Advances in Lipid Research

Volume 15

Edited by

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PREFACE

This volume of *Advances in Lipid Research* is devoted to rather extensive discussions of important subjects which are not often reviewed. The first chapter is devoted to the subject of long-range order in biomembranes. The authors propose and defend the thesis that the proteins and lipids of biological membranes are partitioned into functional and structural aggregates in the plane of the membrane. The second chapter reviews the pharmacology and toxicology of steroids and related compounds. This exhaustive review covers methodology, physiologic mechanisms, transport mechanisms, and effects on the central nervous system, among others. An interesting class of lipids, fungal lipids, is the subject of the third chapter. This review covers all aspects of lipid composition of all classes of fungi. It goes on to discuss the intracellular distribution and biosynthesis of the lipid components of fungi. The final contribution is a comprehensive treatise on the biochemistry of plant sterols, including structure, stereochemistry, biosynthesis, metabolism, and function. The evolutionary role of plant sterols is the subject of thoughtful speculation.

RODOLFO PAOLETTI
DAVID KRITCHEVSKY

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Long-Range Order in Biomembranes

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A violent order is disorder; and a great disorder
is an order. These two things are one.[1]

Wallace Stevens

I. Introduction

Recently, there has been an upsurge of interest in biological membranes. Cell membranes are being studied with respect to their anatomy or gross structures, their physiology or function, and the chemistry of their components. The central problem of membrane structure and its correlation with physiological and biochemical functions is to define the organization of constituent molecules. Thus the organization of membrane constituents in a semiinfinite continuous sheet can be described in terms of the covalent

[1] In "Connoisseur of Chaos" from *The Collected Poems of Wallace Stevens.* Alfred A. Knopf, New York (1957), p. 215–216.

structure of the constituents, the organizational characteristics of lipid/water system as bilayer, and the molecular conformations that determine subtle phase characteristics of lipid bilayer. The possibility remains that the whole can be qualitatively different from the sum of its parts, and yet be dependent upon the organization of its parts for its unique properties. Indeed, we cannot but share the perspective articulated by Teilhard de Chardin (1959): "The farther and more deeply we penetrate into matter, by means of increasingly powerful methods, the more we are confounded by the interdependence of its parts.... It is impossible to cut into this network, to isolate a portion without it becoming frayed and unravelled at all its edges."

In the last 50 years, biomembranes have been extensively studied. The existence of bilayers in biomembranes is firmly established by a variety of physicochemical techniques. It has been shown that the subtleties of organizational and phase characteristics of the bilayer arise from the segmental motion and the transverse, rotational, and lateral mobilities of constituent lipids. These molecular features of lipids in the bilayer organization satisfactorily account for dielectric (capacitance, reflectance), viscoelastic (surface tension, resealability), partitioning, and passive permeability characteristics (Jain, 1972). It has been realized for quite some time now that a simple lipid bilayer structure cannot adequately account for the properties of biomembranes. Experimental evidence indicates that the membrane lipids not only create a barrier to the free entry and exit of molecules into and out of the cell, but lipids also provide a matrix *in/on* which biochemical reactions can take place (Fourcans and Jain, 1974); *through* which certain metabolites can pass selectively; and *with* which recognition, adhesion, aggregation and fusion of cells can be mediated. A molecular explanation of these varied processes would require a detailed description of the interactions among the membrane components.

Most, if not all, of the forces between membrane components are noncovalent, similar to those that hold the molecules of most organic solids and liquids together. The two-dimensional matrix, a lamellar lipid structure, seems to arise from the amphipathic nature of the phospholipid molecule. Since a typical biomembrane has a large variety of lipids and proteins, one may expect a certain degree of suborder or fine structure within its organization. Such aspects of membrane organization and their functional significance are the underlying theme of this review. From a review of the literature pertaining to biomembrane properties, it emerges that the current models of biomembrane structure need elaboration and further modification at least in one important respect. A wide array of experimental data appears to be consistent with a postulate that *the biomembrane continuum is broken up into a number of "plates" that are in relative motion with respect to each other. The ordered and rigid regions may be separated from each other by relatively fluid and disorganized regions. These regions are contiguous and in equilibrium.*

II. The Models of Biomembrane Organization

The plasma membrane has proved to be a durable, dynamic, and functionally complex structure that performs a wide range of physiologic tasks besides delineation of cellular boundaries. A rational explanation of most, if not all, of the above processes requires involvement of specific and selective sites that could result only from proteins present in biomembranes. The organization of proteins in a lipid bilayer lends itself to several possibilities and, therefore, to considerable speculation in the form of membrane models. So far about 50 such models have been proposed to describe membrane organization. Models of necessity oversimplify, and they also tend to overemphasize certain features in order to stress their importance. It may be noted that the main role of models is not so much to explain and to predict—though ultimately these are the functions of science—as to polarize thinking, to establish dialectics, to pose sharp questions, and above all, lead to some radical, undreamed of unifying concept. It must be emphasized at the outset that most membrane models are not mutually exclusive. Indeed, they seem to emphasize different aspects of membrane organization. A short review of the various models that have been proposed to account for the functional and organizational features of biomembranes will be useful in elaborating the major theme of this review. Although a thorough historical review of membrane models is outside the scope of this chapter, a brief summary of some of the major features of membrane organization emphasized in these models is given in this section. The conceptual evolution of the various models can be reduced to three major categories as discussed in sequence: the bilayer hypothesis, the "iceberg" models, and the fluid-mosaic model.

A. The Bilayer Hypothesis

Classical studies of Overton at the turn of the last century showed rather convincingly that the permeability barrier of biomembranes is lipidlike. In the years to follow, the lipids from a variety of sources were purified and their structures were determined. Langmuir in the second and third decades of this century showed conclusively that most lipidic substances orient themselves at an air/water interface such that the polar groups are directed toward water and the hydrocarbon chains toward air, the less polar of the two phases. It has been taken as axiomatic ever since that similar forces govern the organization of lipid molecules in water: the molecules that cannot be compressed at the air/water interface are forced to form their own nonpolar phase. Such a tendency to form an oil/water interface by lipid molecules has been the basic hypothesis in membrane models in general, and in the bilayer-based models (Fig. 1) in particular.

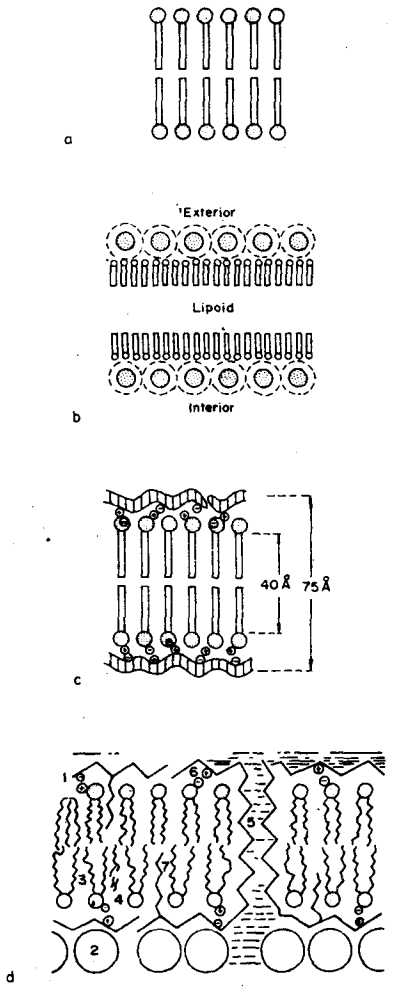


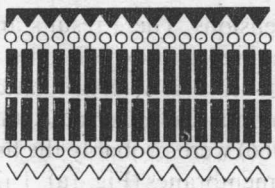
FIG. 1. The "paucimolecular" models of cell membrane. These models essentially emphasize the molecular dimensions of biomembranes. Diagrams are adapted from Mazliak (1971) based on the work of the authors cited: (a) Gorter and Grendel (1925); (b) Danielli and Davson (1935); (c) Davson and Danielli (1943); (d) Stein and Danielli (1956).

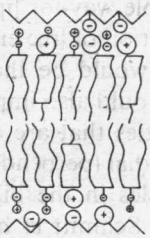
The hypothesis that a living cell had a well-conducting interior, surrounded by a relatively impermeable, poorly ion-conducting region, had by 1921 been well supported by electrical measurements on red blood cells and several tissues. Fricke (1923, 1925) determined electrical capacity of red cell membranes as 0.81 $\mu F/cm^2$. Assuming that the membrane might be an oil with a dielectric constant of 3, the above value of capacity corresponds to a membrane thickness of 33 Å, thus indicating that the biomembrane

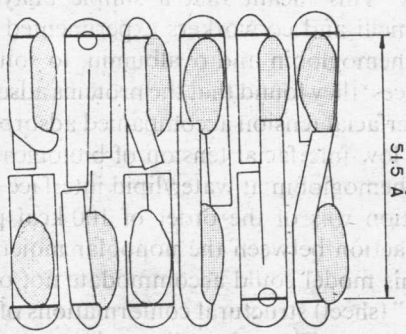
may have molecular dimensions. These results and assumptions have been proved to be correct by subsequent experiments.

Based on their studies on pressure–area relationships for monolayers of lipid isolated from red blood cell membrane, Gorter and Grendel (1925; however, see Bar *et al.*, 1966) were probably the first to suggest the existence of bilayer structure in biomembranes (Fig. 1a). This theme was reinforced by Danielli and Davson (1935) as primarily associated with the physicochemical behavior of amphipaths in water. The bilayer is simply the most efficient and, therefore, the most probable way for lipid molecules to arrange themselves consistent with minimization of free energy. It has been argued that a bilayer arrangement of lipids would be thermodynamically stable under physiological conditions, and would also impart to the membranes the electrical and permeability properties that are observed in living cells. Direct experimental support for the radial (perpendicular to the plane of the membrane) arrangement of molecules in the bilayer came from birefringence measurements (Schmidt, 1936; Schmitt *et al.*, 1936). Inability of a simple bilayer structure to account for a variety of biomembrane characteristics, such as ionic permeability and low surface tension, was realized and appreciated fairly early. This meant that a simple bilayer hypothesis needed modification. Danielli and co-workers experimented with adding globular proteins, such as hemoglobin and ovalbumin, to solutions in contact with lipid/water interfaces; they found that the proteins adsorbed on the interfaces. A reduction in interfacial tension accompanied adsorption. This observation could explain the low interfacial tension of biological membranes. Further experiments with hemoglobin at water/lipid interface indicated that the free energy of adsorption was of the order of 100 kcal per mole, presumably arising from interaction between the nonpolar moieties of the protein and lipid molecule. This model could accommodate not only globular proteins, but also "unrolled" (sheet) structural conformations of proteins in close contact with the lipid layers. Thus, the cell membrane was visualized as "a sort of sandwich—two slices of lipid between two slices of flattened out protein—and probably with a garnish of globular protein over all." Some protein-lined pores were also postulated (see below). All this is consistent with the observation that membranes contain, besides lipids, varying proportions of proteins.

Significant support for the bilayer hypothesis was obtained by electron microscopic studies. These studies confirmed what was suspected from a variety of physiological studies, that the biomembrane is asymmetric and the bilayer structure is a universal mode of organization (unit membrane hypothesis) at least in plasma membranes (Robertson, 1960, 1964, 1967). This theme was further modified by various physicochemical studies that gave indications of the presence of additional features of bilayer organization (Fig. 2). On the basis of electron microscopic studies, Staehelin (1968)







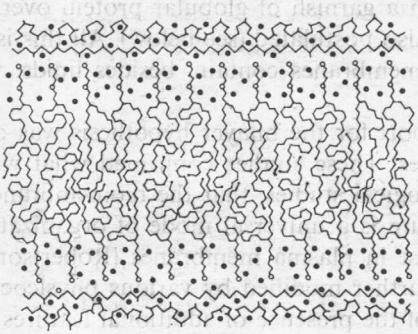
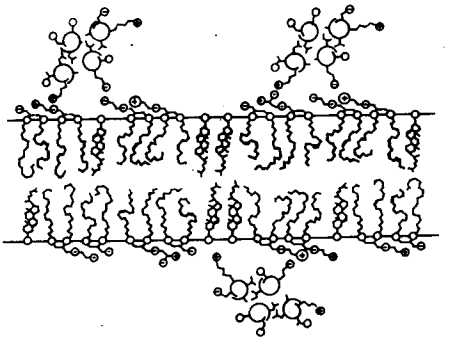


FIG. 2 (a–d).



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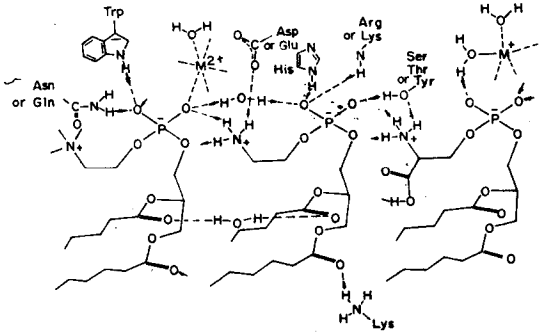

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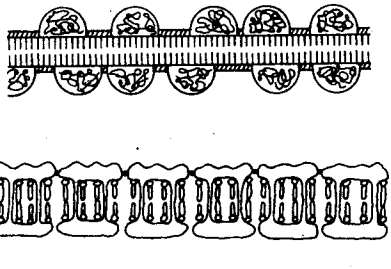

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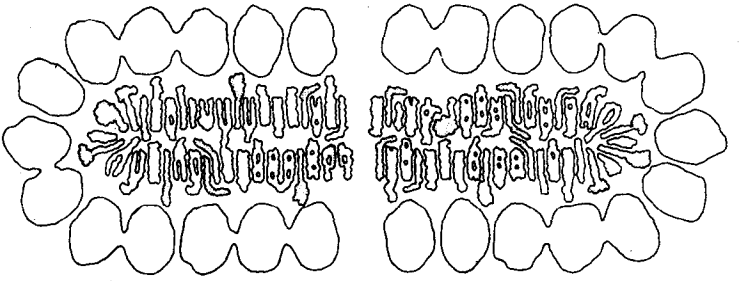
FIG. 2 (e–h).



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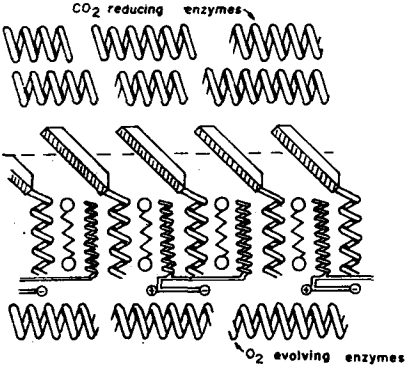

k

FIG. 2. The lipid bilayer-based models that emphasize substructure within biomembranes. The aspects that have been emphasized include asymmetry (Robertson), specific intermolecular interaction (Finean, Vandenheuvel), conformation of polymethylene chains (Stoeckenius, Sundaralingam), presence of specific components (Calvin, Menke), segregation of lipids (Crawford and Sinclair). Diagrams are adapted from Mazliak (1971) based on the work of the authors cited: (a) Robertson (1964); (b) B. Boois and H. G. Burgenberg de Jong (1952, cited in Mazliak, 1971, p. 68); (c) Vandenheuvel (1965); (d) Stoeckenius (1963); (e) Haggis (1964); (f) Sundaralingam (1972); (g) Staehelin (1968); (h) Gross (1967); (i) Menke (1966); (j) Crawford and Sinclair (1972); (k) Calvin (1959).

suggested the presence of globular structures on the surface of the lipid bilayer. B. Boois and H. G. Bungenberg de Jong (1952, cited in Mazliak, 1971, p. 68) emphasized the presence of cholesterol in membranes in stoichiometric proportions. Calvin (1959) proposed the bilayer structure constituted of several overlapping and interdigitated components characteristic to the chloroplast membranes. In accordance with compositional and X-ray diffraction studies, Finean (1953) suggested the presence of several lipid species in the bilayer and a sheet of protein on the interface. The model proposed by Menke (1966) emphasized the presence of globular proteins on the bilayer constituted of diverse lipid components. On the basis of his studies with molecular models, Vandenheuvel (1965) inferred specific interactions between cholesterol and certain phospholipids (see also Finean, 1953; Engström and Finean, 1958; Hechter, 1966), and the model for bilayer organization proposed by him emphasized such aspects of lipid composition and molecular association. Similar studies have been carried out by O'Brien (1967). The cis-trans and gauche-trans conformations in the polymethylene chains of phospholipids were probably emphasized by Stoeckenius (1963) first, and also by Haggis (1964). Sundaralingam (1972), on the basis of his X-ray crystal structure studies of phosphoglycerol derivatives, emphasized the relative orientation and conformations of polar groups that could be involved in the metal binding and hydrogen binding with proteins at the interface of lipid bilayer. Crawford and Sinclair (1972) have emphasized the difference in the length of acyl chains in various species of phospholipids in the membrane. According to their model, differences in the lateral organization of the chains could allow either a "buffering" or an "interlocking" arrangement, which could lead to some special biological advantages by juxtaposition or by relative movement of the opposing bilayers, or by buffering against mechanical shock. Such arguments have also been made by Fergason and Brown (1967).

Some of the basic functions of a biomembrane are associated with its barrier properties, that is, for most solutes the diffusion rate is far less (by a factor of 10^6 to 10^9) than the "free" diffusion rate in the absence of a membrane. This is consistent with the bilayer hypothesis where the biomembrane behaves as a thick wall (about 50 Å) through which various molecules move at a rate predicted from their lipid solubility and size. However, there are some significant exceptions. Abnormally rapid and sometimes uphill (against the gradient) movement of certain solutes was observed across certain cell membranes. These phenomena could be lumped together in the following categories.

1. Generally, fast movement (permeability) of small molecules like urea could be accounted for by assuming the presence of polar pores of about 4 Å radius.

2. Fast movement of certain large polar molecules like sugars and amino acids could be accommodated by assuming the presence of selective carriers in the lipid bilayer.

3. The uphill (active) transport needed to be invoked to account for the existence and maintenance of ionic and metabolite gradients across the cell membrane.

4. Endocytosis (pinocytosis, phagocytosis) and exocytosis, which accounts for the movement of large proteins and bulk material into and out of the cell.

These departures from the behavior of simple lipid bilayer required the presence of "pores" or "active regions" or patches of discontinuity in the sandwich bilayer. Local variations in the arrangement of lipids might exert a significant effect on transport across the lipid bilayer or might even be involved in the formation of these regions of discontinuity. It could be calculated on the basis of several observations that not more than 2% of the membrane surface could be involved in all the above functions. A direct examination of the organization of the pores and patches required for the various specialized membrane function is, therefore, not possible by most experimental techniques. The situation is complicated by the observation that different membranes contain between 20% and 70% proteins by weight, and they are associated in some as yet undefined way with the lipids of the membrane, and together both form a stable-selective barrier at the cell periphery. Stein and Danielli (1956) introduced a few protein lined pores (Fig. 1) in an *ad hoc* fashion, without an explanation as to which proteins they involved or what made them stable. Similarly a continuous unfolded layer of protein would of necessity have many nonpolar residues exposed to water, an energetically unfavorable situation.

Several authors in the early 1960s reported the presence of elaborations (pits, dimples, and wrinkles) in high-resolution electron micrographs. This stimulated suggestions about their origin (Fig. 3). Kavanau (1965), Lucy and Glaubert (1964), Sjöstrand (1963, 1968), and Colacicco (1972) proposed in models of different degrees of sophistication that these substructures in the bilayer could arise from micellar arrangement of lipids and/or from globular proteins within the bilayer. Several other authors have tried to propose plausible models in which a mosaic of lipid and protein could exist (Winkler and Bungenberg de Jong, 1940; Ponder, 1951; Mitchison, 1953). The "patches," "pores," and carriers are particularly hard to visualize in such a micellar mosaic. A hydrophilic opening in an otherwise hydrophobic bilayer would normally be expected to enlarge because of the interfacial tension, to the point where the membrane would be destroyed. This is obviously not the case. Furthermore, a protein layer covering up the hydrophilic residues of lipid is not a generally acceptable situation. Probably