ADVANCES IN IMMUNOLOGY

Volume 19

F. J. Dixon &
Henry G. Kunkel

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PREFACE

There appears little remaining doubt that we are currently in the midst of what might be termed the "new immunology," the study of the lymphocyte. It is likely that in addition to its interest for immunologists, this ubiquitous cell may well become the prototype for the investigation of all eukaryotic cells. The remarkable stimulatory effect of antigens as well as various lectins through surface interactions makes the lymphocyte uniquely suited for a wide variety of studies. A spectrum of membrane markers have been delineated recently and these too have proven of considerable utility. Three of the four articles of Volume 19 deal specifically with branches of this "new immunology."

The first contribution deals with the broader aspects of membranes and covers the work on other cell types in addition to the lymphocyte. The author, Dr. S. J. Singer, is certainly one of the leaders in this field and he is primarily responsible for the fluid mosaic model of membrane structure. The surface markers of human red blood cells are discussed in considerable detail, since these cells are the primary ones utilized in Dr. Singer's studies. The redistribution of components of cell membranes by a variety of externally added agents is emphasized throughout the section and the importance of this phenomenon in biology is clearly apparent. Special stress is placed on various membrane phenomena of interest to immunologists, such as antigenic modulation, capping, and lectin effects on lymphocytes.

In the second article, Dr. Noel L. Warner deals primarily with the problem of membrane receptors for antigen on B and T lymphocytes. This exhaustive review by one of the leading authorities in the field supplements very well the broader consideration of membranes in the first article. The controversial topic of the character of T cell receptors is covered in special detail and the evidence for the concept of the immunoglobulin nature of these receptors, to which the author adheres, is emphasized. Many other questions concerning lymphocytes and other immunologically important cells are considered in great detail, making this contribution a valuable reference for the cellular immunologist. In addition, its very readable character and illustrations make it a useful review for those less familiar with this branch of immunology.

The third article is by Dr. Victor Nussenzweig and deals with the field he initiated, the complement receptor sites on lymphocytes and other immunologically important cells. It is clear that the simple technique of rosette formation utilizing red cells coated with complement offers a very useful procedure for enumerating B cells. Considerable evidence is presented indicating that the complement receptors have important biological significance, particularly in facilitating the interaction of immune complexes with B cells and possibly in T and B cell interactions.

The last article is written by Dr. Hans L. Spiegelberg and concerns the many specific biological activities of the different immunoglobulin classes and subclasses. In view of the great interest in the variable portion of the antibody molecule and its relation to antigen binding, the constant part of the molecule involved in these biological activities has not received the attention that it probably deserves. Dr. Spiegelberg, who has contributed very significantly in this area, has brought together the many and diversified properties that are dependent on the constant areas in a very useful review.

The fine cooperation of the publishers in the production of Volume 19 is gratefully acknowledged.

HENRY G. KUNKEL
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Molecular Biology of Cellular Membranes with Applications to Immunology

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I. Introduction

Events occurring at the level of the plasma and intracellular membranes of lymphoid cells have increasingly come to be recognized as critical to the expression of many immune phenomena. The induction of antibody synthesis by antigen, cell–mediated immunity, histocompatibility and blood group antigenicity, antibody secretion, and complement-induced cytolysis, are only a few of the membrane-associated phenomena of great interest to immunologists. In the last few years, intense research activity has centered on the effects of anti-immunoglobulin antibodies, mitogens, and antigens on the membranes of T and B lymphocytes. While immunologists have been working on these problems, rapid developments have simultaneously taken place in membrane molecular biology. Theoretical and experimental advances have generated new insight into the molecular organization of membranes. This, in turn, has led to novel ideas and speculations about how membranes carry out their manifold functions. The primary object of this review is to discuss the molecular structure of membranes and its bearing on membrane functions as these concepts are presently emerging. In the latter half of this article, the relevance of these concepts to some selected immune phenomena will be discussed.

II. Molecular Organization of Membranes

In two recent articles (Singer, 1971; Singer and Nicolson, 1972), we have presented a detailed analysis of the thermodynamics of membrane systems and of new experimental information which has led us to propose the *fluid mosaic model of membrane structure.* In this review, only a summary of this material will be given; for further details, the reader is referred to the original articles.

A. Thermodynamic Considerations

In many discussions in the past, *ad hoc* assumptions and questionable conclusions derived from electron-microscopic observations have led to arbitrary models of membrane structure. Our own starting point has been thermodynamic. On the assumption that a membrane and its components obey the laws of equilibrium thermodynamics, at least in local domains, we have tried to develop in a systematic fashion a set of thermodynamic criteria, or restrictions, that membrane components must satisfy. For the present purposes, a large body of information about macromolecular interactions in aqueous solutions can be summarized as follows. Three major kinds of interactions must play prominent roles in determining membrane structure: hydrophilic, hydrophobic, and hydrogen-bonding interactions.

1. *Hydrophilic Interactions*

By hydrophilic interactions we mean a set of interactions that is responsible for the preference of ionic and highly polar groups for an aqueous rather than a hydrophobic environment (Singer, 1971). It generally costs an unacceptably large amount of free energy to remove an ionic or highly polar group from water into a nonpolar solvent. For example, about 6 kcal./mole is necessary to transfer zwitterionic glycine from water to acetone (which is still a fairly polar solvent). In terms of membrane structure, this means that in the intact membrane the ionic and polar heads of the phospholipids, the ionic amino acid residues of the membrane proteins, and the sugar residues of the glycolipids and glycoproteins, essentially all have to be in atomic contact with water to yield a thermodynamically stable structure.

2. *Hydrophobic Interactions*

The hydrophobic interactions are responsible for the immiscibility of water and nonpolar substances. As a consequence, it costs free energy to remove a nonpolar residue from a nonpolar environment and transfer it to an aqueous one (Kauzmann, 1959). To transfer a single valine side chain from a solvent as polar as ethanol is to water, for example, takes about 2.1 kcal./mole (Cohn and Edsall, 1943). In terms of membrane structure, this means that in the intact membrane, the fatty acid chains of the lipids and the nonpolar amino acid residues of the membrane proteins have to be sequestered to the maximum extent possible into a hydrophobic environment away from contact with water.

3. *Hydrogen Bonding*

For membrane structure, the important point about hydrogen bonding is that in the intact membrane, hydrogen-bond donor and acceptor groups that are *not* in contact with water (for example, any N—H or C═O groups of the protein polypeptide chains that are buried in the nonpolar membrane interior) should be hydrogen bonded to the maximum extent possible to other acceptor and donor groups, respectively (Singer, 1971). To the extent that such internalized hydrogen bonds do *not* form, the membrane is destabilized by about 4 kcal./mole of potential hydrogen-bonding groups (Klotz and Franzen, 1962).

Other factors, such as electrostatic interactions, should also be considered in any detailed theory of membrane structure, but for the level of approximation of the present analysis, they may be neglected.

These few thermodynamic generalizations might seem, at first glance, to be unlikely contributors of any detailed structural insight about membranes. To the contrary, however, they are quite powerful: they place restrictions on membrane models and allow predictions to be made about protein and lipid structures in membranes, as will be demonstrated after some of the properties of membrane proteins are discussed.

B. Proteins of Membranes

Until relatively recently, most discussions of membrane structure have emphasized the role of membrane lipids. The fact is, however, that of the three major constituents of membranes—protein, lipid, and carbohydrate—proteins have been shown to be the predominant constituent by weight in most well-characterized preparations of functional membranes (Table I). [Among those membrane systems that have been analyzed, myelin is the only exception to this generalization and contains about 4 times as much lipid as protein. But myelin is not a typical membrane; it functions as an electrical insulator rather than as a biochemically active, selective, permeability barrier.] This fact suggests that knowledge of the composition, conformations, and organization of proteins in membranes is of the greatest importance to understanding membrane structure.

TABLE I
CHEMICAL COMPOSITION OF CELL MEMBRANES[a]

Membrane	Protein (%)	Lipid (%)	Carbohydrate (%)	Ratio of protein to lipid
Myelin	18	79	3	0.23
Plasma membranes				
Blood platelets	33–42	58–51	7.5	0.7
Mouse liver cells	46	54	2–4	0.85
Human erythrocyte	49	43	8	1.1
Ameba	54	42	4	1.3
Rat liver cells	58	42	(5–10)[b]	1.4
L cells	60	40	(5–10)[b]	1.5
HeLa cells	60	40	2.4	1.5
Nuclear membrane of rat liver cells	59	35	2.9	1.6
Retinal rods, bovine	51	49	4	1.0
Mitochondrial outer membrane	52	48	(2–4)[b]	1.1
Sarcoplasmic reticulum	67	33	–	2.0
Chloroplast lamellae, spinach	70	30	(6)[b]	2.3
Mitochondrial inner membrane	76	24	(1–2)[b]	3.2
Gram-positive bacteria	75	25	(10)[b]	3.0
Halobacterium purple membrane	75	25	–	3.0
Mycoplasma	58	37	1.5	1.6

[a] From Guidotti (1972).
[b] Deduced from the analyses.

TABLE II
CRITERIA FOR DISTINGUISHING PERIPHERAL AND INTEGRAL MEMBRANE PROTEINS

Property	Peripheral protein	Integral protein
Requirements for dissociation from membrane	Mild treatments sufficient: high ionic strength, metal ion chelating agents	Hydrophobic bond-breaking agents required: detergents, organic solvents, chaotropic agents
Association with lipids when solubilized	Usually soluble free of lipids	Usually associated with lipids when solubilized
Solubility after dissociation from membrane	Soluble and molecularly dispersed in neutral aqueous buffers	Usually insoluble or aggregated in neutral aqueous buffers
Examples	Cytochrome c of mitochondria; spectrin of erythrocytes	Most membrane-bound enzymes; histocompatibility antigens; drug and hormone receptors

As a first step in an analysis of membrane proteins, we have proposed (Singer, 1971) that at least two major categories of proteins be discriminated—they are termed *peripheral* and *integral.* The criteria suggested for distinguishing them are given in Table II. The main point is that certain membrane-associated proteins (peripheral) appear to be only weakly bound to the membrane, so that very mild treatments release them intact into molecular solution in aqueous buffers; whereas, the majority of membrane proteins (integral) are much more strongly bound and require hydrophobic bond-breaking agents to release them. The division into only two classes of proteins may ultimately prove to be inadequate, and the distinction may be more graduated, but in the absence of much evidence on this point, our purpose is served adequately by considering just the two classes. This classification also helps one to recognize that the structural properties of the more readily isolated and characterized peripheral proteins may not apply to the majority of membrane proteins. For example, the complete three-dimensional structure of cytochrome c of mitochondria has been determined by X-ray diffraction; but because it is released in soluble form from mitochondrial membranes by simply increasing the ionic strength (to 3 *M* KCl), it is a peripheral protein. Its structural features may, therefore, be only remotely related to, and may even be radically different from, those of most membrane-bound integral proteins.