Meetings

Membrane Models and Membrane Formation

The rapidly increasing interest in the molecular structure of membranes and their biogenesis was reflected in a NATO Advanced Study Institute devoted to these topics. The conference was held at Frascati (near Rome) in Italy, 11–17 June 1967.

To allow ample time for discussion, the different topics were reviewed by session chairmen, who called on participants only to elucidate certain points rather than give formal presentations of recent results. B. A. Pethica (Port Sunlight, England) summarized the state of our knowledge on the binding and the molecular motion of membrane constituents obtained with spectroscopic techniques, differential thermal analysis, and high-angle x-ray diffraction. Several states of structural and loosely bound water were distinguished. No agreement could be reached on whether hydrocarbon chains of the lipid were more solid- or liquid-like.

D. A. Haydon (Cambridge, England) discussed the electrical properties and permeability of black lipid films. The high electrical resistance of these films was found to be consistent with the view of their interior as a thin, continuous sheet of hydrocarbon. The water permeability could also semiquantitatively be predicted from this model. T. E. Thompson (Charlottesville, Virginia) showed electron micrographs of these lipid films and a preliminary analysis of their composition. The thickness, though not uniform, was shown to be greater than generally assumed —with 140 angstroms perhaps the most reliable value. The molar ratio of hydrocarbon to phospholipid was found to be 10 to 1 (*1*). He also described a new model system consisting of a free-floating spherical lipid film of large area in equilibrium with a bulk phase occupying only a small part of the sphere surface. Values for water permeability and electrical resistance are similar to those in planar films (*2*). A. Lev (Leningrad) discussed the effects of valinomycin which increases the permeability of these films for inorganic ions and is highly selective for potassium. However, it has only a very small influence on the electrical capacitance (*3*, *4*).

F. Lynen (Munich, Germany) reviewed our rather extensive knowledge of the biosynthetic pathways for membrane lipids and pointed out how little is known about important questions where membrane lipids are synthesized in cells and how they are incorporated into the membranes. V. A. Parsegian (Cambridge, Massachusetts) presented a theory of lipid aggregation in the liquid-crystalline state indicating that long-range electrostatic plus short-range interfacial interactions are sufficient to explain the molecular order found in x-ray diffraction diagrams (*5*).

D. J. Luck (New York) summarized present data on the formation of mitochondria and chloroplasts. The discussion was centered on what proteins are synthesized under the control of mitochondrial and chloroplast DNA and whether these proteins could serve as structural components of the membranes and unique determinators of membrane functions, especially if "structural protein" would meet these criteria. It was concluded that final acceptance of this idea would have to await more detailed data on the genetic determination of mitochondrial and chloroplast functions and resolution of the apparent heterogeneity of "structural protein" preparations (*6*).

Coupling factor 4 may represent a more homogeneous preparation with the properties of a "structural protein." E. Racker (Ithaca, New York) showed that coupling factor 4 is necessary for the reconstitution of mitochondrial membranes from their separated lipid protein constituents and for the binding of the mitochondrial adenosine triphosphatase to the membrane (*7*). P. Cerletti (Rome) reported a specific interaction between succinate dehydrogenase and cardiolipid and S. Fleischer (Nashville), presenting work done with W. Stoeckenius (San Francisco), showed that mitochondrial phospholipid and "structural protein" react to form vesicles bounded by a typical "unit membrane."

L. Ernster (Stockholm) led the session which discussed enzyme synthesis and turnover of membrane constituents in the endoplasmic reticulum. Apparently the membranes of the smooth endoplasmic reticulum—at least in the rat liver—are derived from the rough endoplasmic reticulum. Several more detailed models for the assembly of membrane components into the final membrane are still under discussion. Since the different enzymes of the mature membrane appear at different time points during maturation, a one-step, self-assembly process can be ruled out. The different turnover rates of lipids and proteins in the membrane make unlikely the existence of a continuously formed basic membrane into which specialized functional components such as enzyme proteins would be inserted. However, the difficulties of purifying firmly membrane-bound enzymes and the question of homogeneity of endoplasmic reticulum membrane will have to be resolved before an acceptable detailed model can be constructed (*8*).

In a session chaired by W. Stoeckenius (San Francisco), W. R. Loewenstein (New York) described the reformation of a membrane junction after artificial dissociation of cells. He showed that the regions of membrane contact become more permeable than the rest of the surface membrane by several orders of magnitude. This is caused by a reduction in calcium and magnesium ion activity occurring selectively at these sites (*9*). A. Katchalsky (Rehovoth, Israel) drew a parallel to the behavior of certain macromolecules which undergo phase transitions at certain Ca^{++} Mg^{++} activities (*10*).

T. M. Sonneborn (Bloomington, Indiana) spoke about polarization and localization of outer cell membrane growth and differentiation in paramecium. Observations were presented and their bearing on outer membrane growth was pointed out (*11*). It was shown that this growth is longitudinal and polarized and is virtually restricted to

the equatorial zone and even to minutely localized parts of this zone because (i) this membrane is marked by about 8000 fixed points (the insertions of ciliary basal bodies), (ii) an equal number of new basal bodies become inserted in precise orientations during fission, and (iii) nearly all outer membrane growth occurs in association with fission. Experimental rotation of a small part of the surface through 180 degrees reversed the polarity of the growth of this part. A local differentiation of the membrane (the anterior suture), devoid of basal bodies, arises only from part of the cleavage furrow and does not reform during regeneration of an amputated anterior region.

S. E. Luria (Cambridge, Massachusetts) discussed colicins and the bacterial cell envelope. He described a new approach to isolation of bacterial mutants with altered membrane properties. These mutants are isolated as tolerant to the action of colicins and appear to have defects in some of the membrane proteins.

S. Dales (New York) described his attempts to characterize the proteins and lipids composing the envelopes of vaccinia virus and to ascertain the time of their synthesis. Experiments with inhibitors of protein synthesis revealed that pools of protein accumulate in the infected host shortly before the appearance of vaccinia-specific membranes. Judging from the incorporation of choline into lecithin of the membrane, nascent phospholipids are integrated into the viral envelopes at the time of their being assembled.

Two special group discussions were held. One session, organized by A. Mauro (New York), dealt with problems of thin lipid films of the Mueller-Rudin type. Two distinct views concerning the structure of these films emerged. The majority of the workers thought that "additives" (decane, tetradecane, and alpha-tocopherol) are inserted between the fatty acid chain of the phospholipid with their long axes perpendicular to the plane of the film. A minority view favored a structure in which the hydrocarbon additives are concentrated in a central planar thin layer, thus giving rise to a triple-layered structure, phospholipid-hydrocarbon-phospholipid. It was generally agreed that the large variations in electrical resistance observed in these films are most likely due to leakage around the torus of bulk lipid at the edge of the aperture. It was also agreed that the simple diffusion theory, subject to partitioning of water in the oil phase, accounts for the water fluxes observed and that the disparity in the values obtained with tagged-water versus osmotic-flow measurements can be accounted for by inadequate stirring, which results in too low values for the tracer measurements. The equality of the two coefficients is consistent with a continuous lipid phase without "pores" or "channels" (*12*). There was also complete agreement that the films, unless modified, are inert as electrical elements and impermeable to electrolytes. The original observation, that extracts from *Aerobacter cloacae* cultures can reduce the resistance of the films by several orders of magnitude, has now been extended. Cyclic peptides and polyenes, in addition to the fall in resistance, also produce an electromotive force which depends on cations in the case of valinomycin and anions in the case of the polyene amphotericin. It is thought that these compounds may create specific "pores" or act as "carriers." It became clear that the phospholipid-hydrocarbon film is an interesting system for the study of chemistry and physics of ultrathin structures. How useful it will be as a model for biological membranes remains to be seen.

The second group discussion on genetic approaches to functional complexes in membranes was organized by S. E. Luria (Cambridge, Massachusetts). The discussion centered on the question of mutations that affect enzyme functions through altered organization of enzyme complexes. P. Sweetly reported immunological differences in structural protein isolated from wild-type and "petite" mitochondria in yeast. Luria reported on the recent work of Puig and co-workers in Marseilles with mutations that affect the supramolecular organization of enzymes concerned with terminal steps of anaerobic catabolism in bacteria.

The conference was sponsored by the University of Rome and supported by a NATO Advanced Study Institute grant. Additional financial support was received from the Italian Ministry of Public Education, the Italian Ministry of Foreign Affairs, and the Consiglio Nazionale delle Ricerche. Part of the travel expenses for American participants was provided by the National Science Foundation.

W. STOECKENIUS
University of California,
San Francisco Medical Center,
San Francisco 94122

References

1. T. E. Thompson, *J. Mol. Biol.* **24**, 51 (1967).
2. R. Pagano and T. E. Thompson, *Biochim. Biophys. Acta* **144**, 666 (1967).
3. A. Lev, *J. Evolut. Biochem. Physiol.* **2**, 109 (1966).
4. A. Lev, *Cytology* **9**, 102 (1967).
5. V. A. Parsegian, *Trans. Faraday Soc.* **62**, 848 (1966).
6. D. O. Woodward and K. D. Munkres, *Proc. Nat. Acad. Sci. U.S.* **55**, 872 (1966); R. S. Criddle, D. L. Edwards, T. G. Petersen, *Biochemistry* **5**, 578 (1966); D. Haldar, K. Freeman, T. S. Work, *Nature* **211**, 9 (1966).
7. E. Racker, *J. Biol. Chem.* **241**, 2461 (1966).
8. T. Omura P. Siekevitz, G. E. Palade, *J. Biol. Chem.* **242**, 2389 (1967).
9. W. R. Loewenstein, *Ann. N.Y. Acad. Sci.* **137**, 441 (1966); W. R. Loewenstein, *Develop. Biol.* **15**, 503 (1967).
10. A. Katachalsky and M. Zwick, *J. Polymer Sci.* **16**, 221 (1955).
11. T. M. Sonneborn, in *The Nature of Biological Diversity*, J. M. Allen, Ed. (McGraw-Hill, New York, 1963); R. Dippell, *Excerpta Med.* **77**, 16 (1964); J. Beisson and T. M. Sonneborn, *Proc. Nat. Acad. Sci. U.S.* **53**, 275 (1965); Chen-Shan, thesis, Indiana University (1967).
12. A. Cass and A. Finkelstein, *J. Gen. Physiol.* **50**, 1765 (1967).

Antimicrobial Agents and Chemotherapy

New penicillins, cephalosporins, and antibiotics were described at the Seventh Interscience Conference on Antimicrobial Agents and Chemotherapy in Chicago, Illinois, 25–27 October 1967. Of the 1133 scientists registered at this meeting, 113, representing 17 countries, came from abroad.

The successes resulting from chemical modification of antibiotics continue to be reported. Those mentioned at this meeting included: (i) Carbenicillin, a new semi-synthetic penicillin with high activity against *Pseudomonas* and other gram-negative bacteria; (ii) Cephalexin, a semi-synthetic cephalosporin which gives high blood levels and can be orally administered; (iii) two aminoalicyclic penicillins (Wy-4508 and Wy-7953 from the Wyeth Laboratories) which give high blood levels and have low-serum binding coefficients, and are more slowly excreted than ampicillin though they are somewhat less active against gram-negative bacteria; (iv) potassium-6-(D-α-azidobenzyl-acetamido)-penicillin, a new, clinically useful penicillin from Astra Research Laboratories, Sweden; and (v) a series of lincomycin derivatives with antimalarial activity in tests on a variety of animals. All of these studies showed that by rather close cooperation between chemists, microbiologists, and pharmacologists it is possible to prepare new drugs with many desired features. Perhaps, in time, "custom ordering" of antibiotics will be possible.