PROCEEDINGS OF THE BIOCHEMICAL SOCIETY

The **512th Meeting** *of the Society was held at the Medical Research Council National Institute for Medical Research, Mill Hill, London N.W.7, on Friday,* 19 *February* 1971, *when the following papers were presented:*

COLLOQUIUM ON 'THE PROTEINS OF MEMBRANES'

Possible Modes of Organization of Protein Molecules in Membranes

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There is convincing evidence from diverse sources that hydrocarbon chains of lipids of both natural and artificial membranes exist in a liquid crystalline state rather than as an immobile solid phase (Chapman, 1968). For example, a calorimetric study of membranes of *Mycoplasma* grown on media of varying fatty acid composition showed broad reversible phase transitions at temperatures that increased with increasing saturation of the lipid (Steim, Tourtelotte, Reinert, McElhaney & Rader, 1969). The temperature and the enthalpy of the transitions were almost the same whether whole membranes or bilayers of the extracted lipids were examined. This suggested that most of the lipid molecules in the membranes were organized in a bilayer and interacted only to a minor extent with the protein molecules. Attempts to grow the organism at temperatures below the melting temperature of the membrane lipid resulted in lysis; only fluid membranes were viable. On the other hand there is much evidence suggesting that, although some proteins can be removed from the membrane by hydrophilic reagents, most are held by hydrophobic interactions and can be removed only be suitable detergents. It is not difficult to resolve the apparent contradiction between the large amounts of protein held hydrophobically in membranes with the relatively small proportion of lipid apparently involved in direct interaction with it. The protein molecules are about 100-fold larger than the lipid molecules and many project outside the lipid bilayer, so, provided that their tertiary structure is compact, they need immobilize only a fraction of their weight of lipid. Lipid–protein interaction would be still further decreased if protein subunits formed oligomeric structures in the plane of the membrane, though at present there is little evidence for this. This leads us to a picture of a major class of membrane proteins, floating as single molecules or oligomers in a pool of lipid. Like the lipid molecules themselves they will be anchored firmly in the membrane, but at the same time they will be free to rotate and diffuse in the plane of the membrane, provided they are not restricted by polar interactions.

Although this picture is undoubtedly oversimplified it has the merit of consistency with the properties of the membrane lipids, and it suggests further questions about the size of the diffusing units and possible restrictions on their motion. Apart from its emphasis on the mobility of the protein it is similar to that put forward by Glaser, Simpkins, Singer, Sheetz & Chan (1970) to explain their results with phospholipase C-treated membranes. It is worth recapitulating this evidence, since related work on endoplasmic reticulum (Finean & Martonosi, 1965; Trump, Duttera, Byrne & Arstila, 1970), mitochondria (Ottolenghi & Bowman, 1970) and erythrocyte 'ghosts' (Coleman, Finean, Knotton & Limbrick, 1970; Glaser *et al.* 1970) has led to common findings. It was generally observed that the phospholipase C released about 70% of the phosphorylcholine from the phosphatidylcholine of the membrane and that the resultant diglyceride formed membrane-bound droplets, probably retained within the residual bilayer (Trump *et al.* 1970). This parallels a suggested course for the biosynthesis of triglyceride by membrane-bound enzymes (Danielli, 1967). The enzyme neither broke down the macroscopic structure of the membrane nor affected the thickness of the residual bilayer, the area of which was approximately halved. The proteins remained bound to the membrane, and their conformation measured by circular dichroism was not significantly changed. Some enzyme activities were lost but could be restored by added phospholipid. These results showed that a large proportion of the lipid could be removed from the bilayer with only minor effect on the tertiary structure of the proteins, and suggested a fair measure of independence in the organization of these two major components. Both this conclusion and the observation of a decrease in the surface area of the membrane without loss of protein suggests that there is no rigid network of structural protein

in these membranes and that the molecules are free to come closer together as the diglyceride coalesces into droplets. It is informative to apply this hypothesis to the vesicles of the sarcoplasmic reticulum, in which about 80% of the protein appears in negative stain as an irregular array of short rods of diameter 35 Å, projecting 50 Å from the surface (Greaser, Cassens, Hoeckstra & Briskey, 1969). It may be calculated that the observed loss of 70% of the phosphatidylcholine and half the area (Finean & Martonosi, 1965) would just bring these subunits into contact if they are embedded in the outer half of the lipid bilayer, as suggested by results of freeze-etching (Bertaud, Rayns & Simpson, 1970). Examination of freeze-etched specimens of influenza virus has also suggested a structure in which the projecting protein antigens are embedded in the outer half of the lipid bilayer (Bächi, Gerhard, Lindenmann & Mühlethaler, 1969).

A further striking example of the fluidity of membrane structure has appeared in a paper on the surface antigens of hybrid cells (Frye & Edidin, 1970). Cell lines of mouse and human origin were fused and the hybrids were treated with fluorescent antibodies (red for human and green for mouse antigens). At 10 min after fusion most hybrids showed distinct red and green halves, but within 40 min the colours were completely mixed. The process was not affected by inhibitors of protein synthesis or oxidative phosphorylation, but it ceased dramatically at temperatures below 15°C. It was calculated that this high rate of diffusion was consistent with the movement of molecules of diameter 200 Å in a medium of the viscosity of castor oil.

These arguments suggest that the organization of protein molecules in the plane of the lipid bilayer is governed relatively loosely by hydrophobic interactions. The organization in a perpendicular direction is likely to be dominated by more specific protein–protein interactions in an aqueous environment. A combination of electron microscopy with chemical or immunological labelling methods should enable one to decide where particular proteins are located. They may for example be on the inner or the outer surface of a vesicle, confined within the bilayer, or they may penetrate from one aqueous surface to the other. So far, negative staining has shown many examples of proteins that project from one surface or another of a membrane, while the ready cleavage of the bilayer along the central hydrophobic plane in freeze-fractured preparations suggests that proteins that pass through the membrane are relatively rare (da Silva & Branton, 1970).

Whatever the nature of the interactions between proteins on the membrane and proteins in the membrane, it can be said that the observations discussed in this brief survey are inconsistent both with any major role for so called membrane structural proteins and with any network of proteins of the type visualized by Changeux (1969) as allosteric mediators of responses in excitable membranes. If there is any substance in this theory then excitable membranes must be built on rather different principles.

Bächi, T., Gerhard, W., Lindenmann, J. & Mühlethaler, K. (1969). *J. Virol.* **4**, 769.

Bertaud, W. S., Rayns, D. G. & Simpson, F. O. (1970). *J. Cell Sci.* **6**, 537.

Changeux, J. P. (1969). In *Nobel Symp.* no. 11, p. 235. Ed. by Engstrom, A. & Strandberg, B. New York and London: John Wiley and Sons.

Chapman, D. (1968). *Biological Membranes*, p. 125. New York and London: Academic Press.

Coleman, R., Finean, J. B., Knotton, S. & Limbrick, A. R. (1970). *Biochim. biophys. Acta*, **219**, 81.

Danielli, J. F. (1967). In *Formation and Fate of Cell Organelles*, p. 239. Ed. by Warren, K. B. New York: Academic Press Inc.

da Silva, P. P. & Branton, D. (1970). *J. Cell Biol.* **45**, 598.

Finean, J. B. & Martonosi, A. (1965). *Biochim. biophys. Acta*, **98**, 547.

Frye, L. D. & Edidin, M. (1970). *J. Cell Sci.* **7**, 319.

Glaser, M., Simpkins, H., Singer, S. J., Sheetz, M. & Chan, S. I. (1970). *Proc. natn. Acad. Sci. U.S.A.* **65**, 721.

Greaser, M. L., Cassens, R. G., Hoeckstra, W. G. & Briskey, E. J. (1969). *J. cell. Physiol.* **74**, 37.

Ottolenghi, A. C. & Bowman, M. H. (1970). *J. Membrane Biol.* **2**, 180.

Steim, J. M., Tourtelotte, M. E., Reinert, J. C., McElhaney, R. N. & Rader, R. L. (1969). *Proc. natn. Acad. Sci. U.S.A.* **63**, 104.

Trump, B. F., Duttera, S. M., Byrne, W. L. & Arstila, A. V. (1970). *Proc. natn. Acad. Sci. U.S.A.* **66**, 433.

The Proteins Released from Intact Erythrocyte 'Ghosts' at low Ionic Strength

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The methods currently in use for the preparation of haemoglobin-free mammalian erythrocyte 'ghosts' by different research groups vary considerably. This may explain to some extent the overall lack of agreement and co-ordination that exists in the field of erythrocyte membrane proteins. Even though haemoglobin-free erythrocyte 'ghosts' can reasonably be considered as a homogeneous membrane preparation, there can be little doubt that every research group is dealing with a slightly different product from which their protein extracts are made. The preparation method employed by the present author closely follows the procedure developed by Dodge, Mitchell & Hanahan