Proteins and Cell Membranes

from a Correspondent

AN international symposium on interactions of proteins and ligands and their relevance to membrane phenomena, which was organized by the International Union of Biochemistry in Buenos Aires on November 20 to 22, brought together researchers interested in different levels of biological organization—isolated proteins, particulate systems and living cells—in the hope that they would clarify the complex phenomena occurring *in vivo*.

The structural models best suited to explain the functional properties of the cell membrane were discussed by S. J. Singer (University of California). The idea of the cell membrane as a lipid bilayer interrupted or deformed in places by the presence of specific proteins is well known. Singer has introduced the useful concepts of intrinsic proteins, associated with the membrane in a permanent fashion, and peripheral proteins with a more temporary, weaker association. Is there an end-to-end rotation of proteins in the membrane—that is, rotation about an axis parallel to the membrane—as has long been assumed, additional to side-to-side rotation and lateral diffusion? Singer provided proof for the second notion and also discussed the great difficulties associated with the first. Singer's own model amounts to the recognition that rotational or translational motion inside the membrane must be clearly distinguished and has an altogether different physical basis from the diffusion into and out of the membrane.

Several contributors discussed intrinsic membrane proteins; for example, R. Zand (University of Michigan) reported studies on the basic protein of myelin, but most speakers dealt with specific protein receptors. It now seems that these receptors are indeed proteins which are strongly associated with lipid material to the point that they are soluble in water only in the presence of detergents. The study of J. P. Changeux (Institut Pasteur, Paris) of the isolation of the cholinergic receptors from *Electroplax* is a model of what can be accomplished in this area by initial study at the more complex biological level, followed by a systematic simplification of the system and isolation of the active component.

P. Cuatrecasas (Johns Hopkins University) described the isolation of the insulin receptor from adipose tissue cells, a piece of work that must rank with the classical success stories of biochemistry. It is now known not only that the receptor is a protein with the expected solubility and binding characteristics but also that there are no more than ten in each adipose cell, that the insulin molecule need not penetrate beyond the membrane to exercise its effect, but that probably this is mediated through an inhibition of adenyl cyclase directly or indirectly. The insulin molecule, in fact, seems to do no more than carry information much in the same way as does a telegram. It is becoming evident that receptor molecules are just that—specific binding agents capable of "signalling" to neighbouring structures, by some intrinsic property of their own; and that the binding site is free, or occupied. One can venture the guess that when the numbers of receptors per cell are as few as in this case, the following stage must be an amplifying one, which can hardly be anything other than the regulation of an enzyme, the product of which is itself regulatory.

E. G. Lapetina and R. H. Michell (University of Buenos Aires and University of Birmingham) interjected here a most interesting possibility—that cyclic AMP may not be the only result of this stage of amplification. It seems that another cyclic phosphate, 1,2 inositol monophosphate (CIP), is formed upon degradation of phosphatidyl inositol and is itself converted into the linear monoester by a specific phosphatase.

The investigations of E. De Robertis and his colleagues—S. Fiszer, M. Parisi, C. Vasquez, E. Rivas and others—in the University of Buenos Aires, are of great importance to many fields. They have developed a remarkably simple method of isolating receptors by chloroform–methanol extraction that circumvents the lengthy, if more orthodox, procedures of lipoprotein extraction by detergents and fractionation. The overall result of this work—the identification of Folch's proteolipids with definite neuroreceptor properties—must be counted as a most important structure–function correlation in membrane research. This work has received the criticism that the experiments were conducted in an "unphysiological medium", such as in chloroform instead of in water. The preservation of clear biological specificity, however, demonstrated by the binding of bungarotoxin by the acetylcholine proteolipid receptor in chloroform, finally disposes of this objection. Moreover, the finding of specific, binding activity of proteolipid fractions for noradrenaline, and more recently for glutamic and aminobutyric acids, seems convincing.

M. Sonenberg (Sloan-Kettering Institute, New York) described his studies on the interactions of red-cell ghosts with growth hormone. The finding of detectable spectroscopic and enzymatic changes on the addition of amounts of growth hormone no larger than 100 molecules per cell must be, if confirmed, one of the most interesting examples of sensitivity of a biological system that is not actually metabolizing, to an added molecule.

Of the proteins intrinsic to the membrane none has attracted more attention than the Na, K-activated ATPase since its discovery by Skou a few years ago. The convenient purification methods developed by L. Hokin (University of Wisconsin) starting from the salt glands of *Squalus acanthias* promise to open a new chapter on the investigation of the physical properties of the isolated enzyme. Hokin described preliminary experiments which indicate what may be achieved by the combined use of physical methods and fluorescent ouabain derivatives. The properties of this enzyme suggest that it must be the main molecular instrument of membrane ionophoresis. R. L. Post (Vanderbilt University) discussed a series of experiments which demonstrate that the system can be described by postulating only two forms of enzyme. In these, the enzyme is phosphorylated in the same place by either ATP or inorganic phosphate, according to the prevalence of Na or K binding respectively.

The observations of Post on the isolated ATPase provide experimental data about a system in which chemical and osmotic energies are interconverted. Although the strong coupling necessary for the interconversion derives from the existence of a contribution of osmotic energy to the chemical potential, many aspects are not yet well understood. G. Weber (University of Illinois) suggested that through the interaction of bound ligands proteins may perform the addition of chemical and osmotic free energies.

D. Sabatini (New York University) described the integration of functions by which proteins are secreted in the endoplasmic reticulum as their synthesis proceeds. Here can be seen a high degree of complexity because the system involves the activities of ribosomes and mRNA as well as specific associations of these with the membrane of the endoplasmic reticulum. Yet a simple, almost trivial, explanation of these complexities in terms of known entities endowed with well defined properties is now emerging. Only fifteen years ago these entities were ill defined; their functions, and even their names, were unknown; now, the secretory function may be resolved into simple, well understood steps, which can be handled experimentally and conceptually without undue difficulty.