Meetings

Membrane Structure and Function

The study of the structure and function of cell membranes is one of the most active and fruitful fields of biological investigation today. It draws on many scientific disciplines—biochemical, biophysical, and physicochemical—so that workers with widely different trainings and experience are involved. The advances are so numerous and cover so many intertwining topics that it is difficult for an investigator in one field to be fully appreciative of what is taking place in a neighboring one. For this reason many scientific conferences dealing with general aspects of membranology are taking place at the present time.

One such biochemical conference on membrane structure and function was held at Ste. Marguerite, P.Q., Canada, 27 February-3 March 1967. The conference aimed at bringing together some new data and a variety of interpretations concerned with the structure of cell membranes, with the chemical factors involved, and with some of the specific transport processes that are so important for the growth and function of the cell.

What is the pattern of organization of biological membranes? The unit membrane concept, that has evolved during the last decade mainly from studies of developing peripheral myelinated nerve fibers was discussed by its propounder J. D. Robertson (Duke University, Durham). According to this concept, which is a development of the classical Davson-Danielli model, a membrane consists of a single continuous bilayer of lipid with the nonpolar carbon chains at the center of the membrane and the polar ends pointing outward. The polar surfaces of the lipid bilayer are each covered by unimolecular films of nonlipid. The outside surface differs chemically from the inside and, thus, the membrane is a chemically asymmetric structure. Emphasis was given mainly by Robertson to problems of membrane substructure. He considered two kinds of substructure, referred to as granular-fibrillar and globular. The granular structure has a hexagonal pattern of subunit facets, about 90 Å in diameter; each facet contains a dense spot, possibly a mucopolysaccharide. This structure appears to be confined to the hexagonal dense strata of the membrane. The globular substructure results from dense lines crossing the light central core of the membrane, spaced regularly at a period of 90 Å. This is considered by some to be a microspherical globular arrangement of the lipid core of the membrane and is present in retinal rod outer segments. X-ray diffraction studies of these segments do not seem to support, however, the postulated microspherical transformation in the membrane lipids. It would appear that the globular structure might be regarded as an optical artifact derived from the granular substructure, which is itself real and of widespread occurrence.

F. S. Sjöstrand (University of Los Angeles, California) regards the globular structure as a real feature of membrane architecture and indicative of specific molecular arrangements. The term "elementary particle" has been applied to appearance of globular structure of this kind in retinal rod outer segment membranes; similar appearances exist in mitochondrial membranes. The particles of the globular structures are held to be lipoproteins, not necessarily in a rigid state but rather in a dynamic condition and they may be the sites of multienzyme systems. The question of the reality of the substructures described was the subject of much discussion and it seemed to be evident that it is linked with the preparative techniques. Conceivably the appearances under the electron microscope might represent different phases of a nonrigid system.

X-ray diffraction data, obtained in studies of a variety of membrane preparations, were the subject of J. B. Finean (Birmingham University, United Kingdom). He pointed out that a series of diffraction patterns, corresponding to a range of levels of hydration, may be obtained with each preparation. Much of the water can be removed from myelin preparations without damage to the structure. Some of the patterns obtained at relatively high levels of hydration, and presumably some of these approximate to the native state, are simple lamellar patterns. However, those patterns obtained at lower levels of hydration tend to be more complex and to suggest multiphase systems. It is evident that additional data are needed of the biochemical factors involved in isolation procedures and of their influence on the diffraction patterns subsequently obtained. There is considerable doubt as to whether the description of a native, hydrated plasma membrane as an assembly of structural units provides the correct interpretation of present diffraction data. They do not, it was emphasized, eliminate a true segmentation but this may not be so pronounced as would appear from the electron microscope data. The discussion indicated that the x-ray diffraction data were made with fresh material but, nevertheless, the effects of an inevitable time lapse may have to be considered in interpreting the data. J. F. Danielli (State University of New York, Buffalo) felt that it was unwise to refer only to a lamellar structure or to a micellar structure. The free energy involved in the transformation of one structure to the other may be small. Consequently, it would be better to consider the representation in the membrane of both the lamellar and the micellar states. The proportions may well depend on the dynamic state of the system.

Studies have been made of the conditions under which the phospholipids of mitochondria may be organized as a bimolecular leaflet (F. A. Vandenheuvel, Canada Department of Agriculture, Ottawa). In such a bimolecular leaflet the polar groups of phospholipids are turned outward and the phosphorus atoms of the phospholipids, apposed tail to tail, are separated by a uniform distance of 51.5 Å. Protein may also be tentatively located as a thin sheet covering both sides of the bimolecular leaflet. The key to the molecular arrangement, derived from studies of precise stereo models, lies in the existence of common and complementary structural features in other-

wise dissimilar myelin lipids. Confirmational relations between cholesterol and any of the other myelin lipids may account for the formation of stable bimolecular complexes which impart order and stability to the myelin sheet. The organization of myelin lipids might serve as a model for membrane systems such as those of the erythrocyte where cholesterol is a major component. But it should be understood that cholesterol is not present at high levels in many membranes.

Some doubts have been expressed concerning the ability of unsaturated phospholipids to form bilayers as they do not form condensed monolayers as easily as saturated ones. However, Vandenheuvel pointed out that the energy situation in bilayers differs considerably from that in monolayers. There is abundant evidence of the spontaneous formation of micelles of bilayers and single bilayers of phospholipid in an aqueous phase. Moreover, there is evidence to indicate that stability of bilayers depends on the presence of unsaturated groups.

A fully unsaturated layer offers no space through which water molecules and small inorganic ions can pass. Even assuming that a random movement of chains will allow the formation of transient pores, the actual porosity will be low. Partial substitution by saturated chains will increase porosity. The ratio of 1 to 2 of saturated to unsaturated chains in mitochondrial lipids would seem to be optimal to give the necessary porosity, fluidity, and stability to the bilayers.

In discussing the lipids of animal cell membranes, G. Rouser (City of Hope Medical Center, Duarte, California) pointed out that analysis of organs of vertebrates and invertebrates shows that all animal cells contain very similar polar (membrane) lipids. Phosphatidyl derivates of choline, ethanolamine, serine, and inositol occur in all animal cells. Sphingomyelin occurs in cells of all vertebrates and many invertebrates, but in some individuals ceramide aminoethyl phosphonate or a related lipid replaces sphingomyelin. Animal cells also contain glycolipids but in widely varying amounts. Any one organ, such as muscle, possesses, qualitatively and quantitatively, the same of a very similar phospholipid composition for all vertebrates. Since whole organ composition is largely a reflection of the components of the mitochondria and endoplasmic reticulum, it is evident that these organelles show very little species variation for any one organ. The composition of brain lipid of various species is very similar though the amount of sphingomyelin is apt to vary. In marked contrast, however, is the large variability in phospholipid composition of different organs of the same species. This variation is due to large differences in the composition of the endoplasmic reticulum of different organs. It seems that the compositions of mitochondria and nuclei of different organs and species are very similar whereas those of the cell surface membranes and endoplasmic reticulum are variable.

C. L. Hannay (Canada Department of Agriculture, London, Ontario) described methods for the preparation of lipid complexes of globular micelles from mixtures of ovalecithin, cholesterol, and saponin. It was found that all the structures, including the double helices, could be formed from all manner of ovalecithins but that there was no certainty that all the structures would be formed in any particular experiment. One variant, for example, was the degree to which the solvent was removed from the lipids before their suspension in saponin solution. The discussion of this problem indicated the complexity, and perhaps the lack of biological reality, of the saponin mixtures.

Turning to bacteria, R. G. E. Murray (University of Western Ontario, London) indicated their manifold advantages in providing systems suitable for biochemical and biophysical studies of membranes and their functions. He discussed the anatomical features of bacterial membranes and the properties of the cell wall and protoplast membranes. He demonstrated the great enlargement of membranes in the developing spore and the continuous intracellular stacked membranes of the vitrifying organisms; he pointed out the structural differentiation of areas in bacterial membranes. Again the question of reality of the structures presented by the electron microscope arose. Are the cells fixed, he asked, in the dynamic state normal to them or do they assume the nearest stable configuration?

The properties of membrane preparations from halophilic organisms *(Halobacterium halobium)* were the subject of comment by C. McClare (Kings College, London, United Kingdom) who was mainly concerned with the bonds existing between the membrane components. In these organisms,

it was interesting to note, there may be a concentration ratio of potassium ions between cell water and medium of 100 to 1. Whether there is an active ion pump in the bacteria is not yet known with certainty. He showed that the membranes may be isolated by rupturing the cells with glass beads or subjecting them to osmotic shock with 0.02*M* $MgCl_2$. After treatment of the membranes by chloroform-methanol extraction, or by dialysis followed by centrifugation, two types of protein are obtained. However, the major portion of the lipid is found to be associated with one or other of the proteins. This suggested that in vivo lipids are bound to both types of protein by two kinds of bonds which are labile to one or other treatment. One protein has a high proportion of acid (glutamic, aspartic) residues but seems to have a hydrophobic face as it associates with lipids. The other protein type seems to bind the lipids ionically since it is not itself soluble but is taken into chloroform-methanol as a lipoprotein. It would seem that the ionic bond is an intermolecular chelate between the lipid head group, phosphatidyl glycerophosphate, magnesium ions, and a complex of acid and basic groups on the protein. The general properties and similarities of the lipoprotein and proteolipid fractions obtained may be broadly understood in terms of their interactions with water, strong salt solutions, and hydrophobic media.

M. Kates (National Research Council, Ottawa) reviewed the present knowledge of lipid composition in bacterial membranes. Attempts were made to correlate the phospholipid and fatty acid composition with the taxonomic classification of the bacteria. Kates pointed out how there is considerable variation in the composition of bacterial fatty acids and emphasized the dynamic aspects of bacterial membrane composition. This may vary considerably according to the nutritional conditions. Moreover, the degree of saturation of bacterial fatty acids appears to be a function of the time of growth of the organism as well as the temperature. With halobacteria the cell envelopes are high in lipids with but little mucopeptides. Dihydrophytol ethers of D-glycerophosphates are also present in the lipids of all halophilic microorganisms; and such ethers are not present in nonhalophiles.

The phospholipid composition of biological membranes was further dis-

cussed by L. L. M. Van Deenan (University of Utrecht, Netherlands) who described experiments on monolayer interactions between phospholipids and cholesterol. The interactions, though complicated, indicated that cholesterol may contribute to a high degree of molecular organization. Selective lysis of the cell membranes of fungi or erythrocytes, but not bacteria, brought about by application of polyene antibiotics, was studied in mono- and bilayers of different lipid composition. The action of these drugs depends on the ratio of phospholipids to sterols. They bring about reorientation of lipids rich in sterols without affecting lipoproteins such as ATP-ase. Studies were made of the structural requirements of the fatty acid constituents of phospholipids to serve as membrane components. The monoacyl-phosphoglycerides, and possibly lysolecithins, play a role in the regulation of the fatty acid composition of the components. The ratio of differently charged head groups of phospholipids is genetically controlled in mammalian cells such as erythrocytes. In some bacteria (*Staphylococcus aureus* and *Bacillus megaterium*) major changes can be induced in the relative proportions of the phospholipids by changing the *p*H of the nutritional medium. Even the shape and properties of the protoplasts vary according to the *p*H. In *B. megaterium* a glucosamine phosphatidylglyceride is present whose content depends on the nutritional conditions.

A. D. Bangham (Institute of Animal Physiology, Babraham, United Kingdom) discussed phospholipid models for passive diffusion studies. Molecular orientation and dimensions of the membranes are in accord with a bilayer structure. Each membrane forms a closed surface separating one compartment from another. The membranes are permeable to water. However, different phospholipids form membranes exhibiting differential permeabilities to cations and anions, and exchange diffusion can occur. Some membranes can distinguish between K^+ and Na^+; the presence of Ca^{++} may have critical effects on permeability possibly through chelation. Ammonium ions are more freely permeable than K^+ or Na^+.

Flux rates of ions across phospholipid layers were described by J. H. Schulman (Columbia University, New York) who pointed to the high rate of flux of iodide as compared with other ions. He has measured fluxes

from an aqueous phase to oil and thence to an aqueous phase and showed how the fluxes are affected by phospholipid layers. Schulman described the ingenious experiments whereby ions may be moved from an aqueous phase into a phase such as pentanol by the use of carriers such as 0.01*M* lauric acid, stearic acid, or lecithin. While such models are of great importance for our knowledge of diffusion through phospholipid layers, we still recall that proteins are present in natural bilayers and that these may well affect the properties of permeability and diffusion.

Turning to problems of ionic transport, Skou (Aarhus University, Denmark) showed that the membrane-bound ATP-ase sensitive to Na^+ and K^+ fulfills many of the requirements of an ion transport system. It is reasonable to conclude that the enzyme plays a major role in the active transport of cations across the cell membrane. The enzyme apparently has two sites with affinities for cations, one where the affinity for Na^+ exceeds that for K^+ and the other where the affinity for K^+ exceeds that for Na^+. The affinities are influenced, in a manner not yet understood, by adenosine triphosphate (ATP) which increases the affinity of Na^+ for the Na site and diminishes that of K^+ for the K site. ATP also affects affinity of the enzyme for strophanthidin.

The cations at the two sites control the manner in which ATP is broken down. With sodium at both sites the hydrolysis of ATP leads to a phosphorylation of an enzyme component. No such phosphorylation is evident with K^+ at one site and Na^+ at the other. The possible conformational changes brought about in the enzyme protein by the cations, and the changed affinities, are problems of great importance which have to be solved. Such solutions are required before acceptable models can be developed. It should be emphasized that the ATP-ase is a lipoprotein which when treated with phospholipase loses its specificity for Na^+ and K^+.

Studies of the membrane-bound ATP-ase, believed to be involved in coupled transport of Na^+ and K^+ in animal tissues, were the subject of discussion by L. E. Hokin (University of Wisconsin, Madison) who has found that its alkylation by diisopropylfluorphosphonate (DFP) is blocked by ATP and other nucleotides at higher concentrations. Either the cardiotonic ste-

roids or K^+ potentiate the alkylation by DFP, and K^+ antagonizes protection of the enzyme by ATP. Just as ATP affects the affinity of K^+ for ATP-ase, so does K^+ affect the affinity of ATP for ATP-ase. As the steroid or K^+ acts at the outer surface of the membrane and ATP acts at the inner surface, the former presumably brings about a conformational change affecting the relative affinities of DFP and ATP at the substrate site. Conceivably the steroid may also affect the affinity of K^+ for the enzyme strophanthidin-bromo (or iodo) acetate irreversibly inhibits the enzyme by alkylation at the steroid site. This reaction combined with a triple labeling technique has made it possible to isolate a lipoprotein, on chromatography, with suitable labeling. The molecular weight of such a lipoprotein was calculated to be of the order of 175,000. Assuming one strophanthidin site for each ATP site, the protein was considered to be 50 percent pure.

P. G. Scholefield (McGill University, Montreal) discussed the role of Na^+ in transport reactions. He pointed out that Na^+ is not required for translocation as it is not needed for the process of exchange diffusion, which is also independent of ATP. Moreover, this process may not be affected by substrate analogues that block transport. Thus it is evident that the carriers involved in exchange diffusion and active transport are not necessarily the same. Although, as is well known, Na^+ is needed for many forms of transport, it may not be essential for all transport systems. It is clear also that sodium movement may occur without necessarily having an effect on the transport of an amino acid, as for example, that of aminoisobutyric acid in rat diaphragm. Thus it is evident that Na^+ is required more for the operation of some systems than for others. The sodium effect on transport may in fact be due to conformational changes in the carrier protein that may result in changed transport velocities. Its effect on membrane-bound ATP-ase is doubtless a basic mechanism. However, it was pointed out in discussion that in some bacteria no Na-K-dependent ATP-ase is present though concentrative uptake occurs. It was also pointed out that certain bacterial transport systems are not dependent on Na^+. Discussion indicated the pressing problem of throwing further light on the precise relation between the kinetics of ionic

and molecular transport and the molecular architecture of the cell membrane. If there is a relatively small number of transport sites at the membrane they may never be visualized by the electron microscope. It will be necessary to decide as to whether there are active patches for transport or whether there are on the membrane reversible deformational changes that cause changed rates of transport or whether in fact both mechanisms operate.

In considering specific factors involved in molecular transport, E. P. Kennedy (Harvard University, Boston) described work leading to the finding of a protein localized in the membrane-containing fraction of *Escherichia coli* that is an essential component of the lactose transport system. Techniques were devised for labeling this protein. Study of the genetic control of the protein revealed that it is the product of the *y* gene of the *lac* operon. Study of the interaction of the membrane protein with β-galactosides in cell-free systems strongly suggests that the protein must have two distinct sites for binding sugars.

V. P. Cirillo (State University of New York, Stony Brook) described investigations into the mechanism of monosaccharide transport in two strains of *Saccharomyces cerevisiae*, one an asexual diploid and the other a sexual haploid. By use of nonmetabolizable sugars it was found that the mechanism is apparently a carrier-mediated, facilitated diffusion. The process of uptake shows saturation kinetics and exhibits both marked substrate selectivity, and competitive inhibitions, among the transported sugars, as well as counter transport. Both strains have at least two monosaccharide transport systems—a constitutive "glucose" system and an inducible "galactose" system. From analysis of their relative affinity and inhibition constants, the structural requirements for the glucose system and the inducible galactose system were worked out.

Inducibility of the galactose transport system in the haploid strain is under genetic control and in these cells the transport system is equally well induced by D-galactose and by its nonmetabolized analogues D-fucose and L-arabinose.

D. M. Miller (Canada Department of Agriculture, London, Ontario), in discussing sugar transport in human erythrocytes, concluded that the suggestion that the sugar forms a com-

plex with a carrier moving faster through the membrane than the free carrier is inadequate to account for the present evidence. He considered an alternative mechanism, in which simple carrier transport within the membrane is flanked by two first-order processes (such as slow diffusion layers). Each occurs at each side of the membrane, which would account for the results. He also developed a pore theory in a very ingenious manner that would explain his results; however, this suggestion was not greeted with enthusiasm by the morphological membranologists.

S. Fleischer (Vanderbilt University, Nashville) dealt with the role of lipids in the structure and function of the mitochondria. He showed how extraction of mitochondria with acetone-water leads to a block in electron transfer which can be relieved by the addition of coenzyme Q and not the other neutral lipids. Another method of extraction leads to a condition of block of electron transfer which is reactivated by addition of the appropriate phospholipids as well as coenzyme. Thus, for the first time, a functional requirement of the phospholipids in the electron-transfer chain is demonstrable. Phospholipase treatment can also be used for removal of phospholipids in order to demonstrate their requirement in, say, succinate oxidation. It is of course an old observation that phospholipase A can block succinate oxidation in cell preparations but the reconstitution described by Fleischer is a considerable advance. Gross morphology of the mitochondrial inner membrane is not apparently altered after removal of even 95 percent of the lipid. The "unit membrane" is preserved. It would be necessary to postulate, on the Davson-Danielli model, the presence of cross-links holding the proteins apart. The enzymes of the electron transfer systems in the mitochondria are considered to be an integral part of the membrane proteins.

L. Ernster (Stockholm, Sweden) discussed electron transport in intracellular membranes. He showed how three types of electron transporting systems (the respiratory chain, the NADH-cytochrome b_5 reductase, and the NADPH-linked hydroxylase) are associated with intracellular membrane structures. The respiratory chain is present in all animal tissues except nonnucleated erythrocytes and is associated with the inner mitochondrial membrane. The NADH-cyt b_5 reduc-

tase, occurring abundantly in the liver, is associated both with endoplasmic reticulum and with the outer mitochondrial membrane. The NADPH-linked hydroxylase system is associated, in the liver, with the endoplasmic reticulum and in the endocrine glands with the mitochondria. Association with the membrane structure endows the electron transport systems with properties that distinguish them, both quantitatively and qualitatively, from a random mixture of enzymes. These properties are essential for cell function. Membranes play a basic role in the maintenance and regulation of physiologically adequate levels of electron transport systems, as indicated by studies of thyroxin-induced synthesis of the respiratory chain or the drug-induced synthesis of NADPH-linked hydroxylase.

The conference, which included an address by T. Tearell (University of Uppsala, Sweden) on integrative viewpoints in membranology, made all aware, if they were not already aware, that the biological membrane is much more than a sum of its parts and that it is an entity of as profound importance for the life of the cell as any other organized constituent of the cell. It was evident that the generation of membrane components that control the fluxes of cell constituents is geared to the processes of enzyme syntheses and so to the processes of lipid and protein biosyntheses. How this interdependence is brought about, and the precise nature of the phospholipid-protein associations that control membrane function, are some of the main problems in present-day membranology.

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Drug Information

The first of five projected conferences on drug information was held in Princeton, New Jersey, 4–7 June 1967, and dealt with the drug information which members of the health professions and health services require in order to function efficiently. These conferences, organized by Frank Fremont-Smith, are part of the Program of the