology
Tufts University School of Medicine
Boston, Massachusetts

Illustration on front cover: Portion of the plasma membrane of a lymphocyte as visualized by scanning electron microscopy. Courtesy of Dr. P. S. Lin, Division of Radiobiology, Tufts-NEMC, Boston, Massachusetts.

Library of Congress Catalog Card Number 72-85948.

Printed in the United States of America.

ISBN 0-340-17167-7 The English Universities Press Ltd. London.

ISBN 0-387-90047-0 Springer-Verlag New York · Heidelberg · Berlin
ISBN 3-540-90047-0 Springer-Verlag Berlin · Heidelberg · New York

PREFACE

For whom is a book written, why at a particular time and for what reasons? My major incentive is to present a summary of information and concepts about plasma membranes, that a student in Biology or Medicine, an immunologist, geneticist, oncologist or physician might wish to have readily available. It seems particularly opportune to do this at a time, when the many links between membrane biology and other areas of biomedicine are becoming more apparent and when the exploration of plasma membranes attracts not only increasing numbers of biologists, biochemists and biophysicists, but also those whose main interests lie in the clinical sciences.

In view of my personal experiences, the now widespread, multidisciplinary interest in membranes intrigues me. I have been involved in the study of plasma membranes since my medical school pathology course in 1950, when I became fascinated by the possible role of plasma membrane defects in malignancy. My instructors considered these notions somewhat brash and frowned on my enthusiasm to enter research in this area. Indeed, I pursued clinical work for some years, before turning my attention to membrane research at a rather basic level. However, my medical orientation has invariably led me to consider the clinical relevance of what I learned about membranes.

When I was asked to write a short volume about plasma membranes, I decided to make it a tutorial guide, aimed at bridging communication barriers and linking such vital aspects of membrane biology as genetics, immunology and pathophysiology, with key conceptual and experimental areas, such as dynamic membrane models and new membrane technologies. In doing so, I have prepared a large bibliography, which should help those in the field and others wishing to enter it, to overcome the barrier of a vast multidisciplinary literature and to provide new perspectives for a most exciting domain of biomedicine.

Boston, Massachusetts
September, 1972

Donald F. H. Wallach

ACKNOWLEDGEMENTS

It is a pleasure to acknowledge the support of the John Simon Guggenheim Memorial Foundation, which made this volume possible. I am also most grateful to Professor Herbert Fischer, who encouraged me greatly in this venture, and to Mr. Jose Soriano, whose sensitivity to world health problems led me to a much widened view of membrane pathophysiology.

TABLE OF CONTENTS

Chapter 1

INTRODUCTION

1. Historical Development

Membrane biology, more than any other area of biomedicine, is in a major state of ferment, as testified by the many international conferences and the large number of reviews dealing with various topics in the field (1–12). Some of the older "classics" on membrane structure have also been collected in a small volume edited by BRANTON and PARK (13). However, although the topic of membrane biology is a very broad one, its ramifications into pathology and clinical matters have been too little considered. Accordingly, this book will link general membrane biology to the membrane aspects of diseases. It will serve as an *interdisciplinary tutorial framework,* dealing primarily with the plasma membrane and emphasizing those areas which are beginning to link membrane biology to the rapid advances in molecular biology, biochemistry, and biophysics. In this book I envisage the plasma membrane as an *assembly of numerous diverse, but specifically and genetically* regulated functional systems arrayed at the cell periphery, each encompassing defined receptors and playing biological roles far more intricate than their well studied participation in permeability and transport.

Many of the membrane functions now recognized as crucial aspects of biomedicine, *i.e.*,

(1) cell recognition,
(2) discrimination of self from not-self,
(3) binding of drugs,
(4) binding of viruses, toxins and other cells,

as well as the transfer of extracellular information into a code comprehensible to intracellular machinery, presumably involve specific biochemical cell surface specializations such as predicted much earlier by EHRLICH (14) and WEISS (15).

The field of "membranology" being so broad, not all of it can be

treated in depth here; instead I shall provide extensive reference lists for those who wish to pursue a given topic further.

Awareness that membranes are vital cellular structures began with the *plasma membrane* concept, whose history has been reviewed by SMITH (16). It had its origins in the work of NAGELI (17), who showed by microscopic observations that the surface layer of many plant cells is a viscous, *semi-liquid boundary,* impermeable to dyes (*e.g.,* the pigments in "cherry juice") and that surface wounds are self-healing. He coined the term "Plasma Membran" for this surface layer. At about the same time PRINGSHEIM (18) concluded that the limiting membrane of the cell is responsible for its osmotic responses. The crucial role of the plasma membrane in the interactions of cells with their neighbors and their physiological environment was brilliantly envisaged very early by Paul Ehrlich, particularly the now all-important concepts of specific immunologic, pharmacologic and transport receptors on cell surfaces (14).

Myelin, a plasma membrane derivative, laid down by the satellite cells of nerve cell axons, historically followed the plasma membrane in attracting attention to the extent that much of its overall organization

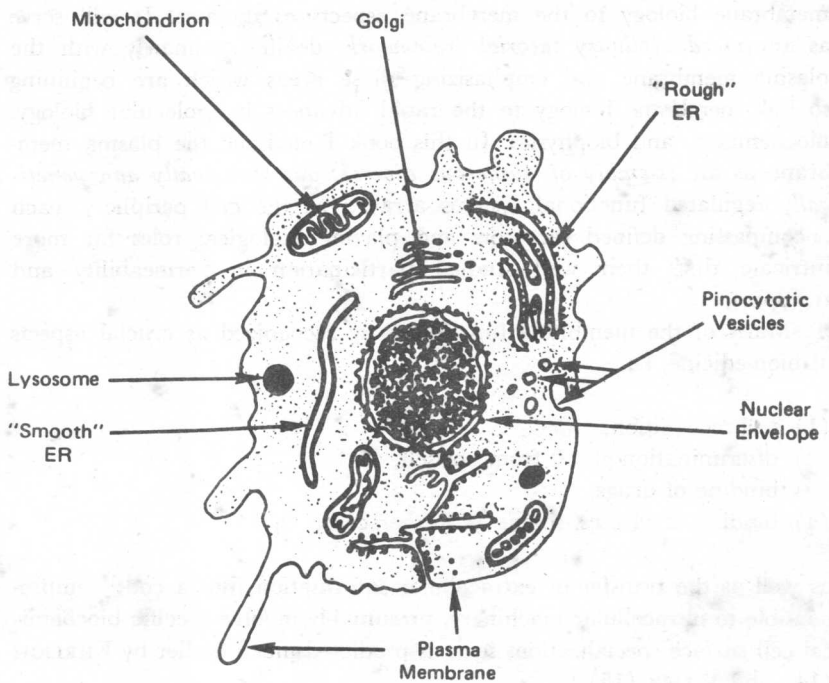

Fig. 1. The plasma membrane and major intracellular membrane systems.

had been worked out by optical and X-ray techniques (19–24) well before its origin and biogenesis was elucidated through the electron-microscopic studies of GEREN (25).

The recognition of *intracellular* membrane systems came in a later burst, spurred by refinements in electron-microscopic techniques. After SJÖSTRAND (26, 27) discovered that the *outer segments of retinal photoreceptor cells* comprise stacks of double membrane discs, it quickly became clear to micromorphologists that the animal cell is not a sack of enzymes, surrounded by a boundary membrane. Rather, its interior comprises a labyrinth of distinct, functional compartments, all separated by membranes (Fig. 1). The *endoplasmic reticulum* was defined by the micromorphologic studies of PORTER (28, 29), and the *Golgi* complex, a probable extension of the endoplasmic reticulum, was characterized almost simultaneously (30) as an array of flattened, membrane-bounded cisternae, with associated membrane vesicles. *Lysosomes* were recognized as distinct, membrane organelles by DE DUVE and associates (31), and the membraneous character of *mitochondria* was discovered independently by PALADE (32, 33) and SJÖSTRAND (27).

2. Some General Membrane Properties

Exploration of membrane structure has accelerated greatly with the development of *high-resolution electron microscopy,* and the field was indeed long dominated by this technique. Indubitably, electron microscopy has contributed substantially to the recognition and mapping of various membrane systems and to our present awareness of the topological diversities of biomembranes, but it has, as yet, failed to provide reliable detail about the *molecular* organization of biomembranes; also the technique remains primarily *qualitative.*

In *fixed, dehydrated, thin tissue sections, stained with heavy metals,* cellular membranes appear as two electron-opaque layers, separated by a lucent zone (Fig. 2). This "unit membrane" image has long been interpreted to represent the structure proposed by DANIELLI and DAVSON (34). That is, it was thought to be a bimolecular lipid layer with protein adsorbed at the membrane water interfaces (35); but it can assuredly represent other molecular arrays. The image reflects the binding of the electron-dense metals used for contrast enhancement and part of this may occur at the polar ends of the lipid molecules, as shown by STOECKENIUS for artificial phospholipid bilayers (37). However, KORN's critique (2) proves that electron microscopic staining is by no means precise or exclusive in localization. Thus, oxidizing heavy metal stains (*e.g.,* osmium tetroxide) bind covalently to certain unsaturated fatty acids and there-

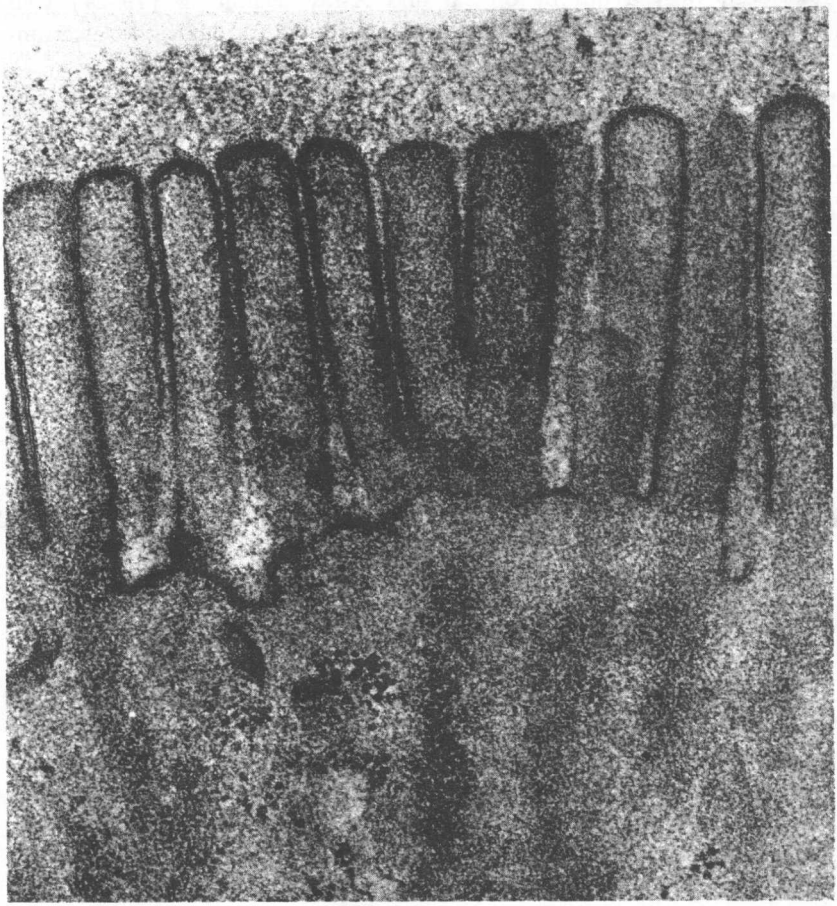

Fig. 2. The classical "unit membrane" seen by high resolution electron microscopy in thin sections of cat intestinal absorptive cells. The material was fixed in OsO_4 and stained with uranyl acetate and lead citrate. The two electron opaque bands, separated by a lucent zone, have often been equated with the "paucimolecular" membrane model described in Chapter 5. 120,000 ×. (Courtesy of Prof. S. Ito, Harvard Medical School, Boston).

fore localize, at least partially, in apolar lipid regions. Moreover, many studies indicate that much of the stain deposits on/in membrane proteins—which is not surprising, since membranes other than myelin contain more than 60% protein by weight. Finally, recent studies (37, 38) showing that most of the lipid can be extracted from certain biomembranes without affect on their electron microscopic appearance, demonstrate that staining is not restricted to lipids.

The electron-opacities producing the "unit-membrane" image vary with stains and staining conditions. Their 5 to 50 Å (the last in "double staining") resolution depends upon grain size, and if this were within 5 to 10 Å, stain-binding sites on/in protein molecules or small lipid micelles should be discernible. However, initial stain-deposition often creates nucleation centers which precipitate additional heavy metal, thus reducing resolution. Protein staining is most likely maximal at *polar* amino acids, and *non-polar* side chains are unlikely to furnish good contrast. But since X-ray analyses of many globular proteins show that polar amino acid residues lie at their *surfaces,* while the non-polar side chains locate both in their interior and at the surface, such proteins are expected to stain more intensely at their periphery than at their center. *Stained sections* of globular proteins should therefore exhibit an electron-opaque periphery with a lucent core, and globular lipid micelles should yield a similar image. Thus, while present high-resolution staining techniques might distinguish between predominantly polar and non-polar regions in protein molecules and lipid aggregates, they cannot discriminate between these possibilities. The "unit membrane" image cannot signify more than that membranes contain a core, occupied mainly by non-polar material, with the electron-dense layers at the membrane surfaces arising from a concentration of polar residues. The image could represent a purely protein membrane, with polar amino acid side chains concentrated at the surfaces, a phospholipid bilayer, or various combinations. Finally, the technique lacks the resolution to detect molecular "pores" in membranes.

The morphology of membranes can now be probed in a more *three-dimensional* fashion, through the rather new *freeze-cleaving methods,* combined with selective sublimation of water, or *freeze-etching.* This approach has been severally reviewed (39–41) and will be repeatedly illustrated in this volume. First, cells are frozen to —180°C, under conditions allowing their live recovery. They are then split mechanically; the fractured surfaces are shadowed with metal vapor (both at —180° *in vacuo*) and the resulting replicas are examined electronmicroscopically. Sometimes, some water is sublimed off (etched) before shadowing. Normally the "fracture planes" tend to be guided for long distances by predominantly apolar domains *within* the membranes, which in membranes other than myelin are populated with numerous, globular "membrane-associated particles" (Fig. 3); these are probably protein in nature (42). Etching, on the other hand exposes "true" external surfaces (Fig. 4).

All of the membrane organelles defined by electron microscopy can now be isolated in varying degrees of purity for purposes of biochemical, biophysical, and immunologic study, but the important field of *membrane*

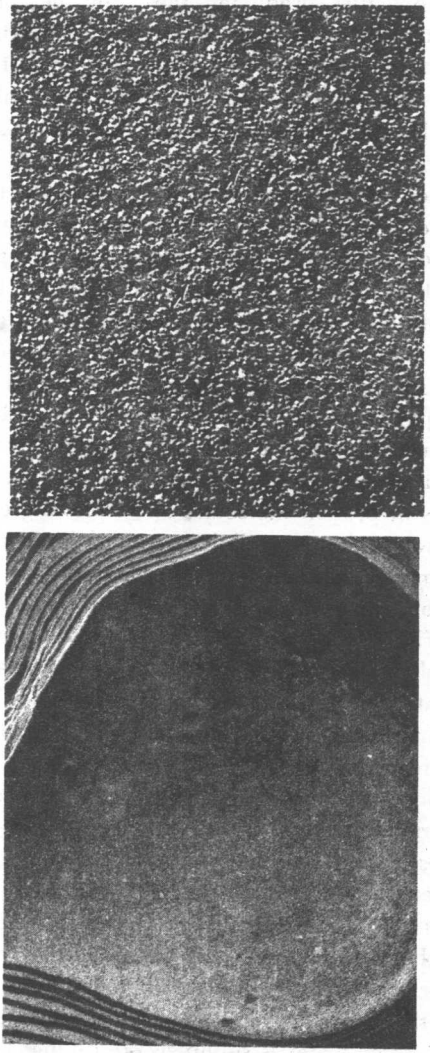

Fig. 3. Freeze-cleaved plasma membrane and myelin. Top: human erythrocyte membrane. The fracture face originates from the interior of the membrane and is covered with numerous 85 Å membrane-associated particles (arrows). 80,000 ×. Bottom: appearance of a tubular myelin figure prepared from mixed brain phospholipids. An eccentric cleavage has revealed fracture faces of concentrically wrapped lamellae. In contrast to other biomembranes, the surfaces are smooth and devoid of membrane-associated particles. 50,000 ×. (Courtesy of Drs. R. S. Weinstein and N. Scott McNutt, Massachusetts General Hospital, Boston.)

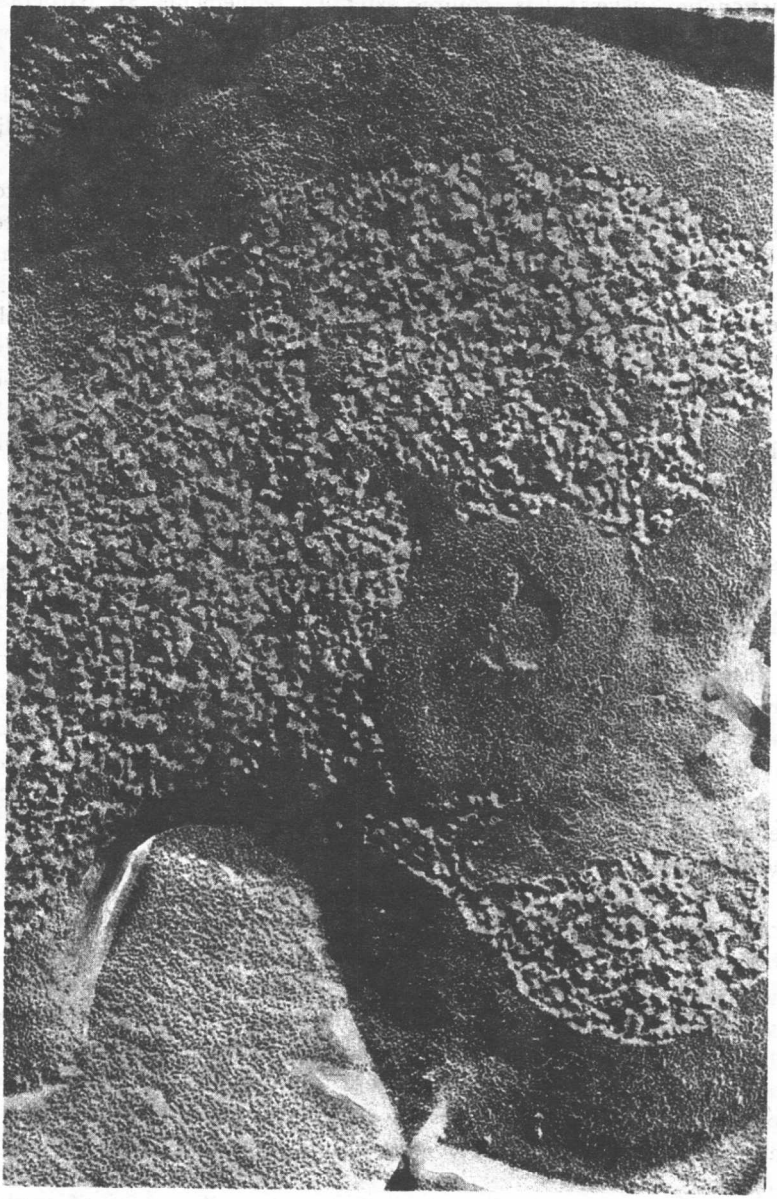

Fig. 4. Freeze-etch appearance of an erythrocyte plasma membrane. The cleavage plane reveals some of the membrane core with its membrane-associated particles. The appearance of the outer membrane surface has been brought out by freeze-etching, (*i.e.*, lyophilization of the volatile suspension medium). Note the lack of defined particles on this surface. 130,000 ×. (Courtesy of V. Speth, Max-Planck Institut für Immunbiologie, Freiburg.)

fractionation nevertheless requires extensive refinements. In particular, the purification of plasma membranes and the subfractionation of diverse membranes into their functional domains still face major obstacles (43). Moreover, analysis of membranes and their components at the molecular level requires their isolation, purification, and fractionation; but, as discussed below, such procedures may create artifacts.

Present views of *membrane structure* represent syntheses of cell physiological data and micromorphologic observations. They originated with the *permeation* experiments of OVERTON (44), who showed that small molecules penetrate plant cell walls, the quicker the more apolar. He also found that the rate of penetration of many polar molecules could be augmented by the addition of residues, increasing solubility in non-aqueous solvents. He concluded that the cell surface was oily in nature. From Overton's studies, and many others, one can make the following generalizations about the *passive* translocation of molecules through membranes:

(1) biomembranes exhibit certain *filter* effects, and small particles generally penetrate more readily than large ones;

(2) for substances with molecular weights greater than 80, *lipophilic* particles penetrate more readily than *hydrophilic* ones.

Electrical *resistances* and *capacitances* exhibited by cell surfaces of diverse animal and plant cells further indicate that *plasma membranes* function as insulators, or, more specifically, as low-dielectric barriers, to the passage of polar molecules. However, this gives no firm information about molecular organization; thin films of polytetrafluorethylene can behave similarly. In addition, there are few facts about intracellular membranes.

As far as plasma membranes are concerned, the *electrical resistance* across the neuronal surface, measured by microelectrodes, is about 400 ohms/cm^2 (45). The electrical resistance across the wall of the squid giant axon is closer to 1000 ohms/cm^2 at rest, but this figure reflects not only the properties of the plasma membrane of the axon but also that of the intricately folded satellite-cell membrane system; importantly, the resistance across this complex drops to 25 ohms/cm^2 during impulse conduction (46). Higher values have been reported for other cells; *e.g.*, the plasma membrane of *Drosophila* salivary glands, (10,000 ohms/cm^2; 47).

The *capacitance* of plasma membranes, including those of erythrocytes is generally about 1 μF/cm^2. Higher values (*e.g.*, 6 to 8 μF/cm^2) have been reported for sarcolemma, but this "membrane" includes several complex connective tissue layers (43). Mitochondrial membranes have a capacitance of 0.5 to 0.6 μF/cm^2.

Table 1.
Protein and Lipid Content of Membranes[a]

Membrane	Protein/Lipid (wt/wt)	Cholesterol/ Polar Lipid (mole/mole)	Major Polar Lipids[b]
Myelin[c]	0.25	0.7–1.2	Cer, PE, PC
Plasma membranes			
Liver cell	1.0–2.3	0.3–0.5	PC, PE, PS, Sph
Ehrlich ascites cells	2.2		
Intestinal villi[d]	4.6	0.5–1.2	
Erythrocyte ghosts[e]	1.5–4.0	0.9–1.0	Sph, PE, PC, PS
Endoplasmic reticulum	0.7–1.2	0.03–0.08	PC, PE, Sph, PC, PE, Plas
Mitochondria[f]			
Outer membrane	1.2	0.03–0.09	
Inner membrane	3.6	0.02–0.04	
Retinal rods	1.5	0.13	PC, PE, PS
Chloroplast lamellae	0.8	0	GalDG, SL, PS
Bacteria			
Gram-positive	2.0–4.0	0	DPG, PG, PE, PGaa
Gram-negative		0	PE, PG, DPG, PA
Halophilic	1.8	0	Ether analogue PGP
Acholeplasma	2.3	0	

[a] From (78), unless indicated otherwise.

[b] Abbreviations: Cer, cerebrosides; DPG, diphosphatidylglycerol; GalDG, galactosyldiglyceride; PA, phosphatidic acid; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PGaa, amino acyl esters of phosphatidylglycerol; Plas, plasmalogen; SL, sulfolipid; Sph, sphingomyelin.

[c] Peripheral nerve myelin has relatively more protein and sphingomyelin than that of the central nervous system.

[d] As much as 50% of the protein of intestinal villi may be non-membraneous.

[e] Comparison of erythrocytes from 10 mammalian species revealed a constant ratio of cholesterol to polar lipids but great variation in glycolipids and phosphatidylcholine (79, 80).

[f] Outer and inner membranes prepared by phospholipase A treatment are said to have identical lipid content and composition (81), but the above figures have been confirmed for digitonin-prepared inner membrane (82). Inner-membrane preparations contain considerable matrix protein.

The electrical properties of plasma membranes and their *permeabilities* to small ions and water-soluble molecules are conveniently described in terms of apolar barriers penetrated by "pores" allowing free diffusion of polar molecules. This "equivalent pore" concept describes some membrane properties, but there is no evidence that membranes, other than the nuclear envelope (48), bear fixed perforations of defined dimensions. However, the concept has heuristic merit and, in the case of an erythrocyte plasma membrane, 10^5 diffusion channels, accounting for perhaps

10^{-3} of the surface area, would describe the observed ion-permeability characteristics (49). With uniform distribution, this would mean one "pore" per 10^4 Å^2 (*i.e.*, the "pores" would be 100 Å apart).

This concept has also been applied to the surface membranes of nervous system cells; however, if the loss of electrical resistance across the axon wall during an action potential is not due to an extensive breakdown of membrane structure (49), its explanation in terms of "equivalent pores" would require the opening of new "pores"—now much closer—or a drastic change in existing "pores."

If their permeability and electrical properties show that membranes possess an *apolar core,* this information yields few clues as to membrane architecture at the molecular level. True, the non-polar membrane attributes have traditionally been ascribed to the hydrocarbon moieties of lipid bilayers and/or micelles—largely because membranes contain the necessary lipids and, until recently, only the named lipid systems seemed to have the approximate properties of biomembranes. This simple view is now no longer justified, since the X-ray analyses of the last years show that the interiors of many soluble globular proteins are also very apolar (50) and that the *only* characterized, biologic "pore" of molecular dimensions is the polar channel penetrating the hemoglobin tetramer (50), which contains no lipid at all.

All hypotheses must also deal with the genetically determined and specific differences in behavior toward closely related ions and their partition across membranes by *active transport,* as well as by related pharmacologic phenomena. Thus, drugs such as tetrodotoxin, inhibit the Na^+ influx normally following axon stimulation, but do not influence K^+ efflux (51); however, K^+ permeation can be blocked independently by other agents. There are 10^9 to 10^{10} tetrodotoxin-sensitive sites per cm^2, probably located on the outside of the axon membrane (52). Alkali cation specificity is also exhibited by a number of non-membrane associated proteins (52) and by numerous cyclic compounds of microbial and synthetic origin (53). It is probable that discrimination between alkali cations occurs only after these are stripped of their hydration shell.

Transport systems are not limited to ions but also mediate the active translocation of numerous biologically important *organic* substances, both large and small, via specific, genetically determined processes, involving specific enzymes and "carrier mechanisms." The large field of membrane transport has been often and extensively reviewed (54–55), but certain, selected aspects will be discussed in this monograph.

Low-angle *X-ray diffraction* analyses have significantly elucidated the organization of lamellar membrane arrays such as myelin and retinal-rod outer segments (cf. reviews by Schmitt; 56–59). These

methods are now being extended theoretically and practically to lipid micelles and membrane fragments in aqueous dispersion (60–62), but such efforts face formidable difficulties. The presence of lipid bilayers in suspension can readily be identified, but interposed globular proteins can be distinguished only by parallel stacking of the membranes (63). Applying this approach to the membranes of *Acholeplasma laidlawii,* ENGELMAN (62) has shown the existence of phosphatide bilayers in these structures and has demonstrated alterations in low-angle X-ray scattering with temperature, concordant with those observed by differential calorimetry (64) on organisms of corresponding lipid composition and thought to mirror a lipid phase transition. However, there are many uncertainties in interpreting the techniques employed (65).

Another major obstacle to current X-ray work derives from unknown behavior of membrane proteins, which account for more than 60% of the mass of most membranes, including those of *Acholeplasma.* Thus, hydrophobic regions of protein are likely to have an electron density as low as that of the hydrocarbon moieties of phosphatides, and it is possible that certain peptide chains with suitable sequences might be tightly packed into structures difficult to discern from lipid bilayers. Indeed, certain synthetic, non-lipid polymers do form such arrays (66). Attempts to increase resolution by centrifugally packing *Acholeplasmal* (62) or erythrocyte (63) membranes into parallely stacked arrays have not been successful. Indeed, ENGELMAN (62) cannot really describe the disposition of the *Acholeplasmal* membrane protein, except to say that it does not distribute symmetrically between the two surfaces of a phosphatide bilayer; that it might penetrate the bilayer, as suggested by others (67–69); and that, if it does, the lipids would not be regularly packed in such perforated regions. It would seem that at present, modern X-ray diffraction studies on membranes only add to other information which suggests that membranes are mosaic structures containing regions of phospholipid bilayer. This matter will be discussed further in the section on membrane models.

The increasing sophistication of micromorphologic methods has created an awareness that the morphologic differences between membranes are really greater than the similarities. The common observations of globular substructure in the electron microscopic image of many membranes (Fig. 3), the increasing spectroscopic evidence that membrane proteins are globular (67–71), the awareness that the interactions in membranes of phospholipids and proteins are largely entropically driven, and the probable importance of protein-protein (as well as lipid-protein) interactions, are all contrary to a monolithic interpretation of membrane structure—particularly if this is only in terms of their lipid constituents. Indeed, the recent studies of membranes *in situ* by optical activity studies,