spectra with the opal glass method (*12*) before and after illumination of bean leaves, which are more than 10 percent green, after periods of growth in the dark. Thus, after the leaf gains photosynthetic power, the existence of another pathway to chlorophyll *a*, perhaps via chlorophyll *b*, is not ruled out (*13*).

D. W. KUPKE
JUDITH L. HUNTINGTON*
Department of Biochemistry, School of Medicine, University of Virginia, Charlottesville

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* Present address: Lindenwood College, St. Charles, Mo.

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Quantitative Molecular Approach to the Permeability Changes of Excitation

Abstract. *Functional relationships, available from only a few monolayer studies, can be applied to a relatively simple model of the excitable membrane to give permeability-potential curves quite similar to the conductance-potential curves obtained experimentally in voltage-clamped giant axons. Contrary to the usual view in terms of "carrier systems," the present model considers the permeability to sodium and potassium to be reduced by the increase in the surface pressure induced by large lipophilic cations and anions in the outer layer of the lipoidal bimolecular leaflet constituting the living membrane; hence, the increase in permeability during depolarization, for example, is due to a decrease in the amount of the organic anions in this layer, whereas the decrease in sodium permeability during inactivation is caused by a rise in content of organic cations. The present proposal has the advantage that it is in keeping with known phenomena observed in simple physico-chemical systems as well as in excitable systems; moreover, the current actually transferred by the postulated lipophilic ions can be negligible compared to that transferred by the inorganic cations they control. The latter situation, as well as the steepness of the permeability-potential relationships obtained, have been pointed out to be critical requirements of a satisfactory molecular hypothesis.*

Recent monolayer studies provide a basis for the labile permeability properties of living membranes. Shanes and Gershfeld (*1*) called attention to the correlation between the increase and decrease of membrane permeability in veratrum alkaloids and local anesthetics, respectively, and the ability of these drugs to induce a corresponding decrease or increase in the surface pressure of monolayers of a fatty acid. It was suggested in consequence of this, as proposed earlier (*2*) on the basis of Skou's monolayer work (*3*), that surface pressure may govern ion permeability by determining the proximity of adjacent membrane molecules in the regions of ion passage, a greater proximity due to a higher surface pressure reducing ion entry and vice versa. Archer and La Mer (*4*) and La Mer and Barnes (*5*) have actually shown the dependence of the rate of penetration of molecules through monolayers on surface pressure. They have demonstrated, moreover, that in the case of fatty acids the presence of small amounts of calcium, which changes the state (that is, the relative solidity and packing) from the liquid condensed to the solid condensed, has two effects: a substantial decrease in the penetrability to water molecules for the same surface pressure, and an increase in the range of surface pressure over which a change in penetrability may be induced. These offer a basis for the well-known dependence of membrane ion permeability on calcium in the medium (*2*) and for the enhancement by this ion of the permeability changes during excitation (*6*). It must be stressed that surface pressure of itself in the absence of proper alignment or strong interactions between neighboring molecules may not affect monolayer penetration by water vapor and other gases (*7*). Moreover, the presence of small amounts of weakly interacting molecules may also greatly increase penetrability (*4*, *8*).

It has been pointed out qualitatively, on the basis of Guastalla's demonstration (*9*) of the enhanced accumulation of lipophilic ions at the oil-water interface because of an electric field, that the redistribution of such ions within the living excitable membrane, brought about by changes in transmembrane potential, offers a possible basis for the well-known dependence of membrane permeability on the potential (*10*). A mathematical approach to this, based on the application of known monolayer properties to a model in which the concentration of lipophilic ions varies continuously with distance through the membrane, has given permeability-potential curves resembling those found experimentally (*11*).

A somewhat simpler set of relations giving an even better fit with the experimental permeability-potential curves has now been obtained by considering the possibly more realistic situation wherein, by virtue of the bimolecular structure of the lipoidal part of the membrane, large lipophilic ions (of the order of one membrane layer thickness in length) are regarded as residing either in one or the other of these layers, their polar group being only at either the inner or outer membrane-water interface. In the insert of Fig. 1 the situation is depicted for an organic anion. Equation 1 given in Fig. 1 represents the application of Boltzmann's principle to this type of distribution Z is the valence of the anion; E, the transmembrane potential; F, Faraday's constant; R, the universal gas constant; and T, the absolute temperature. The possibility of a work function for the transfer of the anion between I and M of the membrane is neglected and C_I, the content of the anion in the inner layer, I, is regarded as constant by virtue of an intracellular source of the anion with which it is in equilibrium. Variations in C_M, the content of the organic anion in M, are considered responsible for the permeability changes of the membrane. Thus, an increase in C_M will increase the packing of the membrane molecules in M and hence its surface pressure, S. The interaction

of large molecules with myelin monolayers (*12*) suggests that S may vary almost linearly with C_M, from which Eq. 2 in Fig. 1 follows. ΔS is the increment in S due to C_M.

Penetrability, p, of lipoidal monolayers by gases is governed by an equation of the form (*4, 13*)

$$p = Ce^{-U/RT} \qquad (3)$$

where U is the experimental activation energy, which includes the sum of the interaction energies of the individual CH_2 groups, $U(CH_2)$, and of the polar and nonpolar end groups; R and T are the usual thermodynamic coefficients and C is the frequency constant. The last may also incorporate any small or invariant energy or entropy terms (*13*).

From Eq. 1 it follows that the penetrability to a given molecular species will change from p_1 to p_2 when U_1 changes to U_2, the relative change being

$$p_1/p_2 = e^{(U_2 - U_1)/RT} \qquad (4)$$

A possible relation between U and S is provided by data on long chain alcohols (*14*); according to this, for S up to about 15 dyne/cm, $U(CH_2) = 16nS$ in calories per mole, where n is the number of CH_2 groups, above 15 dyne/cm,

$$U(CH_2) = 2.5\,n\,(S - 15) + 240\,n$$

the second term being $16n$ [15], or that governed by the lower part of the U-S curve. Thus, if the minimum value of p is associated with a maximum value of S of 15 dyne/cm and only $U(CH_2)$ is considered to change with S, p relative to the maximum p, p_m, may be written

$$\ln(p/p_m) = -(16nbC_I/RT)\; e^{ZEF/RT} \quad (5)$$

This combines Eqs. 1 and 2 (Fig. 1) and Eq. 4. The number of CH_2 groups, n, may be taken to be about that of stearate, namely, 16. From Eqs. 1 and 2 (in Fig. 1), bC_I may be lumped as a single constant; for a given ion species, it is ΔS when E is zero, and this may be assigned a value by deciding how large ΔS is when E is 100 mv. If ΔS at 100 mv is 40 dyne/cm, bC_I is about 0.8 dyne/cm; if ΔS is 16 dyne/cm, bC_I is about 0.3 dyne/cm. The choice of ΔS is dictated, in turn, by the magnitude of the relative permeability change to be described. In the case of the squid axon membrane this has been found to be about 100-fold for the potassium ion and about 1000-fold for sodium (*15*).

In Fig. 1 the solid curves are the experimental curves relating the maximum membrane conductance due to potassium, G_K, and that due to sodium, G_{Na}, to the transmembrane potential of the voltage-clamped squid giant axon (*15*). The resting potential is taken as 60 mv (*15*). The two broken curves are the corresponding hypothetical permeability-voltage relations. That for sodium has been calculated directly from Eq. 5; in the case of potassium, allowance has been made for the increment of $U(CH_2) = 2.5n(S\text{-}15)$ when ΔS is above 15 dyne/cm. Sodium and potassium are considered to pass through different regions of the membrane. In the regions for potassium passage, the permeability is assumed to be governed by a lipophilic anion with a valance of one, and ΔS induced by 100 mv is 40 dyne/cm; the corresponding figures for the sodium regions are taken to be 2 and 16 dyne/cm, respectively. For simplicity, the number of CH_2 groups in the membrane molecules in both the sodium and potassium regions of ion passage is taken as 16. The calculated curves so obtained conform well to the experimental ones. They also cross each other at low transmembrane potential, as in the case of the experimental curves.

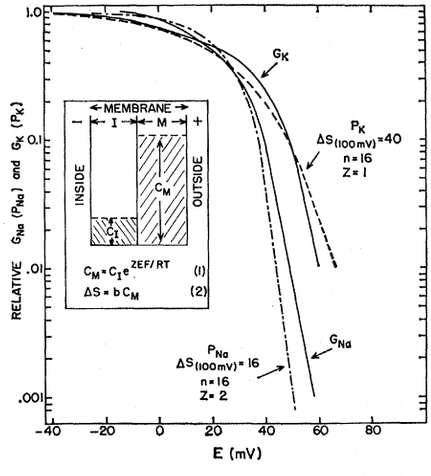

Fig. 1. (Insert) Hypothetical distribution, under the influence of the transmembrane potential E, of a lipophilic anion at concentration C_I in the inner layer, I, and at concentration C_M in the outer layer, M, of the bimolecular lipoidal portion of an excitable membrane. Equation 1 shows the application of Boltzmann's principle to this situation and Eq. 2 the increment in surface pressure, ΔS, of M as a function of C_M. (Curves) Solid lines are the experimental relations between the maximum sodium conductance, G_{Na}, and potassium conductance, G_K, and E of voltage-clamped squid giant axons (*15*). Resting potential is taken as 60 mv (*15*). Broken lines are the corresponding hypothetical ones for sodium permeability, P_{Na}, and potassium permeability, P_K, computed from the values of the parameters shown and as given in the text.

In addition to providing permeability-potential curves similar to those that have been described experimentally for conductance, the proposed approach appears to offer a basis for understanding other characteristics of excitable membranes. For example, the effectiveness of stabilizing blocking agents is reduced by anodal polarization (*16*); this result is to be expected from the proposed model because increased surface pressure is known to squeeze out such molecules from lipoidal monolayers (*1, 17*). Another phenomenon explicable in terms of the model is the increased effectiveness of stabilizers with successive action potentials (*18*). Thus, while S is low during the course of an action potential, increased entry of stabilizer can be expected, thereby increasing the degree of stabilization for the next impulse. From this standpoint, the prolonged refractory period induced by stabilizers (*19*) could reflect the squeezing out of the stabilizer taken up by the membrane during the impulse.

Available monolayer studies offer no data for formulating a quantitative approach to the kinetics of the permeability changes. However, two features of the present model are consistent with the known time-dependence of P_{Na} and P_K. Thus, the large ΔS proposed as necessary for the P_K change with E means that during a depolarization clamp the initial rise in P_K occurs over the range $\Delta U(CH_2) = 2.5n(S\text{-}15)$, and only later over $U(CH_2) = 16nS$; this implies a delay in the rapid phase of the increase in P_K, as actually observed (*15*). Conversely, ΔS required for the P_{Na} change is postulated to occur only over the range $U(CH_2) = 16nS$, hence little delay need be expected in the onset of the increase in P_{Na} during a depolarization; moreover, the higher valence suggested for the lipophilic anion controlling P_{Na} should make P_{Na} change substantially faster than P_K. Such differences in the response of P_{Na} and P_K to a depolarization have been described (*15*).

The approach presented may also be applied as a basis for the phenomenon known as "inactivation," that is, the decrease in sodium transport that reverses the increase during a depolarization. This can be looked upon as a secondary decrease in P_{Na} resulting from the transfer of an organic cation from I to M in the sodium regions of the membrane by virtue of the decrease in E. Thus, an equation similar to Eq. 5, but with the E-containing exponent negative

because of the involvement of a cation rather than an anion, can serve to describe the maximum P_{Na} as a function of E. In Fig. 2 a curve so calculated (designated by $w = 0$) is compared with experimental curves obtained for the squid giant axon in media at different calcium concentrations (*20*). The latter curves have been put on an absolute potential scale by assigning a resting potential of 50 mv; this low resting potential is employed because in the calcium studies the fibers were short and the change in membrane potential for 50 percent inactivation, at normal calcium levels, was about 10 mv higher than in preparations which had a resting potential of 60 mv and gave the experimental curves in Fig. 1 (*20, 21*). The calculated curve was obtained with bC_I assigned 25 dyne/cm. It can be seen in Fig. 2 to deviate from the experimental curves in a manner to be expected from the use of a medium very low in calcium.

One way in which calcium might act in the living membrane is by introducing a threshold for C_M; below this concentration the lipophilic anion fails to penetrate M. This could come about through the conversion of the state of the sodium sites from a liquid condensed type to the solid condensed

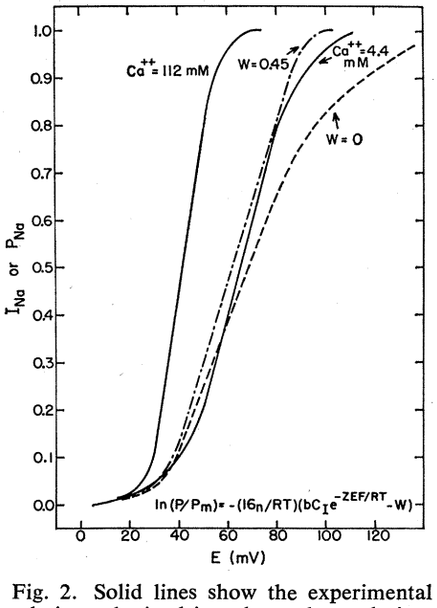

Fig. 2. Solid lines show the experimental relations obtained in voltage-clamped giant axons between the maximum inward (sodium) current, I_{Na}, obtained after prolonged polarizations at the various transmembrane potentials, E, at two different concentrations of calcium in the medium (*20*). Resting potential of these preparations is taken as 50 mv (see text). Broken lines are the corresponding curves in terms of P_{Na}, calculated from the equation shown, for $w = 0$ and $w = 0.45$ dyne/cm.

form, as occurs with stearic acid monolayers on a calcium-containing substrate (*22*). In any case, the introduction of this factor—as the term w in the equation given in Fig. 2—can be seen to lead to an inactivation curve very similar to the experimental one obtained in low calcium when w is taken as less than 0.5 dyne/cm. Increase in w will shift the theoretical curve further to the left, as obtained experimentally by increasing the calcium concentration of the medium.

The present proposal has the advantage that it is in keeping with known phenomena observed in simple physicochemical systems as well as in excitable systems; moreover, the current actually transferred by the postulated lipophilic ions can be negligible compared to that transferred by the inorganic cations they control. The latter situation, as well as the steepness of the permeability-potential relationships, have been pointed out to be critical requirements of a satisfactory molecular hypothesis.

The agreement so far obtained for hypothetical and experimental curves by no means establishes the details of the model. The functional relationships that have been employed are still arbitrary in the sense that they had to be taken from the few monolayer studies available; as such studies are extended to other lipoidal species or mixtures, these relationships may prove less unique. An actual working model is also desirable, especially to explore the possibilities with respect to the kinetics of the permeability changes which have had to be neglected for lack of physicochemical data to approach this problem. Moreover, besides model studies which can of themselves be misleading, research should be carried out on living membranes to test for mechanisms such as have been proposed. One approach would be to compare the effect of different concentrations and types of stabilizers and labilizers on the resting and active permeabilities of the squid giant axon with their effect on, say, gas permeation of monolayers prepared from lipid extracts of axon sheaths. Still another approach would be to add different types of lipoidal molecules to solutions used to perfuse the interiors of giant axons (*23*) in a search for synergistic effects on the permeability changes normally occurring during voltage clamp (*24*).

ABRAHAM M. SHANES
Department of Pharmacology,
University of Pennsylvania
Schools of Medicine, Philadelphia 4

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Inherited Variant of Erythrocyte Carbonic Anhydrase in Micronesians from Guam and Saipan

Abstract. *A variant of one form of red cell carbonic anhydrase was discovered in "Chamorro" inhabitants from the islands of Guam and Saipan. Segregation of this trait in four pedigrees indicates that it is under the control of a single autosomal gene.*

Human erythrocyte carbonic anhydrase has been shown, by the use of electrophoretic and chromatographic separation procedures, to be present in at least two distinct molecular forms (*1, 2*). Because these forms, after separation by starch gel electrophoresis, were first detected in our laboratory by their esterase activity, they were designated as *D* esterases (*3*). When it was demonstrated, however, that these *D* esterases behaved enzymatically as carbonic anhydrase (*4*), they were re-