CELL MEMBRANES
Structure, Receptors, and Transport

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Cecilia Fenoglio, M.D.
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ADVANCES IN PATHOBIOLOGY 1

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College of Physicians and Surgeons of Columbia University
New York

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Contributors: Cell Biology/Membrane Seminar July 8–13, 1974

Fritz H. Bach, M.D., Laboratory of Medical Genetics, University of Wisconsin.

Joseph M. Davie, M.D., Ph.D., Department of Pathology and Microbiology, Washington University School of Medicine

Edward C. DeLand, M.D., Department of Surgery, University of California School of Medicine.

Michael Edidin, M.D., Ph.D., Department of Biology, Johns Hopkins University.

Elwood V. Jensen, Ph.D., Ben May Laboratory for Cancer Research, University of Chicago.

Vincent Marchesi, M.D.. Ph.D., Department of Pathology, Yale University.

Bruce S. McEwen, Ph.D., Rockefeller University.

Keith R. Porter, Ph.D., Department of Molecular, Cellular and Developmental Biology, University of Colorado.

S. J. Singer, Ph.D., Department of Biology, University of California

Foreword

This is the first publication of the Given Institute of Pathobiology and initiates a series of monographs covering a wide group of subjects in biology and medicine.

Although we hope to preserve the informality and spirit of small group discussions of the Pathobiology conferences, which have been held in Aspen for over ten years, the rapid advances in knowledge and the need for interdisciplinary exchange make it important to establish a written record of the seminars.

The first seminar of the summer of 1974 was arranged for M.D./Ph.D. candidates, supported by the Institute of General Medical Sciences, grant #GM 21478–01A1, and covered the broad area of membrane structure, transport, and receptors.

A complete list of the topics for the 1975 season is included elsewhere in these pages.

Donald West King

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Introduction

It is now generally agreed that a significant aspect in the behavior of individual cells involves interactions or communications between them by direct contact or via diffusible substances. It is also accepted that the cell surface, i.e., the plasma membrane with its associated extra- and intracellular structures, plays a most important role in this communication: in cell recognition; immunologic competence, regulation of growth control, in transport mechanisms of fluid, electrolytes, drugs, hormones and nutrients; and also in determining the identity of individual cells of similar or dissimilar ontogenic origin, specific antigenic determinants and receptors to immunoglobins, hormones and drugs or binding sites for viruses and lectins.

Many of the contemporary ideas about the molecular structure of biologic membranes are based on the experiments of Gorter and Grendel [1] who extracted the lipids from erythrocytes and spread them as a film on a trough at low pressure. Their findings indicated that the film covered an area which was twice as large as the one expected according to calculations made for the intact cell surface area. They proposed that the intact cell membrane was composed of a bimolecular layer of lipid, with the hydrophobic ends apposed at the center and the hydrophilic ends facing inside and out.

Davson and Danielli in their classic model [2] postulated the presence of a protein layer attached to the polar ends of both sides of the lipid bilayer. The presence of these proteins would contribute to the stability and strength of the lipid and would also explain the low surface tension of the interface.

Strong support for the Davson-Danielli model came from electronmicroscopic studies (EM) in which the cell membrane appeared as a thin band composed of two dark lines separated by a light zone. This trilaminar structure seen at the EM level was defined as the unit membrane. Most of the early EM studies on the cell membrane were carried out on the myelin sheath which surrounds peripheal nerve fibers [3,4].

Robertson [4] then proposed that the bilipid membrane had structural polarity, namely, that the inner surface was covered by polypeptides while the outer surface of the bimolecular leaflet was covered by mucopolysaccharides or mucoproteins.

With the advent of improved electron microscopic technics and with studies carried out on membranes of various systems (mitochondria and chloroplasts) there emerged observations which described the presence of repeating subunits in various membrane preparations composed of regular arrangements of repeating transverse densities in the unit membrane [5]. The report of Benedetti and Emelot on rat liver membranes [6] indicated the presence of an array of hexagons and some pentagons in the membrane in addition to globular knobs

which were dispersed over the membrane surface. The hexagonal pattern was found in sections prepared at 37°C but not in those prepared at low temperatures.

These observations of subunits resulted in a model proposed by Green [7] which suggested that biologic membranes are formed by the association of repeating structural units of proteolipids.

The views that followed tended to regard the cell membrane as a dynamic structural organization rather than a static one. Reports on the rapid turnover of membrane constituents [8] seemed consistent with such a dynamic model.

One of the current proposals for the structure of cell membranes is that of the fluid mosaic model of membrane structure postulated by J. Singer [9,10] and discussed here by him. The model considers that cell membranes are similar to other biologic structures in that they have evolved into a structural organization which allows for their essential cellular function under optimal thermodynamic conditions. In this fluid mosaic model, the basic structure of the membrane, namely, the matrix of the plasma membrane, is composed of "fluid" lipid bilayer (phospholipids) with integral membrane proteins firmly intercalated into this "fluid" lipid. These components are amphipathic in that they are asymmetric with respect to the hydrophilic portions of the structure. Their hydrophilic ends are oriented towards the aqueous environment on both the outer side of the membrane and on the inner region (cytoplasm). One of the integral proteins which has been characterized is glycophorin in the human erythrocyte membrane (see Marchesi's presentation). Its protein portion is integrated firmly into the lipid bilayer and its glycopeptide portion with its binding sites for virus and some lectins is directed into the aqueous phase, possibly extending for some distance off the membrane bilayer.

Similarly it can be visualized that antigenic determinants, proteins, glycoproteins, oligosaccharide and glycolipid haptens (see papers by Davie, Bach, Edidin) or hormone receptor sites (see Jensen, McEwen) are intercalated into the membrane with their oligosaccharides portion (when present) or their extended peptides radiating out from the outer surface of the membrane.

In the fluid mosaic membrane model on the cytoplasmic side of the plasma membrane matrix (phospholipids and integral proteins) and to some extent on the outer surface, there are the peripheral proteins (enzymes, etc.) which are loosely bound to the integral proteins. On the cytoplasmic side there is an additional region of contractile proteins which are loosely associated with the membrane. Spectrin is one of these proteins in the human erythrocyte [11]. It is associated with the cytoplasmic side of the membrane and has been shown to be loosely bound to the glycophorin by noncovalent bonds [12]. These contractile elements exert a restricting function on the mobility of the integral protein. As discussed by Porter, the microtubles and microfilaments are important in determining the shape of the cell and some of its surface features. Their

integrity is essential for cell division. When a normal cell converts to a neoplastic state, the distribution of these contractile proteins is altered. This would, according to Singer's model, consequently modify the mobility of the integral proteins and their saccharide and protein projections, thus resulting in a change in cell to cell interactions.

While the fluid mosaic model of membrane structure does account for the variety of receptor sites and immunologic determinants on the cell surface, its postulation of the process of active transport of hydrophilic small molecules differs from that proposed by some physiologists, wherein, a carrier protein (ATPase) bearing sodium and potasium ions is rotated from one side of the membrane to the other [13]. According to Singer's model (see Singer), these ions are transported through "pores" which are formed by a spatial conformation of certain integral proteins, considered here as a transport protein rather than a mobile carrier. During transport, the molecular conformation of the pore is altered by energetic interactions, and a pore which was open to the outside now closes off and opens to the inner surface of the membranes. Arguments and suggested proof of the pore theory are in Singer's presentation.

It is our hope that this symposium on the cell membrane will serve to demonstrate a variety of approaches used in membrane research and some current views on the structure and function of the cell membrane and the regulatory mechanisms controlling them.

We would like to acknowledge the assistance of those M.D.-Ph.D. candidates who acted as scribes: Steven C. Quay, Brian Storrie, Owen Witte, Barry Wolfe, Stephen A. Morris, Thomas Moss, Valerie Petit and Dennis Miura. We also acknowledge the aid of Dr. Gerda Nette, who helped with photographic work, and the secretarial assistance of Karin Peterson and Anne Anderson.

The Editors

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Structure of Membranes

S. J. Singer, Ph.D.

Witl in the last few years new ideas on the molecular organization of membranes have brought about great progress in the field of the molecular biology of membrane structure [18,21]

THE FLUID MOSAIC MODEL

The prevailing concept in the fluid mosaic model of membrane structure [18,21] asserts that the basic structure of biologic membranes is a two-dimensional solution of globular integral proteins dispersed in a matrix of fluid lipid bilayer. This theory has received experimental support of three major kinds. First, there is much evidence that membrane proteins can move about rapidly in the plane of the membrane. Initially this was demonstrated for the H–2 antigen in the membranes of mouse-human cell heterokaryons by Frye and Edidin [4]; this translational mobility of membrane proteins has since been observed with many surface antigens and receptors in a wide variety of cells [20] and even in myelin [9]. Secondly, there is evidence that many proteins are deeply embedded in membranes, as revealed by freeze-fracture methods in electron microscopy [1,10,14] and by structural studies of integral membrane proteins such as cytochrome b_5 [5,22], cytochrome b_5 reductase [23] and glycophorin [16] (see the following discussion by Marchesi). Thirdly, the latter three proteins appear to be amphipathic proteins, as had been postulated [8,25], with their hydrophilic ends protruding from the membrane, and their hydrophobic segments embedded in the membrane.

MEMBRANE ASYMMETRY—PROTEINS

There is substantial evidence that the integral proteins of membranes are generally asymmetrically distributed and oriented across the bilayer. Certain

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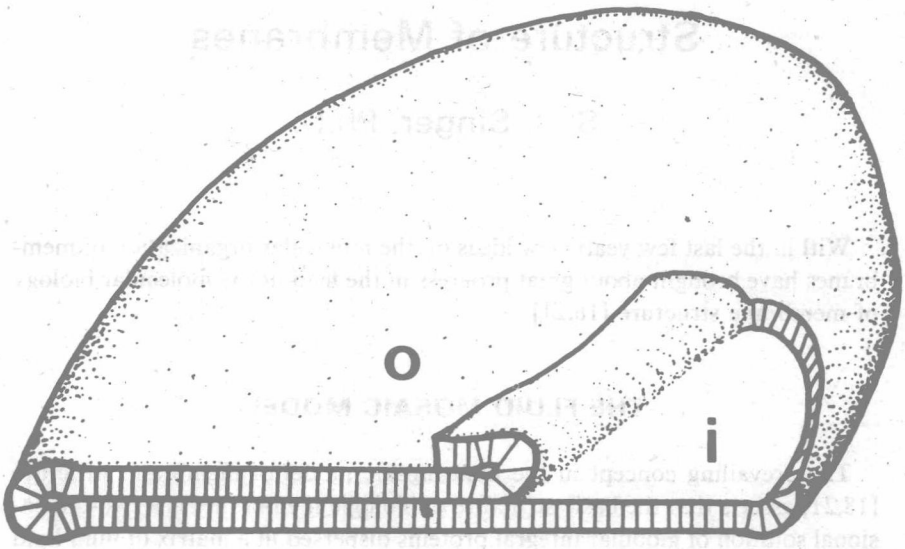

FIG. 1a. A schematic diagram of a flattened red cell ghost membrane which has been partially peeled back to expose the internal face of the membrane (o-outer surface of membrane, i-inner surface of membrane). (Reproduced from [12].)

proteins protrude primarily from one face of a membrane, others from the opposite face, and still others from both faces but with a certain preferred orientation perpendicular to the plane of the membrane. This asymmetry can be graphically illustrated by the example of influenza virus binding to the membrane of the red cell ghost (Figs. 1a and b). It is yet unknown which fraction of the molecules of any given protein is oriented in a particular preferred direction; whether the asymmetry is absolute (this seems unlikely); and if the orientation varies upon perturbing the membrane or its environment.

Let the asymmetry co-efficient *(a)* be the ratio of the numbers of molecules of a particular integral membrane protein which are oriented in one direction in the bilayer to those oriented in the other. Most methods currently used to demonstrate this asymmetry, such as chemical methods comparing right-side-out and inside-out membrane vesicles [24], cannot determine a to a degree better than $\pm$ 10%. Therefore, values of a $>$ 10 cannot be accurately measured at the present time.

There are two possible explanations of protein asymmetry: (1) The distribution is an equilibrium one. There is a difference in the chemical potential (μ_p) for a particular membrane protein oriented facing one way compared to the other; i.e., $\Delta\mu_p = -RT \ln a$. The rates of flipping of the membrane protein from one orientation to the other must be sufficiently rapid to maintain this equilibrium. (2) The distribution is not an equilibrium one. The newly synthesized protein is inserted into the membrane in the appropriate orientation.

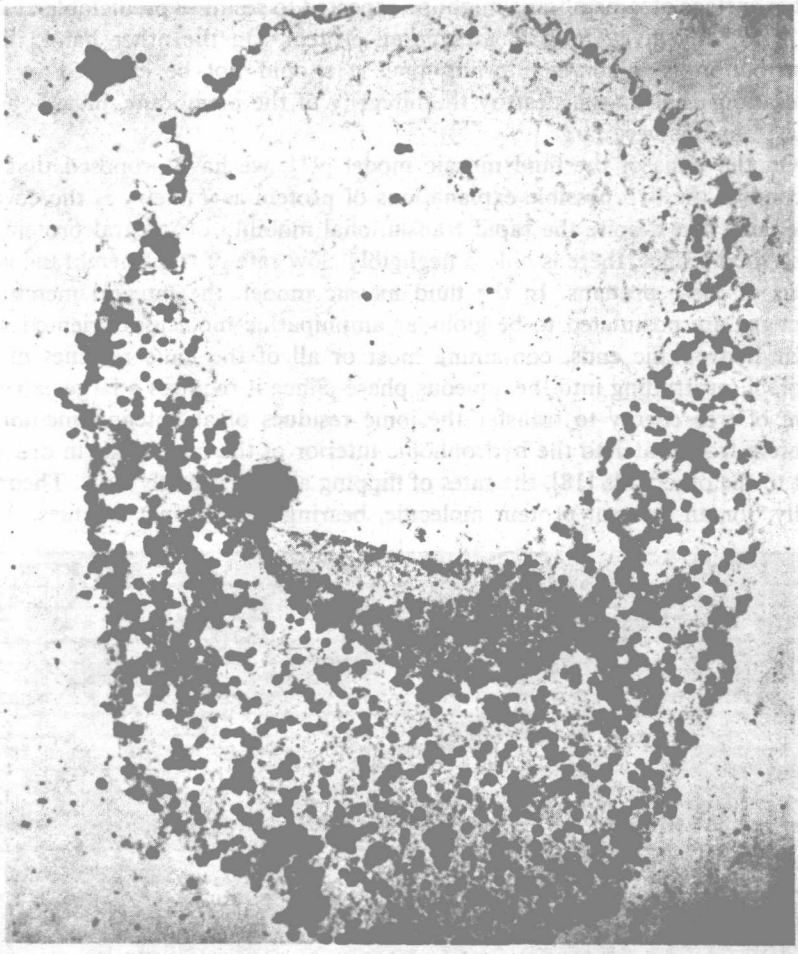

FIG. 1b. When a human red cell ghost is prepared as diagrammed in Figure 1a and is reacted with influenza virus, the virus, which binds to the red cell glycoprotein by its sialic acid groups, becomes attached exclusively to the outer surface of the membrane and does not bind to the inner part. (Reproduced from [13].)

Thereafter, for a time longer than the half-life for the turnover of that protein in the membrane, there is essentially no flipping of the protein to the other orientation; i.e., $\Delta\mu_{\rho}^{act}$, the free energy of activation for the flipping process, is very large. Since we have no data whatsoever which unambiguously favor either of these possibilities we cannot be certain of the basis of protein asymmetry in membranes. However, it is a problem which is experimentally approachable if a can be accurately measured. For example, if the distribution is an equilibrium one, then altering the physical or chemical features of one or the

other surface of a membrane might be expected to result in predictable change in a for any given integral membrane protein. On the other hand, if the distribution is a non-equilibrium one, a should not be changed by any alterations that do not destroy the integrity of the membrane, or as long as $\Delta\mu_{\tilde{p}}^{act}$ remains very large.

On the basis of the fluid mosaic model [21], we have proposed that the second of the two possible explanations of protein asymmetry is the correct one, and that despite the rapid translational mobility of integral proteins in fluid membranes, there is only a negligibly slow rate of transmembrane rotations of these proteins. In the fluid mosaic model, the integral membrane proteins are postulated to be globular amphipathic molecules oriented with their hydrophilic ends, containing most or all of the ionic residues of the protein, protruding into the aqueous phase. Since it requires a large expenditure of free energy to transfer the ionic residues of an integral membrane protein from water to the hydrophobic interior of the membrane in order to get to the other side [18], the rates of flipping are exceedingly slow. Theoretically, for an integral protein molecule, bearing 20–30 ionic residues, $\Delta\mu_{\tilde{p}}^{act}$

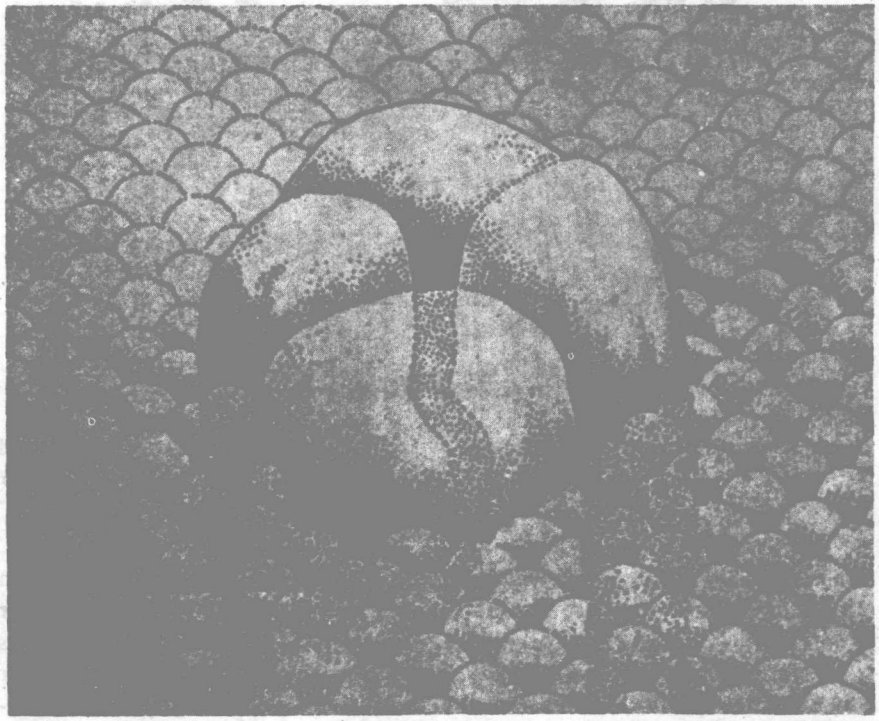

FIG. 2. Diagram of an integral membrane protein present in the fluid mosaic membrane as a subunit aggregate. (Reproduced from [19].)

might well be of the order of magnitude of 100 kcal/mole [18]. Even the most hydrophobic membrane proteins known contain at least as many ionic residues as that [20].

For the reasons given above, the mechanisms for active transport of hydrophilic small molecules which postulate the rapid rotation of a carrier protein from one surface of the membrane to the other are not likely to be correct. We suggest instead that a transport protein exists as a subunit protein aggregate which spans the membrane (Fig. 2), and that the mechanism of transport involves a quaternary rearrangement of that aggregate (Fig. 3). Dr. Jack Kyte [7] has prepared specific antibodies to the major polypeptide chain of ATPase from canine kidney proximal tubule membranes. This enzyme, which is sodium (Na^+) and potassium (K^+) dependent, is the protein involved in the active transport of Na^+ and K^+ in opposite directions through a given membrane. The stoichiometric binding of the antibodies to the enzyme in membrane vesicles has no detectable effect on the K_M or V_{imax} of the ATP hydrolysis by the enzyme. The ATP hydrolysis and the translocation of Na^+ and K^+ ions by this enzyme are widely thought to be expressions of the same, or closely coupled, events [7]. The results of these antibody studies, therefore, provide evidence that a transmembrane rotation of this transport protein cannot be the mechanism for its translocation of Na^+ and K^+ since it is inconceivable that the binding of one or more molecules of antibody to the enzyme in the membrane would not have some effect on its transmembrane

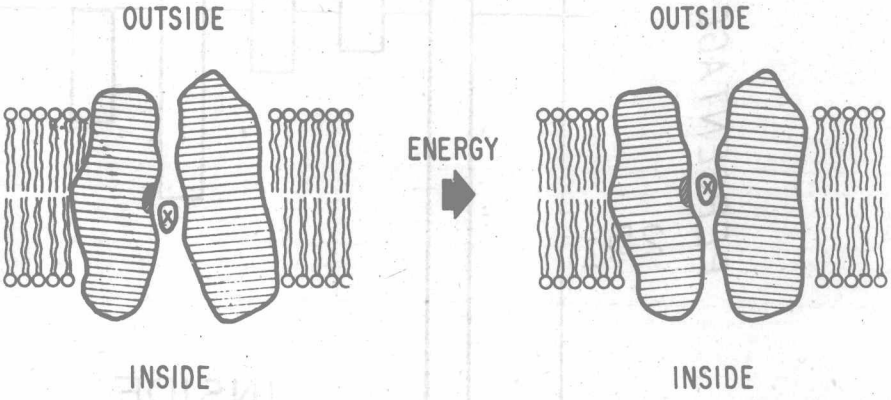

FIG. 3. Diagram of a proposed mechanism of active transport. A subunit aggregate of integral proteins forms a narrow water-filled "pore" (Fig. 2). There is a binding site for the transportable ligand X on the surface of one or more of the subunits. Some energy-producing event might produce a quaternary rearrangement of the subunits and translocate X across the membrane. Reversal of the energy-yielding process then restores the aggregate to its initial state. (Reproduced with permission from SJ Singer, "The Molecular Organization of Membranes," Annual Review of Biochemistry, Vol. 43. Copyright © 1974 by Annual Reviews Inc. All rights reserved.)

rotation. On the other hand, a translocation mechanism that involved a quaternary rearrangement in a subunit aggregate in the membrane (as in Fig. 3) might not have its rate seriously affected by the binding of antibody molecules to one or more of the subunits.

If the asymmetry of proteins in membranes is the consequence of an initially asymmetric insertion of newly-synthesized protein into the membrane, then it is essential that the principles which govern such insertion processes should

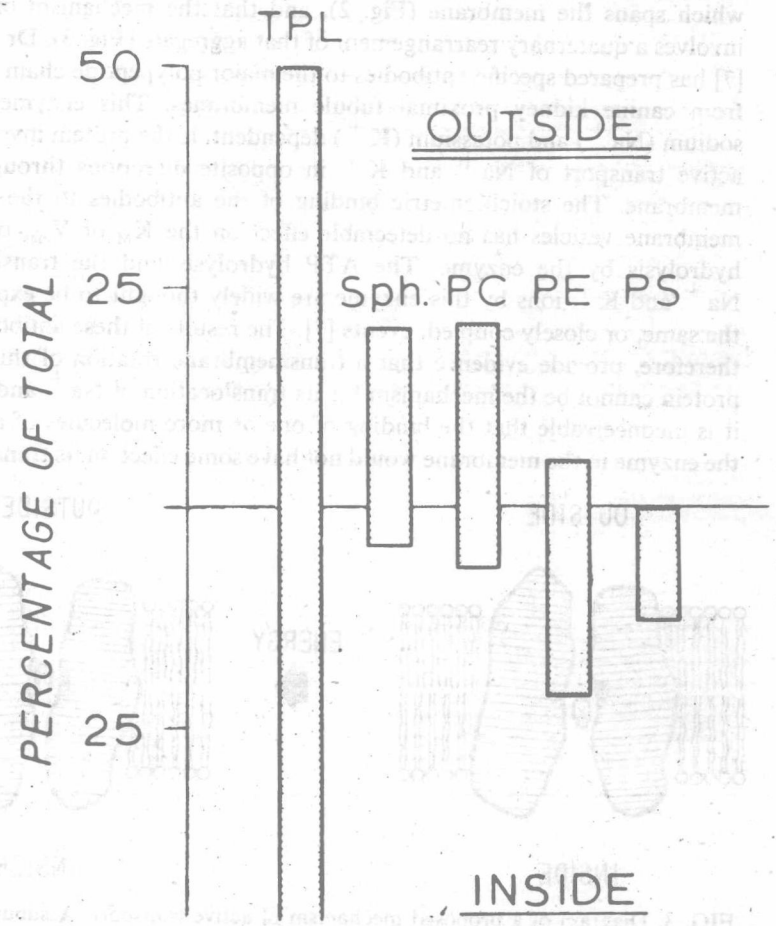

FIG. 4. Diagram of distribution of phospholipids in erythrocyte membrane. These are asymmetrically distributed in the two halves of the bilayer. Syphingomyelin (Sph) and phosphatidylcholine (PC) are predominantly in the outer layer. Phosphatidylethanolamine (PE) and phosphatidylserine (PS) are predominantly in the inner layer. (Reproduced from [26].)

be understood. It is not known at the present time which factors determine that the molecules of protein A are to be inserted into the membrane so that they protrude from one membrane surface, that those of protein B are to protrude from the other, and those of protein C will protrude from both surfaces in a preferred orientation.

MEMBRANE ASYMMETRY—LIPIDS

Evidence has recently been published [23,24] which supports Bretscher's suggestion [2] that the phospholipids of the human erythrocyte membrane are asymmetrically distributed in the two halves of the bilayer, with phosphatidylcholine and sphingomyelin predominantly in the exterior layer, and phosphatidylethanolamine and phosphatidylserine predominantly in the interior layer facing the cytoplasm (Fig. 4). The same two possible explanations which were considered in the previous section on protein asymmetry apply here: (1) the asymmetric distribution of lipids is either an equilibrium one, with rapid flipping of the phospholipids from one-half of the bilayer to the other, or (2) it is a nonequilibrium one, which is generated by different enzyme-catalyzed lipid synthetic reactions in the two halves of the bilayer and is maintained because the rates of flipping of the lipids is slow relative to the rates of lipid replacement in the membrane. The rates of flipping of spin-labeled phospholipids in synthetic bilayers [6] and in electroplax membrane vesicles [11] have been measured by spin-label technics, but their significance is not clear, since these rates were reported to be very slow in the former case and fairly rapid in the latter. More work must be done to settle this very important point.

The lipid asymmetry in the erythrocyte membrane suggests many possible consequences. As one consequence, we propose that the human erythrocyte membrane may behave as a *bilayer couple* [17] in rough analogy to the behavior of a bimetallic couple. It is suggested that under various perturbations, the two halves of the bilayer may be differentially expanded or contracted, producing corresponding evaginations or invaginations of the membrane. It is well known that low concentrations of a variety of amphipathic molecules can produce characteristic shape changes in intact human erythrocytes [3], causing the cells to become either crenated (evaginated) or cupped (invaginated) (Fig. 5). Most of the substances that are crenators are *anionic* amphipathic molecules (such as dinitrophenol and free fatty acids), while most of the cup-formers are *cationic* amphipathic molecules (such as chlorpromazine and other tranquilizers, tetracaine and other local anesthetics) (Tables 1 and 2). There is good evidence that these compounds are taken up by the membrane and expand its surface area by a few per cent [15]. We suggest that the