inability to get together and bring a measure of cooperation and uniformity into the business. One example of this failure is the demise in 1962 of the Joint Blood Council, which was set up in 1955 for the purpose of coordinating the goals and activities of the Red Cross, the AABB, the American Hospital Association, the American Medical Association, and the American Society of Clinical Pathologists.

Nonetheless, many observers believe that things are improving on the blood banking scene. For example, a court in Billings, Montana, recently set a precedent by holding Blood Services of Arizona, the country's largest nonprofit community blood banking network, negligent for having supplied a unit of blood (from a paid donor) to a patient who later came down with hepatitis. In California, Blue Shield has decided to reimburse patients only for blood processing and transfusion costs. The 10-year-old council of Community Blood Centers, a group of AABB members who were originally regarded as a mercenary lot, recently came out with a statement calling for the encouragement of volunteer donors, and the elimination of blood credits and replacement fees. The Red Cross, in its first comprehensive statement on national blood policy, on 22 February put itself on record as being for nationwide voluntarism and uniform medical, technical, and administrative standards.

Despite these encouraging signs, it is unlikely that the blood banking complex will be able to significantly increase coordination and efficiency without a prod from the federal government. A second article will discuss new initiatives in Congress and the executive branch, and what people in the blood business think should happen next.

—CONSTANCE HOLDEN

RESEARCH NEWS

Cell Membranes: A New Look at How They Work

Numerous biochemical events take place on or in the plasma membrane of a cell. Once presumed to be a relatively static entity whose principal role was the simple delineation of cellular boundaries, the plasma membrane has proved to be a dynamic and functionally complex structure that performs a wide range of physiologic tasks. Central to virtually all biological processes, the plasma membrane commands the attention of scores of investigators who are impressed and encouraged by the gains made in membrane analysis during the last decade. Nevertheless, there is no generally accepted explanation of biological membrane structure that provides a fundamental understanding of how membranes carry out their diverse functions. Membranes may be to the 1970's and 1980's what nucleic acids were to the 1950's and 1960's.

The apparent current enthusiasm for membrane studies is the outcome of work that has taken place within the last few years during which investigators have made use of innovations in instrumentation and technique to resolve at least one debate that has dominated membrane research for half a century and to raise new questions that might yield to sophisticated probes. The debate that has been settled involves the issue of the structure of lipids within membranes. The new questions concern membrane proteins.

Today, the picture of membranes that is emerging is one of a dynamic structure approximately 80 angstroms thick with a lipid core. Its surface may be a mosaic of patches of lipid, areas of protein, and carbohydrate sites where the sugar end of a glycoprotein sits on the outer membrane. (As far as is known, the carbohydrates in membranes occur only as glycoproteins or glycolipids and never as free molecules.) At points, proteins and glycoproteins penetrate the lipid core. A variety of techniques, including electron spin resonance, x-ray diffraction, immunofluorescence, and photodichroism suggest that within, things are fluid. Says Daniel Branton of the University of California at Berkeley, "We're beginning to imagine a sea of lipid in which other molecules swim. We're recognizing a great mobility of membrane components undergoing continuing reorganization." This is quite unlike the more subdued picture of membranes that used to prevail.

The classic model of membrane structure is of a lipid bilayer or biomolecular leaflet, the Danielli-Davson model proposed in the early 1930's. According to this model, the backbone of the membrane is formed of lipids with hydrophobic tails apposing each other within the bilayer and with hydrophilic polar heads pointing outward. Globular proteins were postulated to cover the lipids in layers, thereby making the membrane a protein-lipid sandwich. While generally accepted as correct, the lipid bilayer model had its limitations, the most serious being, in the opinion of many investigators, its failure to account for the striking functional diversity of various types of membranes; hence, model building continued, and new models were proposed and debated as experimental evidence accumulated. Models were presented that offered twists of one kind or another (one reversed the protein-lipid-protein assembly of the Danielli design and suggested that lipids formed the outer layers) and, in 1957, the unit membrane hypothesis captured widespread attention and support.

The unit membrane, as proposed by J. David Robertson of Duke University, retained the biomolecular leaflet of the Danielli model but stated that the protein existed not in globular configurations, either as alpha helices or random coils, but as slightly flattened molecules spread across the lipid bilayer in uninterrupted sheets. Further, it was held that the unit membrane structure was characteristic of all types of membranes, including those of intracellular organelles, as well as of cell surfaces. Experimental evidence for this concept rested heavily on data obtained from electron microscopy and x-ray diffraction of myelin.

Viewed under the electron microscope, myelin exhibits a characteristic railroad-track image, two major dense lines with lighter intraperiod lines interpreted as protein covering a continuous lipid core. X-ray diffraction patterns added to the picture. A mathematical synthesis of the patterns made by x-rays that were diffracted off a specimen of

myelin pointed to prominent repeating units consistent with the model. However, as is the case with virtually all of the techniques that are used in membrane analysis, electron microscopy and x-ray diffraction have their limitations —the former because of changes in natural membrane structure that may result from preparative methods, the latter because it is most suited to examination of molecules that can be crystallized (membranes cannot) or to examination of those that occur naturally with a comparatively ordered structure.

Herein lies both the strength and weakness of x-ray diffraction data from myelin. This membrane, which forms a sheath around nerves and functions primarily as an insulator, has the advantage of being an ordered structure and the disadvantage of being atypical of most plasma membranes. By composition, myelin is at least 75 percent lipid in contrast with red cell membranes, which usually are about 50 percent protein and 50 percent lipid and which have a range of physiologic functions that would be impossible for myelin. (At the other end of the scale are bacterial and mitochondrial membranes which are even more active than plasma membranes, and are the site of oxidative phosphorylation and nucleic acid synthesis; the composition of membranes is about 75 percent protein.)

The question of the representative nature of myelin was thus raised by many scientists early in the 1960's, and the unit membrane concept which had won acceptance faced reevaluation. Also contributing to the feeling that it was time for a reassessment was the fact that a body of new data, much of it derived from the application of optical systems to analysis of membrane proteins, was favoring the school that believed membrane proteins to be primarily globular rather than in extended beta configuration.

Optical techniques can be applied to membranes under conditions in which they remain enzymatically functional, thereby offering an advantage in structure-function studies. At the same time, however, these methods are subject to a high incidence of artifacts that complicate experiments and the interpretation of data. Optical rotary dispersion and circular dichroism (CD) are among the biophysical techniques that are now being used to analyze proteins in membranes. In the latter method, circularly polarized light is focused on a membrane specimen in suspension. Proteins yield characteristic CD spectra according to their conformation; for example, certain bands of the spectrum are a mark of an alpha helix. Similarly, beta protein structures show typical CD patterns.

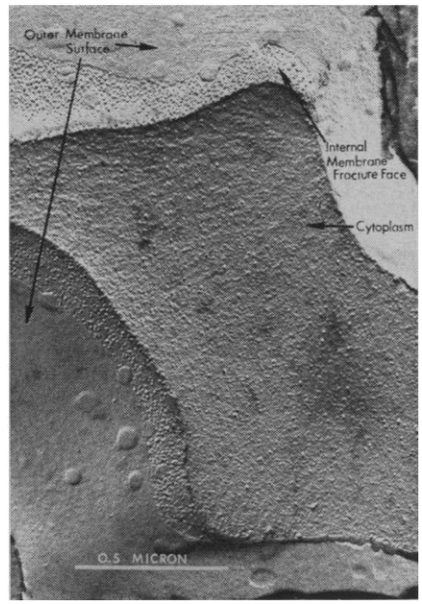

Fig. 1. Freeze-etching of the membrane of sheep red blood cells. [R. G. Kirk, Duke University]

Most investigators agree that a significant portion of membrane proteins are alpha helices. Dan W. Urry of the University of Alabama Medical Center at Birmingham believes, "contrary to previous reports," that comparative CD studies of different membrane systems go so far as to support the idea that the amount of alpha helix protein present can be correlated with membrane function. He examined mitochondrial membranes, red blood cell membranes, and membranes from sarcotubular vesicles and axons and reported recently, "There are marked differences in the CD patterns of biological membranes which correlate with membrane function." The first two membrane systems, with extensive functional capacities, show a higher proportion of alpha helices than the latter two in which proteins appear to be present in more flattened beta configuration.

Application of a technique known as freeze-etching to membrane studies has also contributed important new information about structure (see Fig. 1). In freeze-etching a specimen, often a red blood cell membrane, is rapidly frozen to liquid-nitrogen temperature and fractured in a vacuum. Ice is sublimed from the specimen which is then shadowed and replicated with platinum and carbon. What is revealed is the hydrophobic or internal plane of the membrane, according to Daniel Branton, who points out that there has been some debate over whether the fracture faces are those of the membrane surface or of its inner regions. In one experiment designed to resolve this debate, he and his colleagues labeled red cell ghosts on both surfaces with covalently bound ferritin, a relatively large protein that, once attached to the membrane surface, will not easily break away. These labeled ghosts were then subjected to freeze-etching. "Ferritin molecules were never observed on fracture faces, thus indicating that fracture does not show membrane-surface detail," he reports. Rather, the fracture occurs along the inner hydrophobic core of the membrane to expose the interior plane. (Although the primary interest in freeze-etched membranes is in what they reveal about proteins, the fact that the fracture occurs as it does is, of itself, supportive of the idea that a lipid bilayer as described by Danielli constitutes the predominant lipid structure.)

As with other analytical methods, freeze-etching is subject to artifacts that can complicate the picture; but it has its own advantages. X-ray diffraction and optical measurements provide data that is based on mathematical averages. By contrast, freeze-etching is not an averaging technique and, therefore, may prove to be a way of getting at the small regions within a membrane that are important to its specialized functions. "The focus of attention now," Branton says, "is on what a small number of components are doing." For example, there is evidence that there are about 300 virus receptors on a bacterial cell. The number of sites for potassium ion transport in the human erythrocyte membrane may be as low as 100 or 200. Thus, the activities of membranes appear to be conducted by molecules that occupy a neglible fraction of the total surface and that may be unidentifiable by averaging techniques.

The most striking feature of a freeze-etched red cell membrane examined under the electron microscope is the presence of large, globular particles some 75 angstroms in diameter. They may span the membrane, penetrating both outer and inner surfaces. These particles appear to occupy no more than 10 to 20 percent of the total membrane; yet, some researchers speculate, they may contain 30 to 40 percent or more of the membrane protein. Demonstrable by freeze-etch studies, these large, penetrating particles have also been identi-

fied by chemical analyses. A few months ago, Mark S. Bretscher of Medical Research Council's Laboratory of Molecular Biology in Cambridge, England, showed that there is a major erythrocyte glycoprotein that spans the lipid bilayer rather than merely being attached to the surface. The glycoprotein Bretscher described has a molecular weight of about 31,400 and, he says, carries much of the carbohydrate and most of the sialic acid of the cell surface. (In red cells, sialic acid molecules, one of several classes of carbohydrates, bind exclusively to membrane proteins, whereas other sugars appear to be distributed evenly between proteins and lipids.) Another, second major protein component with a molecular weight of about 105,000 occurs in close proximity. He identified these components with the use of a new labeling reagent, ^{35}S-formylmethionylsulfone methyl phosphate (FMMP) which cannot get through the erythrocyte membrane barrier. Therefore, the proteins must be labeled from the outside. Using the radioactive label, Bretscher finds approximately the same number of proteins in a membrane as Branton finds particles by freeze-etching. Investigators speculate that there are probably 10 or 12 important membrane proteins in erythrocytes. While trying to identify and characterize them, scientists are asking another question: Do they move? At present, there is little data on the subject but some say that there is considerably more movement within membranes than had been supposed.

As the debate over membrane proteins took on new dimensions during the last couple of years, the debate about the predominant lipid structure was brought to a close with the virtually unanimous opinion that the array of sophisticated data that has accumulated from experiments of all types vindicates the theory that has predominated all along; namely, that the Danielli bilayer is essentially correct. Among the latest data in this regard is that reported recently by Nobel laureate Maurice H. F. Wilkins and A. E. Blaurock of Cambridge University, and Donald Engleman of Yale University, who have expanded the uses of x-ray diffraction to include examination of various types of membranes.

Recognizing the restraints that diffraction studies of ordered membranes such as myelin or retinal rods place on one's ability to generalize to other membrane systems, the team extended x-ray analysis to membranes that are not arranged in arrays to provide what it describes as "direct evidence that a phospholipid biomolecular layer is a major structural component of membranes of widely differing types." To avoid the requirement of artificially stacking membranes to achieve an orderly array, they developed a method of obtaining diffraction data from random dispersions of membrane fragments. To test the method, they first looked at phospholipids dispersed in water to form biomolecular layers. "The diffraction patterns of these model systems show features recognizable in patterns from natural membranes; this helps to interpret membrane patterns and to obtain electron density profiles," they observed.

X-ray Diffraction Patterns

Diffraction patterns of membrane dispersions sealed in a thin-walled glass capillary were then recorded for membranes from two types of *Mycoplasma laidlawii*, simple organisms frequently used in membrane studies, from red cell ghosts, and from nerve-ending plasma membranes from rat cerebral cortex, as well as from myelin and retinal rods. "A basically similar pattern is given by all the membranes although they differ considerably in biological function and composition, for example, the ratio of lipid to protein," the investigators conclude, adding the qualification most contemporary researchers attach to statements about the universality of the lipid bilayer: there is no necessity that the bilayer be continuous. In other words, it may be interrupted in various places by functionally active protein sites without negating its central role, which is to form the structural backbone of the membrane and to serve as its primary permeability barrier.

Thus, discovering the molecular architecture of membranes is, according to investigators, necessary to answering the question of how a single biologic entity can have so many functional properties. The plasma membrane functions as the boundary to the cell, the permeability barrier that separates the internal and external milieu of the cell. If the internal environment of the cell is disrupted, cellular viability is lost. The plasma membrane regulates that environment, acting as a gatekeeper to allow, through various mechanisms of active and passive transport, the passage of ions, of nutrients, and of other chemicals into and out of the cell. (Membrane transport mechanisms constitute another major research area today as scientists explore the role that specific proteins may play in this regard.)

All chemical and electrical information that reaches the cell does so through its membrane. Hormones, insulin for example, initiate their effects through interactions with receptor sites on the cell surface. But precisely how they pass information through the membrane and what specific effects the hormone–cell surface interaction has on intracellular events remains unclear. Many drugs act through cell surface contacts, muscle contractions are triggered by electrical stimuli acting on membranes, and a host of enzymatic activities are carried out on the cell surface.

When aberrant cell behavior occurs, as it does during malignancy, membrane disorders usually exist, and may account for the observation that tumor cells cannot obey the law of contact inhibition; that is, they cannot read or understand whatever instruction it is that tells cells to stop growing when they come in physical contact with each other. Cancer cells continue to proliferate and pile up on each other to form a tumor. Immunological specificity is another product of the molecular structure of the plasma membrane, for that is the location of the antigens which immunologically distinguish one man from another (so-called histocompatibility antigens) and also distinguish the cells of the heart from those of the liver and so on. Therefore, surface antigens are of interest not only to immunologists, but also to biologists concerned with questions about cellular differentiation and the mechanisms by which it occurs.

Studies of membrane lipid and protein structure are only a beginning. The matter of carbohydrate structure also will have to be resolved, as well as questions about the special types of lipids, proteins, and carbohydrates that compose membranes of various types in various combinations. In addition to the three principal components, membranes also contain ions, various small molecules, and modest amounts of RNA. So little is known about what these molecules do that researchers are reluctant to even guess. The questions of membrane composition, therefore, must be resolved hand-in-hand with those about structure.—BARBARA J. CULLITON

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