Meeting Report

BIOPHYSICAL STUDIES OF CELL MEMBRANES

A REPORT OF THE BRITISH BIOPHYSICAL SOCIETY MEETING HELD IN BIRMINGHAM ON 15 AND 16 APRIL 1969

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1. Introduction

This well-attended meeting, organised for the Society by J.B.Finean, was primarily concerned with the physical chemistry of lipids and membranes. The first day, under J.B.Finean's Chairmanship, was devoted to lipids, lipid-water systems and the interactions of lipids and proteins. On the second day, the contributions reported studies made by optical and X-ray diffraction methods on a variety of biological membranes; V.Luzzati and Audrey M.Glauert were in the Chair for successive sessions.

2. The structure of lipid-water systems

The initial paper entitled "Polymorphism of Lipids" was presented by V.Luzzati, T.Gulik-Krzywicki, E.Rivas, A.Tardieu, F.Reiss-Husson and R.P.Rand (Centre de Génétique Moléculaire, C.N.R.S., France, and Brock University, St. Catherines, Canada). It surveyed X-ray diffraction investigations on the crystallographic behaviour of a wide variety of lipid-water systems, ranging from simple soaps to extracts of mitochondrial lipids. Although numerous different phases have been observed, depending upon the nature and concentration of lipid, certain common features have been found. Thus each phase contains a paraffin region, in which different species of lipid molecules tend to lose their individual identity, and a water region: the two regions being separated by an interface that is covered by the polar groups of the lipid. Within the paraffin region, various conformations of the hydrocarbon chains occur: fully ordered (e.g. in soap crystals), highly-disordered and liquid-like giving a diffuse X-ray diffraction band at 4.5 Å, and intermediate states having stiff or helically coiled paraffin chains that exhibit rotational disorder (e.g. bilayers of mitochondrial lipids exhibit a region of disorder in the middle of each layer that apparently facilitates the accommodation of paraffin chains of differing lengths). However, lipid-water systems display long-range organisation in one, two or three dimensions even though they may exhibit highly disordered short-range conformations within the paraffin region. The structural elements involved in the long-range order may be either lamellar or rod-like and, whenever a topological distinction may be drawn between "inner" and "outer" volumes, the paraffin chains may occur either inside (Type I) or outside (Type II) the structural elements. Small changes in temperature or chemical structure can readily produce changes in the lattice structures of lipid-water systems, demonstrating that lipid systems are capable of the allosteric behaviour to be expected of the constituents of biological membranes, and suggesting that phase changes within membranes may be of physiological and biochemical importance [1,2].

In a second contribution by this group (A.Tardieu, T.Gulik-Krzywicki, F.Reiss-Husson, V.Luzzati and R.P.Rand) the structures of some cubic phases observed in lipid-water systems were described in detail. Three types of cubic phase were discussed. For the first, which is exhibited by strontium soaps, deter-

gents and lecithin, it would appear that the most satisfactory structure is an arrangement of rod-like elements in which the rods are joined three-by-three into two three-dimensional networks that are mutually interwoven but are otherwise unconnected. In some cases the rods are filled by the paraffin chains, in others by the polar moieties [3]. A second type of cubic phase occurs in certain lipid-water systems containing an organic solvent, while a third has been observed in phosphatidyl ethanolamine-water preparations at low pH.

A complementary contribution from E.Junger and H.Reinauer (Institut für Biophysik und Elektronenmikroskopie and Institut für Physiologische Chemie, University of Dusseldorf, Germany) described studies of some liquid crystalline phases of lipid-water systems by electron microscopy. They employed the negative staining technique (2% sodium phosphotungstate) with four different lipid-water systems (cardiolipin, phosphatidyl ethanolamine, lecithin and lysolecithin) at different states of swelling. Lipid samples were suspended in excess of water and a sample was taken for microscopy immediately, followed by others at later times. The micrographs obtained revealed globular, rod-like and lamellar structures. Globular micelles arranged in a hexagonal array occurred in a phase of crystalline appearance observed with preparations of lysolecithin having a high water content. A hexagonal array of rods of indefinite length, with a centre-to-centre spacing of 70 Å, which was comparable to a Type I hexagonal lattice (paraffin chains inside the rods), was found with lysolecithin preparations (concentration 50 to 70%) at 37°C, while structures comparable to Type II hexagonal patterns were observed with preparations of phosphatidyl ethanolamine (fig. 1) and with the disodium salt of cardiolipin. Linear patterns were also seen that were thought to be due to hexagonal structures lying at a tilted angle to the electron beam (fig. 1). These findings provide complementary information on the structure of lipid-water systems to that obtained from X-ray diffraction and increase the validity of the negative staining method for lipid structures [4].

Work on multilayers of phospholipid was described in the next paper, which was given by Y.K.Levine, Anita I.Bailey and M.H.F.Wilkins (M.R.C. Biophysics Research Unit, King's College, London). Multilayers of phospholipids had been built up on solid surfaces by repeatedly dipping a teflon or wax surface through a monolayer that was near to its collapse pressure, and diffraction photographs were then obtained of the multilayers at different degrees of hydration. The lamellar spacing and Fourier syntheses indicated that the repeat unit in the layers is a bimolecular leaflet, and that the multilayer consists of stacked bimolecular leaflets that are separated by water and lie parallel to the solid surface. Orientated bimolecular leaflets were also prepared by evaporating a solution of egg lecithin or a lecithin-cholesterol mixture on to a solid surface. The diffraction band at 4.6 Å was oriented at right angles to the lamellar reflections; Fourier syntheses indicated that the terminal CH_3 groups of the hydrocarbon chains are located in the centre of the hydrocarbon region of each leaflet, and interdigitation of the hydrocarbon chains appeared not to occur.

3. Bilayers of lipid

R.J.Cherry (Molecular Biophysics Unit, Unilever Research Laboratory, Welwyn) next gave the first of three talks on optically-black, lipid bilayers. Black films, which were initially described by Mueller and Rudin and their collaborators [5] have attracted a great deal of attention in recent years from both physical chemists and biochemists since they provide one of the best currently available experimental models for biological membranes [6]. This paper described refined optical methods for the determination of the thickness of a lecithin bilayer. It was pointed out that the determination of bilayer thickness by electrical capacitance measurements has two disadvantages: the method depends on a knowledge of the dielectric constant of the film which cannot be measured directly and, since the capacitance is determined by the hydrocarbon layer of the film, the procedure cannot yield data on the polar regions of the lipid bilayer. Experiments on the optical properties (Brewster angle) of a black film prepared from egg lecithin indicated that such films are not optically isotropic and that they should be considered as having two refractive indices: n_3, the refractive index parallel to the optic axis, and n_1, the refractive index perpendicular to the optic axis. A value of 1.464 ± 0.004 was obtained for n_1. An independent determination of n_1

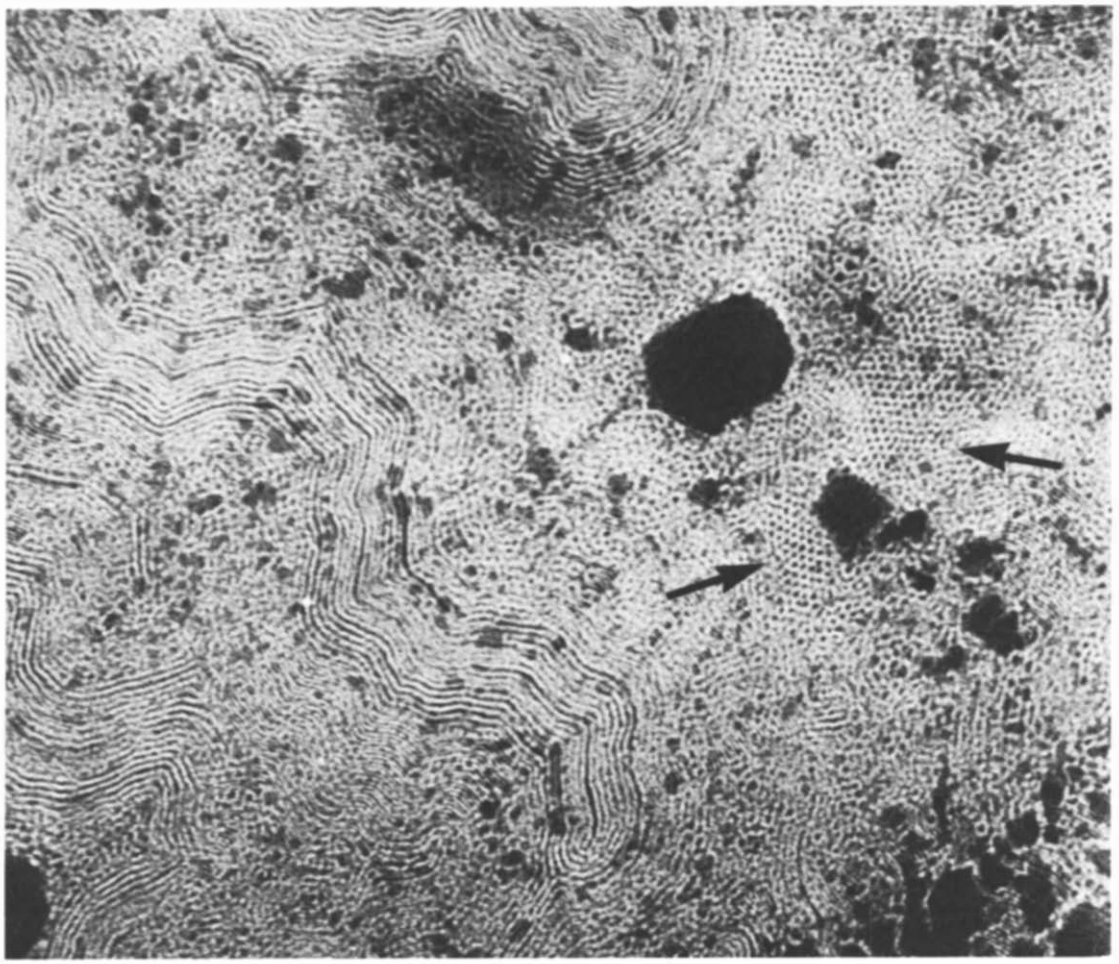

Fig. 1. An electron micrograph of a negatively-stained preparation (2% sodium phosphotungstate, pH 7) of phosphatidyl ethanolamine dispersed in water. Hexagonal arrays (arrows) of water cylinders separated by the lipid phase are observed that may be correlated with the hexagonal phase Type II observed by X-ray diffraction [12]. X 160,000. Reproduced by kind permission of Dr. E.Junger.

by reflectivity measurements gave a value of 1.454 ± 0.002, from which it was calculated that the total thickness of the film was 62 ± 2 Å: a value compatible with the polar groups being either parallel to the film or possibly in a fluctuating configuration, but not with their being perpendicular to the film.

The second paper on lipid bilayers was by D.Rosen and A.M.Sutton (Biophysics Laboratory, Chelsea College of Science & Technology), who have investigated the change of permeability of lipid bilayers brought about by ionising radiation because small changes in the permeability of natural membranes induced by radioisotopes might be of considerable biological importance. Bilayers were prepared from egg lecithin, and were irradiated at up to 600 rads/min. The electrical conductance, which was determined before, during and after irradiation, was highest during irradiation by a factor of from 4 to 12 times the basal level. This increase in conductance was dependent both on the dose rate, and on the nature of the cation in the aqueous medium – the effect for Ca^{++} being greater than $K^+ > Na^+$. Anions were without effect. Experiments with cations of differing sizes indicated that conduction occurred by

an ionic movement through holes of about 5–6 Å in diameter; pulse radiation experiments indicated that the half-life of such holes in the bilayer was approximately 15 milliseconds. It was tentatively suggested that the phenomenon may involve destruction of lipid molecules by the irradiation, or the creation of an ionised pathway between lipid molecules in the bilayer, or a heating effect that would disturb the membrane sufficiently to allow the passage of ions. This last suggestion was of particular interest to the writer since a local rise in temperature might facilitate a transition from a continuous bimolecular leaflet to a membrane containing a small proportion of globular micelles [2]. Such a membrane would be expected to exhibit an increased conductivity. Furthermore, if the chemical composition of the membrane is unchanged by irradiation, the micelles may be relatively unstable since the free energy of formation of spherical micelles from an uncharged phospholipid bilayer is about + 12,000 calories [7].

The final contribution on lipid bilayers was entitled "Recent Physical Studies of Lipid/Alamethicin Interactions" and it was by M.C.Phillips (Molecular Biophysics Unit, Unilever Research Laboratory, Welwyn). Alamethicin is a surface-active, cyclic, predominantly hydrophobic, molecule containing 19 amino acid residues, which complexes with cations under all conditions of pH; at neutral pH it is negatively-charged. At the air-water interface it exhibits a collapse pressure of 30 dynes/cm, and a minimum area per molecule of approx. 200 $Å^2$ that indicates tilting or folding of the molecule occurs at the surface. Alamethicin penetrates into lipid films, e.g. phosphatidyl serine, to form a mixed monolayer. The critical micelle concentration of alamethicin is 2.4 μM. In water, it forms aggregates (M.W. approx. 13,000) at neutral pH: in ethanolic solution it is present as a monomer. Treatment of black films with alamethicin results in a decrease in the resistance of the membrane, and the development of a voltage-dependent cationic conductance. At low applied voltages alamethicin may exist in monomer form and behave as a cation carrier within the bilayer, while at high voltages some molecules of alamethicin may aggregate to provide a trans-membrane pore. X-ray diffraction studies of lecithin-water systems revealed that an increase in the lamellar repeat distance, from 61 to 66 Å occurs on adding alamethicin, while nuclear magnetic resonance experiments indicate that alamethicin penetrates into the interior of the lipid layer and reduces the movement of the hydrocarbon chains.

4. Interactions of lipids and proteins

In a paper entitled "Interactions of Lipids and Proteins: an X-ray Diffraction Study" T.Gulik-Krzywicki, E.Shechter, V.Luzzati and M.Faure (Centre de Génétique Moléculaire, C.N.R.S., and Institut Pasteur, Paris) described interactions in several lipid-protein-water systems, including those of lysozyme, cytochrome and ribonuclease with phosphatidyl inositol, cardiolipins, phosphatidic acids and total mitochondrial lipids. Several different phases were observed, depending on the nature and the concentration of the components. The structure of most of the phases was lamellar (smectic), though some two- and three-dimensional structures were also seen. As a rule, there was no indication of tangential order in the lamellar phases. This result, which is somewhat surprising in view of the very high protein concentrations used, indicates the presence of a continuous lipid layer, and suggests that the outer boundaries of the protein molecules are somewhat blurred. Some of the lamellar phases appear to fulfil one of the requirements of the Danielli-Davson model, namely the presence of a lipid bilayer that is coated by protein molecules, which are bound to the lipid by polar bonds. With cytochrome *c* and phosphatidyl inositol, electrolytes dissociated the complex into protein and lipid; this complex exhibited a smectic type arrangement in which the paraffin chains were disordered, and the lipid lamellae were intercalated with layers of protein/water. Either one or two layers of densely-packed protein molecules could occur between the lipid layers. In other lamellar phases, the interactions of lipids and proteins involved hydrophobic contacts in addition to weak polar interactions. Thus although the lysosome-cardiolipin-water system at pH 6–7 has a smectic organisation with liquid paraffin chains, the presence of excess protein results in a reduction of the thickness of the lipid layer and some of the lipid hydrocarbon chains may penetrate into clefts of the protein contained in the aqueous layer. More than ten different phases were observed with the lysozyme-phosphatidyl inositol-

water system, and chemically specific interactions between the lipid and protein were thought to be responsible for this large number of different phases.

The first day ended with a paper by E.Shechter, T.Gulik-Krzywicki and V.Luzzati (Paris) on optical absorption and circular dichroism measurements made on the lipid-protein complexes discussed in the immediately preceding paper. Isotropic, transparent, non-turbid preparations were used for the optical studies in which the observed absorption was due only to the protein. In most cases, perturbations were noted in the regions of aromatic and peptidic absorption, as well as in the Soret region (in the system containing cytochrome *c*), which pointed to the existence of well-defined conformational states and which were consistent with the results of the corresponding X-ray diffraction analyses.

5. Studies on membranes by nuclear magnetic resonance and electron spin resonance

The second day began with a paper on "N.M.R. Spectroscopic Studies of Membranes and Lipoproteins", given by V.B.Kamat in the absence of D.Chapman (Molecular Biophysics Unit, Unilever Research Laboratory, Welwyn). Membranes investigated by this group include those from erythrocytes, myelin and from *Halobacterium halobium*; both high and low density lipoproteins were also studied. Model systems had also been investigated and comparisons made between the behaviour of the model systems and natural membranes. It was observed that the organisation of the lipids in myelin and in erythrocyte membranes appeared to be lamellar. With the red cell membrane there were indications of interactions between non-polar amino acids and the hydrocarbon chains of the lipid, but this was not found in myelin where cholesterol seems to regulate hydrocarbon mobility. Treatment of membranes with lysolecithin appeared to yield a complex type of micelle containing lysolecithin, membrane lipids and membrane proteins (cf. [8]).

Experimental applications of "spin labels" in studies on natural membranes were discussed by J.C.Metcalfe (M.R.C. Molecular Pharmacology Research Unit, Cambridge), in "Magnetic Resonance Studies of Cyto-Membranes". A "spin label" is a molecule containing a chemical group that has an electron spin resonance (ESR) spectrum [9]. The unpaired electron of the nitroxide group ($>N \rightarrow O$) has a simple ESR spectrum, and this group can be introduced into a wide range of molecules including analogues of membrane lipids. When labelled molecules are inserted into natural membranes, they can be used to provide information on the dielectric nature of the micro-environment of the label, and on the orientation and rotational movement of the label in the membrane. Furthermore perturbations in the structure of membranes caused by exogenous agents, such as anaesthetics, can be investigated by studying the corresponding changes in the environment of the spin label. As an example, treatment of erythrocyte membranes with concentrations of benzyl alcohol in excess of 100 mM (sufficient to cause lysis) induced a spin label analogue of cholesterol to bind so strongly to membrane proteins that a new spectral component could be distinguished. Since one molecule of spin label, e.g. TEMPO (2,2,6,6-tetramethyliperidine-1-oxyl), to 100 molecules of membrane lipids is normally used in such experiments, it was suggested during the discussion of this paper that changes in membrane structure might be caused by the presence of the spin label. It was pointed out, however, that the concentrations of spin labels used had no measurable effect on the hypotonic lysis of red blood cells.

6. Optical properties of membranes

D.F.H.Wallach (Biochemical Research Laboratory, Massachusetts General Hospital, USA) contributed a paper entitled "Optical Activity of Cellular Membranes". Studies of the plasma membranes of human erythrocytes and Ehrlich ascites carcinoma cells by circular dichroism and optical rotatory dispersion techniques [10] were found to provide evidence of the presence of short helices of protein, and of the location of some peptide chromophores in a highly polarizable, apolar environment. Treatment of plasma membranes with lysolecithin or with phospholipase A results in changes in the structure of membrane proteins (cf. the paper by Kamat discussed above), which indicate that the structures of membrane proteins normally depend upon lipid-protein interactions and/or lipid-sensitive, protein-protein interactions. Interest-

ing studies on the infrared spectra of membranes revealed no evidence of a β-configuration in the proteins of red cell membranes, but the inner membranes of mitochondria did show some indication of β-structure. Although the findings with mitochondria may have resulted from the preparative freeze-drying of the specimens, it was thought that the observed results were probably valid because the degree of β-conformation was distinctly greater in comparable preparations of membranes isolated in Respiratory State 4. Membranes from mitochondria in State 3 showed no evidence of the β-conformation.

7. X-ray diffraction studies of biological membranes

The final group of papers was concerned with studies made by X-ray diffraction studies on a wide variety of preparations of biological membranes. The first of these, on "X-ray Structure Research on the Photosynthetic Membrane", was by W.Kreutz (Technische Universität Berlin, Max-Volmer-Institut für Physikalische Chemie), described investigations both on green leaves of plants and, for comparison, on white leaves obtained from mutant species. It was found that the photosynthetic membrane has an asymmetric construction comprising a protein layer, a pigment layer and a lipid layer. A highly complex model for the photosynthetic membrane was described that had been developed by combining the results of X-ray diffraction techniques with data obtained from other physical studies. In this model, the protein layer is built up of small two-dimensional crystallites that form linear aggregates, and cytochromes are located between the rows of crystallites. The porphyrin rings of chlorophyll are arranged in groups of dimers in the pigment layer. Finally, lipids form a monolayer along the inner surface of the membrane, with the hydrophilic groups being at the surface of the membrane and the hydrocarbon chains being associated with the chlorophyll.

"X-ray Diffraction from Membranes of *Mycoplasma laidlawii*: Thermal Phase Transitions" was the title of a progress report from D.Engleman (Department of Biophysics, King's College, London). Membrane preparations from this organism contain two phospholipids and two glycolipids, and membranes can be obtained in which the fatty acids are either fully saturated or unsaturated depending on the growth medium. X-ray diffraction studies of fully-hydrated membrane preparations from cells grown in the presence of palmitate revealed the existence of a thermal transition, occurring over the range 35–42°C. On reducing the temperature over this range, a diffraction band at 4.5 Å diminishes while a relatively sharp reflection appears at 4.2 Å. This transition occurs both with membranes containing saturated lipids and with those containing unsaturated lipids. It is also found in whole cell preparations; the organism does not grow well below the transition temperature. One possible interpretation of the observed phase change is that a large number of "icebergs" of relatively highly-ordered paraffin chains are formed in the membrane on reducing the temperature.

The paper by R.E.Burge (Department of Physics, Queen Elizabeth College, London), dealt with combined X-ray diffraction and electron microscope studies on isolated cell walls and some wall components of the Gram negative bacteria *P. vulgaris* and *E. coli*. The interpretation of the results was discussed in terms of the electron density distribution through the walls; some remarks were also made on the relationship between the resolution of electron density distributions derived with the aid of electron micrographs and the resolution of the electron micrographs. It was suggested that the electron microscope can only be of value with the first three or four orders in phase determinations for diffraction studies. Considerable discussion subsequently developed in this connection; it was emphasized that densities observed in electron micrographs of fixed and stained sections of membranes need not necessarily be related to the electron density distributions of the natural components of membranes that are observed in X-ray diffraction studies on unfixed preparations.

The final afternoon session started with three consecutive contributions by J.B.Finean and his colleagues (Department of Biochemistry, University of Birmingham). Each paper was concerned with X-ray diffraction studies on "condensed" preparations of biological membranes. These condensed preparations had been dehydrated to produce stacked arrangements of membranes suitable for X-ray diffraction studies, and investigations were made to determine whether structural modifications are caused by the drying process before suitably condensed preparations

are obtained. In "Studies of Condensed Membrane Preparations" by J.B.Finean, S.Knutton, A.R.Limbrick and R.Coleman, it was concluded that modifications occurred in some instances but not in others. With a variety of membrane preparations, well-defined diffraction patterns normally became apparent when the water content of the membrane sample had been reduced to 30–50% with respect to final dry weight. These initial patterns frequently featured diffraction orders of lamellar repeats, and the diffraction intensities of the lamellar patterns had been interpreted in terms of the electron density profiles through the layers of membranes. Below 20–30% water content, diffraction changes attributable to modifications of membrane structure have been observed. These changes are considered to represent a partial breakdown of lipoprotein structure that results in the separation of an independent lipid phase or phases; this interpretation was reached from diffraction data on membrane preparations that were modified by treatment with enzymes before applying the condensation procedure. For example, a condensed preparation (containing only 5% water) of membranes from red cell ghosts behaved as a multi-phase system at room temperature and exhibited repeating units at 83, 53 and 43 Å. The spacings observed at 53 Å and 43 Å are considered to be due to lipid since the 53 Å repeat is progressively shifted to ~ 40 Å on raising the temperature to 100°C, while that at 83 Å is probably lipoprotein since this is unaffected by the temperature changes but can be modified by treatment with trypsin. The reflections at 53 and 43 Å are removed by treatment with phospholipase C, but the 83 Å lipoprotein peak persists.

In the paper given by A.R.Limbrick and J.B.Finean, entitled "X-ray Diffraction Studies on the Brush Borders of Guinea Pig Intestinal Epithelial Cells", diffraction bands (38, 43, 60, 75 and 100 Å) that were obtained from the hydrated membranes were orders of a lamellar repeating pattern of approximately 300 Å that comprised two membrane units. (When the water content was reduced below about 25%, reflections were observed at 43, 60, 73 and 245 Å.) No diffraction bands were recorded that would be indicative of an arrangement of subunits in the plane of the membrane. By referring to electron micrographs of these condensed membrane preparations, an electron density profile of the brush border membrane had been evaluated; it was considered that the data obtained fitted quite well with the bimolecular leaflet structure for membranes.

Further data on the drying of red cell preparations was given by S.Knutton, J.B.Finean and A.R.Limbrick in "Structural Studies of Erythrocyte Membrane Preparations". Rat and human red cell membranes were prepared by a procedure based on that of Dodge et al. [11], in which the residual haemoglobin is dependent on the ionic strength of the preparative buffer used. Hydrated membrane preparations that were made using ionic strengths below 3 mM, and which contained some residual haemoglobin, yielded diffraction bands at 37, 56 and 112 Å thought to result from a single membrane repeat of 112 Å. By contrast, preparations made at ionic strengths greater than 3 mM but less than 10 mM, which were relatively haemoglobin-free, behaved as if a separation of different phases occurred very early in the drying procedure, i.e. before a good lamellar pattern appeared. Glutaraldehyde partially stabilized these preparations against changes due to drying, while osmium tetroxide prevented the apparent separation of independent phases. Residual haemoglobin was considered to be responsible for the relative stability of preparations made with buffers of ionic strengths below 3 mM – an interpretation that was supported by the finding that added haemoglobin stabilized preparations made using buffers between 3 mM and 10 mM.

A.E.Blaurock and M.H.F.Wilkins (M.R.C. Biophysics Research Unit, King's College, London) in "X-ray Diffraction Studies on some Natural Membranes" reported the results of experiments on frog sciatic nerve and retina, and on dispersions of rat cerebral cortex vesicles and red blood cell preparations. Diffraction was regularly observed at 10.5 Å (12.5 Å for myelin) and at 4.7 Å. With retina and myelin, the 10.5–12 Å reflections were oriented and consistent with a regular repeat parallel to the membrane surfaces. The large spacing of nerve myelin was found to increase in each of several sucrose solutions ranging from pure water to 1.8 M sucrose. This offered an opportunity to find the average electron density of the myelin membrane, the modulus of the Fourier transform of the membrane, and the phases of the transform. The average density of the membrane was observed to be near that of 0.25 M sucrose. Models of the electron density profile of the membrane were

tested in relation to the parameters of membrane dimensions and absolute electron densities. The results obtained were considered to confirm the bimolecular leaflet model for nerve myelin: the best-fitting model containing a low-density layer near the centre of the membrane that was just under 20 Å wide with an electron density of 0.25 e/Å^3. In the discussion it was pointed out that, although the electron density in the centre of the bilayer cannot be due primarily to the presence of protein, the possibility remains that certain protein side chains, of relatively low-density, may occur in the interior of the membrane.

A related paper by M.H.F.Wilkins and A.E.Blaurock, "X-ray Diffraction Studies on Membrane Suspensions", was of particular interest in that it described a technique that may become of considerable value in biochemical investigations. Previous X-ray diffraction studies of membranes have been largely confined to specimens in which membranes are stacked in a parallel manner and regularly spaced (e.g. as in nerve myelin) thus giving sharp Bragg reflections. The studies on partially-dried, condensed preparations of membranes studied by Finean and his collaborators represent one way of attempting to overcome this limitation. Wilkins and Blaurock consider that it does not seem to have been adequately realised that suspensions of disorientated randomly-arranged membrane fragments can also be used to give useful diffraction data. This method clearly has the advantage that it can be applied to a wide variety of different types of membranes, and it also avoids the possible hazards of the effects of drying on membranes, although careful experimental procedures are required with suspensions of membranes since the diffraction is continuous. The method has been tested on suspensions of lecithin and of various membranes. A major peak in the continuous Fourier transform has been observed at about 50 Å with membrane preparations, while dispersions of egg lecithin gave a continuous transform in which the main peak was located at a spacing of about 40 Å.

"The Effects of Protective Agents on Cell Membrane Structure" was the title of a short contribution by R.T.Joy, G.K.Rennie and M.Ellis (Department of Zoology, University of Nottingham and School of Biological Sciences, University of East Anglia), who reported experiments on the effects of glycerol and dimethyl sulphoxide on preparations of nerve myelin subjected to freezing and thawing. X-ray diffraction data indicated that, although glycerol did not give protection, dimethyl sulphoxide effectively stabilized nerve myelin in a slightly modified form during this treatment.

8. Postscript

One of the valuable aspects of the meeting was the participation of contributors from France, Germany and the U.S.A., who gave the meeting an international air. Wallach, an overseas contributor, commented that he considered that the use of optical measurements on biological membranes lies in following functional changes rather than in determining membrane structure, in view of the difficulties involved in developing a quantitative method for establishing the absolute structures or configurations of the components of membranes. As biochemists, we can look forward to the more widespread use of physical techniques in studies on functional changes in membranes, and in the light of this meeting it would seem that optical methods, "spin labels", and X-ray diffraction on suspensions of membranes may be of particular application in this field.

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