Meetings

Ion Flow in Membranes

It is almost 20 years since the experiments of Hodgkin and Huxley yielded a virtually complete and quantitative explanation of how the action potential arises in normal axons. In the intervening years a great deal has been learned from studies in which the internal and external environments of the axon have been deliberately altered. In addition, extensive experience with a variety of pharmacological agents has served to further expand the range of experimental conditions encompassed (often with only minor modifications) by the quantitative treatment of Hodgkin and Huxley. In spite of the completeness of the experimental information, the molecular nature of the processes controlling ion flow in the membrane has remained elusive.

The idea of a conference concerned with nerve excitation arose because it was thought useful to survey the field as it presently exists and to stimulate both experimental and theoretical effort toward the molecular identification of substances responsible for excitation phenomena and toward an appropriate theoretical treatment of the molecular physics of the substances involved in ion transfer across excitable membranes (*1*).

One of the needs perceived was to interest eminent theoreticians in the problems of nerve excitation and the following participants were invaluable for their discussions of both experimental and theoretical matters: G. Adam, W. Brattain, M. Delbrück, F. Dodge, B. Hamel, T. Hill, R. Hoyt, L. Onsager, M. Starzak, L. Y. Wei, and J. W. Woodbury. It is difficult to summarize current theoretical work; most individuals appeared to be working on the K^+ system of the squid axon because it is supposedly simpler phenomenologically. Cooperative schemes for the sensitivity of K channels to membrane voltage have been tried but have serious difficulties in matching experimental results. One of these difficulties is that the K currents must arise with a delay that is dependent on membrane hyperpolarization (the Cole-Moore effect). Using the Hodgkin-Huxley equations requires an exponent of 25.

One goal of membrane excitation research is to extract Na- and K-carrying systems (if these exist as molecular entities) from membranes and to show that they have appropriate properties when incorporated into artificial systems—this has not been accomplished as yet. Important contributions to this discussion were provided by R. C. Bean, G. Ehrenstein, and D. A. Haydon. Much progress has been made in understanding how small polypeptides that form channels are incorporated into lipid bilayers, but the physical mechanisms involved in ion penetration through channels are not entirely clear. Efforts to incorporate large protein molecules into lipid bilayers appear, in the main, to have been unsuccessful. Much more work on relatively simpler substances such as gramicidin will be necessary before an appropriate model for pore penetration can be developed. Gramicidin appears to increase lipid bilayer conductance as a power function of its concentration in the bathing solution, so that channels may require a polymer of this substance. Present "channel forming" polypeptides confer little ion selectivity on the channels they form while carrier-type polypeptides with high ion specificity seem less likely as models.

Membrane chemistry was another topic that received extensive discussion. The experts in this area were V. Luzzati, W. Stoeckenius, and R. Villegas, but the discussion was wide-ranging and involved most participants. The picture of the cell membrane which emerged from these discussions is that of a lipid bilayer with discrete, but not continuous, protein patches on both sides. If (as electron microscopic evidence suggests) some of these proteins extend through the bilayer, they would become attractive as possible routes for ion penetration. Studies of surface films of lipids with defined composition have revealed that substances such as tetrodotoxin (TTX) and DDT have strong and apparently specific interactions with some membrane lipids, although binding constants are very much less than physiological.

The ionic currents that flow during excitation were discussed with regard to the following: (i) optical changes associated with excitation, (ii) effects of altered ionic environments, and (iii) the effects of pharmacological agents. For (i), it seems likely that changes in light scattering and birefringence relate to virtually instantaneous changes in the membrane dielectric, although there are long time constant phenomena as well. With fluorescent probes in the axon membrane there are changes in fluorescence emission with the action potential, but it is too early to tell whether these relate to ionic currents or to reorientation in the dielectric. Discussion on this topic was led by R. D. Keynes and I. Tasaki.

New information about the behavior of axons when both their internal and external environments are markedly changed was a topic for lively discussion (W. J. Adelman, Jr., and B. Hille). On the one hand, K-free solutions inside and outside the axon interfere with Na inactivation, while the use of Na substitutes on the frog node has made it possible to characterize the requirements for Na selectivity of the "fast" channel, in the sense that the ions involved must be smaller than K^+ and waterlike in what would correspond to their first hydration shell. Total dehydration (such as with K^+-valinomycin) seems impossible because then Na^+ and guanidinium$^+$ (a Na^+ substitute in which $-NH_2$ corresponds to H_2O) would not have steric properties in common. In preparations such as the barnacle muscle fiber, Ca^{2+} is a depolarizing current carrier while Sr^{2+} and Ba^{2+} can substitute as penetrating ions; the channel can be blocked La^{3+} (S. Hagiwara). This system is clearly different from the Na system of nerve and indeed in *Aplysia* there are neurons which have both Na^+ and Ca^{2+} systems that carry depolarizing current; one is blocked by TTX and the other by La^{3+}. Another preparation, the *Myxicola* axon, was described (L. Goldman); it has a conventional Na-K system but a significantly larger axon membrane–Schwann cell space. The attractiveness of the preparation lies in the readily available nature of the organism as compared with squid, although its stiffer axoplasm has not made for easy internal perfusion.

The pharmacological response of axons to certain substances (C. M. Armstrong, J. W. Moore, T. Narahashi) has led to estimates of the number of

Na channels in the squid membrane (experimental estimates agree on ~50/μm^2) and to a rather detailed description of the K channel and its gate. Both tetramethylammonium ion (TEA^+) derivatives and Cs^+ inside an axon inhibit K currents in a time-dependent manner. By testing for the recovery from inhibition it has been possible to work out the kinetics of channel blocking and of channel recovery after the membrane has been repolarized. The model that emerges is one with a voltage sensitive gate at the inside by the membrane. A region under the gate must supply ion selectivity both for blocking substances and for K^+.

Two additional topics relating to voltage clamp currents were discussed in some detail: (i) single versus separate channels for the Na and K currents and (ii) the problem of gating (or carrier) currents. Although a poll was not taken, it is likely that most investigators are impressed by the pharmacological specificity of TTX and TEA for the Na and K channels. Most theorists started their work with a coupled Na-K channel but have now shifted to an emphasis on separate channel models. Some evidence was offered that the Na channel may be a coupled system in the sense that the Hodgkin-Huxley conductance parameters m and h are not independent. Experimental support for this depends on high precision measurements of h versus (E_m). Mainly such a shift of models has been occasioned by an appreciation of the difficulties posed by the results of the Cole-Moore exeriments, that is, the continuous delay of K currents with increasing hyperpolarization. The Na currents are unaffected by values of membrane potential more negative than ~75 mv.

Gating current—that is current that should precede the start of ion conductance change—has never been observed experimentally. Yet most investigators thought that there must be such a current. Those who thought that it was possible to devise a model showing a voltage sensitivity of ion conductance but no gating current were challenged to come up with a model; no such model was offered during the conference. It was also suggested that gating currents could well be hidden in the capacitative transient. A further experimental attack on the problem seems possible by the use of signal averaging equipment.

One session was devoted to tracer

methods of measuring ion fluxes (T. I. Shaw and R. E. Taylor) and optical methods for following Ca^{2+} fluxes (A. L. Hodgkin). Recent measurements of ^{22}Na flux in voltage clamped axons at a variety of potentials have shown that the transference number for Na (as early current) is very close to unity. Some of the difficulties in using perfused axons for active transport studies were discussed. It was noted that the use of dextran in the interior of the axon seems to stabilize the membrane against what appears to be some sort of an irreversible uncoupling of the Na pump. If aequorin, a compound that emits light in the presence of Ca^{2+}, is injected into squid axons, it is possible to follow changes in $[Ca]_i$ (and therefore Ca entry) by measuring light emission from the fiber. An early Ca^{2+} entry that is synchronous with Na entry has been observed as well as a late entry seen in TTX-treated fibers. This latter process is especially interesting as it may be related to transmitter release phenomena which ordinarily are observed only in nerve terminals.

L. J. MULLINS

Department of Biophysics,
University of Maryland
School of Medicine, Baltimore

Note

1. The organizers of the conference were D. E. Goldman, chairman, K. S. Cole, and L. J. Mullins. There were about 30 participants, but no formal lectures. The conference was held 5 to 9 April at Ann Jordon Farm, Alexander City, Alabama, a conference center of the University of Alabama. Participants were welcomed by Dr. R. Hill, vice president for health affairs of the University of Alabama in Birmingham and were entertained for dinner by President J. F. Volker. The local arrangements were handled by Dr. W. Rehm, Chairman, Department of Physiology and Biophysics University of Alabama, Birmingham. Financial support for the conference was provided by the Grass Foundation, Quincy, Massachusetts, International Business Machines Co., and the University of Alabama at Birmingham. Support for the expenses of NIH personnel was provided by the National Institute of Neurological Diseases and Stroke, Bethesda, Maryland. The organizing committee is grateful to the University of Alabama in Birmingham for providing for the needs of the conference and to the agencies whose financial support made the meeting possible.

Membrane Structure

The theme and perspective for the Conference on Membrane Structure and Its Biological Applications, which was sponsored by the New York Academy of Sciences and held 2 to 4 June 1971, was set by the first two presentations, given respectively by G. Vanderkooi and J. Singer. Vanderkooi presented de-