Some Implications for Biology of Recent Theoretical and Experimental Studies of Ion Permeation in Model Membranes

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This paper is an early, and possibly premature, examination of some consequences of different mechanisms of ion permeation in the hope of providing a basis for progressing beyond the present treatments of bioelectric phenomena (*cf.* Hodgkin, Huxley, and Katz, 1952*a*, *b*, *c*, *d*, *e*) which, in the absence of knowledge of the details of the mechanism by which ions cross biological membranes, have necessarily been phenomenological.

Theoretical Studies (*Conti and Eisenman, 1965a, b, c*)

At the outset it seems important to distinguish whether ion permeation utilizes a fixed-site, mobile-site (*i.e.* "carrier") mechanism, or neither. Some criteria can be deduced for simple models of charged membranes. As a model for membranes having fixed sites, we have chosen a system of two solutions separated by an ion exchange membrane having fixed (but not necessarily uniformly spaced) sites, under the restraints that the membrane is solely permeable to cations or anions, no flow of bulk solution occurs, and the solutions contain no more than two permeant monovalent ionic species. In our model for a membrane having mobile sites it has been necessary to assume in addition that all ions are completely dissociated, behave ideally, and have constant mobilities. It has not been necessary to assume a particular electrical field to carry out our analyses. Rather, it has been deduced that a constant electric field is characteristic only of those fixed-site ion exchangers which have a homogeneous site distribution. Some relevant relationships derived from these models are given below for "ideal" systems in which ionic activities within the membrane are identical to the ionic concentrations (Conti and Eisenman, 1965*a*, *b*, *c*, for more general cases).

The "resting" (*i.e.* zero current) potential of membranes, regardless of whether sites are fixed or mobile, is:

$$V_o = \frac{RT}{zF} \ln \frac{a_1' + \frac{u_2}{u_1} Ka_2'}{a_1'' + \frac{u_2}{u_1} Ka_2''}, \tag{1}$$

where a_1 and a_2 are the activities of species 1 and 2 in solutions (′) or (″), z is their charge, u_2/u_1 is the ratio of their mobilities in the membrane, and K is the equilibrium constant for ion exchange at the membrane-solution interfaces. This equation is formally equivalent to the Goldman-Hodgkin-Katz equation as applied when the membrane is permeable solely to species of one sign, so that one can define the permeability ratio P_2/P_1 in terms of mobility ratio, u_2/u_1, and ion exchange equilibrium constant, K, as:

$$\frac{P_2}{P_1} = \frac{u_2}{u_1} K. \tag{2}$$

When the current is not zero, the potential difference between solution (″) and solution (′) for any fixed current is:

$$V = \frac{RT}{zF} \ln \frac{a_1' - \frac{J_1}{J_2} \frac{u_2}{u_1} Ka_2'}{a_1'' - \frac{J_1}{J_2} \frac{u_2}{u_1} Ka_2''}, \tag{3}$$

where J_1 and J_2 are the fluxes of species 1 and 2. Again the calculated potential difference is not influenced by whether sites are fixed or mobile. We will return to this relationship in discussing mosaic membranes.

The fixed- and mobile-site membranes are distinguished by the current-voltage relationship for singly charged counterions and sites. For a fixed-site membrane, the current is:

$$I = -\frac{zFRT}{S} \frac{1 - \xi}{\alpha' - \alpha''\xi} \left(\ln \xi + \ln \frac{\alpha''}{\alpha'} \right). \tag{4}$$

while for a membrane with mobile sites it is:

$$I = \frac{4zFRTu_1 \sigma}{d^2} \cdot \frac{1 - \sqrt{\frac{\alpha''}{\alpha'}} \xi}{1 + \sqrt{\frac{\alpha''}{\alpha'}} \xi} \cdot \frac{\frac{1}{\alpha'} (1 - \xi)}{1 - \frac{\alpha''}{\alpha'} \xi}. \tag{5}$$

In (4) and (5), $S = \int_0^d \frac{dx}{C_0(x)u_1(x)}$, $\sigma = \int_0^d C_0(x)\, dx$, d is the membrane

thickness, $C_o(x)$ is the concentration of sites at x, $u_1(x)$ is the mobility of counterion 1 at x, and:

$$\xi = \exp \frac{zF(V - V_0)}{RT};$$

$$\alpha' = \frac{1 + K\frac{a_2'}{a_1'}}{1 + \frac{u_2}{u_1}K\frac{a_2}{a_1'}}; \qquad \alpha'' = \frac{1 + K\frac{a_2''}{a_1''}}{1 + \frac{u_2}{u_1}K\frac{a_2''}{a_1''}}.$$

The current I_1 borne by counterion 1 for a fixed-site membrane is:

$$\frac{I_1}{zF} = -\frac{RT}{S}\left[\ln \xi + \ln \frac{\alpha''}{\alpha'}\right] \frac{\left(1 + \frac{u_2}{u_1}r''\right) - \left(1 + \frac{u_2}{u_1}r'\right)\xi}{(1 + r')\left(1 + \frac{u_2}{u_1}r''\right) - (1 + r'')\left(1 + \frac{u_2}{u_1}r'\right)\xi}. \quad (6)$$

while for a mobile-site membrane it is:

$$\frac{I_1}{zF} = \frac{4RTu_1\sigma}{d^2} \frac{1 - \sqrt{\frac{\alpha''}{\alpha'}}\xi}{1 + \sqrt{\frac{\alpha''}{\alpha'}}\xi} \cdot \frac{\frac{1}{1 + r'} - \left(\frac{1}{1 + r''}\right)\frac{\alpha''}{\alpha'}\xi}{1 - \frac{\alpha''}{\alpha'}\xi}. \quad (7)$$

In (6) and (7):

$$r' = K\frac{a_2'}{a_1'}, \qquad r'' = K\frac{a_2''}{a_1''}.$$

COMPARISON OF THE STEADY-STATE PROPERTIES OF MEMBRANES HAVING FIXED VS. MOBILE SITES

A number of relationships, such as Equations 1 and 3, are independent of the distribution of sites in fixed-site membranes. All such relationships are also independent of whether or not the sites are fixed or mobile.

On the other hand, those relationships in fixed-site membranes (Equations 4 and 6) which contain S depend on the distribution of sites and are different in fixed-site and mobile-site membranes. The differences are illustrated in Fig. 1 for the current-voltage relationship and in Fig. 2 for the relationship between individual ionic fluxes (or currents) and voltage. Notice that displacements of the voltage of less than ± 125 mv from the resting potential do not clearly distinguish fixed- from mobile-site membranes.

The resting potential difference (V_o) across an ion exchange membrane having fixed sites is independent of time, once boundary equilibria have been established. This suggests that one could interpret permeability ratios meas-

ured during a transient such as the action potential in terms of a steady-state theory (Hodgkin and Katz, 1949), provided that there were sites involved which behaved as if they were fixed during the time of the transient and that current flow was negligible. On the other hand, the calculated time independence of the resting potential indicates that an experimentally observed time independence of a membrane potential cannot be used to rule out the presence of a significant diffusion potential (*cf.* Ling 1962, 275–278).

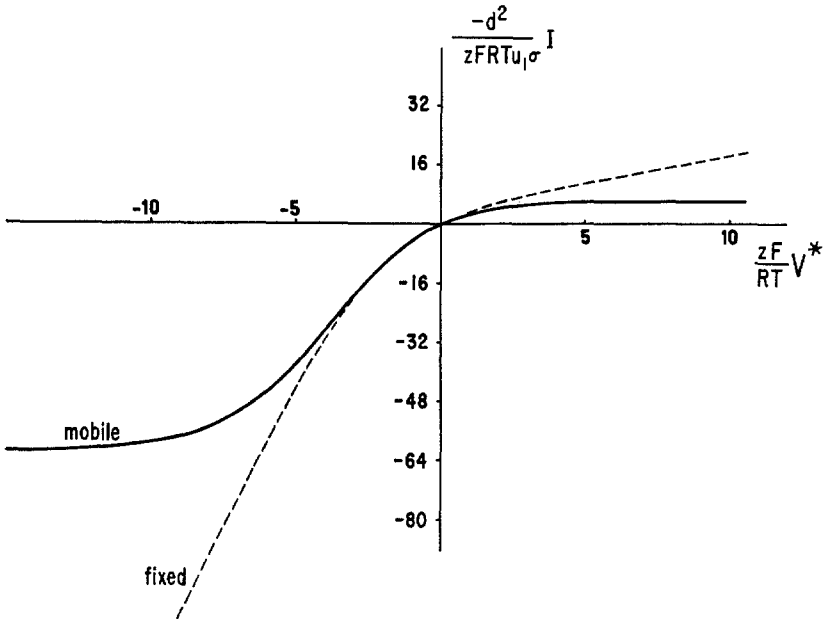

FIGURE 1. Current-voltage relationship (Conti and Eisenman, 1965*c*). $-Id^2/zFRTu_1\sigma$ is plotted as a function of V^*zF/RT for the values of the parameters $u_2/u_1 = 30$, $r' = 1$, $r'' = 0.02$ for the case of a mobile-site ion exchange membrane (continuous line) and for a membrane with fixed uniformly distributed sites (dashed line). $V^* = V - V_o$.

The principal formal difference between the properties of the membrane assumed by Hodgkin and Katz (1949) and those of a fixed-site ion exchanger with uniformly spaced sites results from the different conditions chosen to describe the ionic concentrations at the membrane-solution interfaces. If one replaced Hodgkin and Katz's boundary condition 3, "that the concentrations of ions at the edges of the membrane are directly proportional to those in the aqueous solutions bounding the membrane," by the boundary condition appropriate to an ion exchange membrane, the treatments would be identical for the case of zero anion permeability. It is therefore desirable to test which of these boundary conditions is more consistent with the properties of biological membranes. For example, the different boundary conditions lead to a

difference in expectation for the I-V behavior when the ionic strength of the solutions on either side of the membrane is varied. Thus, if the ionic concentrations of the external or internal media are diluted, the Hodgkin and Katz boundary condition requires that the membrane conductance vary proportionally with the solution concentration; while the ion exchange boundary condition requires that membrane conductance be constant.

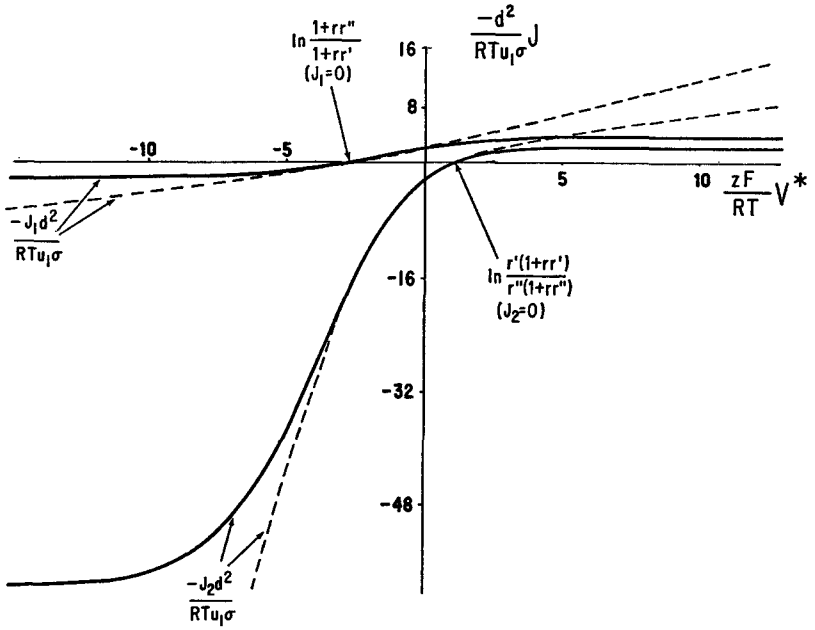

FIGURE 2. Flux-voltage relationship (Conti and Eisenman 1965*c*). $-J_1d^2/RTu_1\sigma$ and $-J_2d^2/RTu_2\sigma$ are plotted as functions of $V^* zF/RT$ for the values of the parameters $u_2/u_1 = 30$, $r' = 1$, $r'' = 0.02$ for a mobile-site exchange membrane (continuous lines) and for a membrane with fixed uniformly distributed sites (dashed lines). Notice that the potentials for which J_1 or J_2 becomes zero are the same for fixed- and mobile-site cases, being respectively the equilibrium potentials for species 2 and 1. $r = u_2/u_1$; $V^* = V - V_o$.

The design of experiments for such a confrontation requires, among other things, recognition of the fact that biological membranes generally are not solely permeable to either cations or anions. It may therefore be necessary to carry out experiments using anions (or cations) to which the membrane is not permeable. Alternatively, if anions and cations permeate biological membranes through a mosaic of separate "pathways," such a system would be characterized by local eddy currents even when net membrane current is zero. The treatments of Conti and Eisenman (1965*b*, *c*) provide a method of analysis of this more complex situation; for this complication may be met by assuming that the total potential difference between the two aqueous solutions is the same across each permeation pathway. Under this assumption,

one can deduce in the case of fixed-site pathways, for example, that when a single salt is present at activities a' and a'' on the two sides of the membrane, the resting potential, E_o, is:

$$E_o = \frac{S^- - S^+}{S^+ + S^-} \frac{RT}{F} \ln \frac{a''}{a'}, \tag{8}$$

where S^- and S^+ are the values of S for the anion and cation "pathways," respectively. Equation 8 indicates that one would expect the resting potential to have a linear slope, less than that of the Nernst equation, as a function of dilution of the solutions on either side of the membrane.

Equation 3 indicates that under non-zero current conditions the apparent permeability ratio is given by:

$$\frac{P_2(\text{apparent})}{P_1(\text{apparent})} = -\frac{J_1}{J_2}\frac{P_2}{P_1}. \tag{9}$$

Should cations and anions cross biological membranes through separate pathways, the effect will be to produce local circuits of current in the absence of any net membrane current. In this case, the permeability ratio would not be constant as in the Goldman, Hodgkin, Katz equation but would vary as a function of the membrane potential. (Notice that for the particular currents on Fig. 2 for which J_1 or J_2 is zero the apparent permeability ratio of species 2 to 1 becomes zero or infinite, respectively; and Equation 3 reduces to the Nernst (*i.e.* "equilibrium") potential for species 1 or 2.)

Experimental Studies in Membranes with Fixed Sites (Eisenman, 1965)

Let us now turn from theoretical considerations to some experiments specifically directed to the question of the origin of specific ion permeabilities in a typical fixed-site membrane, the glass electrode. The Na^+ to K^+ permeabilities of five well aged electrodes of about 1 cm^2 surface area and 0.1 mm thickness from two different batches (designated types I and II) of K^+-selective NAS 27-4 glass were characterized by measuring the potentials in mixed aqueous solutions (see Eisenman, 1965, for methods). The self-diffusion coefficients of Na and K in the hydrated glass surface were then measured by exposing the electrodes to Na^+ and K^+ solutions labeled with Na^{24} and K^{42} and studying the uptake of tracer as a function of time and of solution composition. The experimental methods and detailed results are given elsewhere (Eisenman, 1965), but certain major conclusions are worth mentioning here. The observed diffusion and ion exchange properties of NAS 27-4 glass indicate that its chemical properties are sufficiently uniform over the entire hydrated thickness for the theory of Conti and Eisenman (1965*a*) to be applicable. Other glasses (notably those having high Na^+ selectivity) show a more

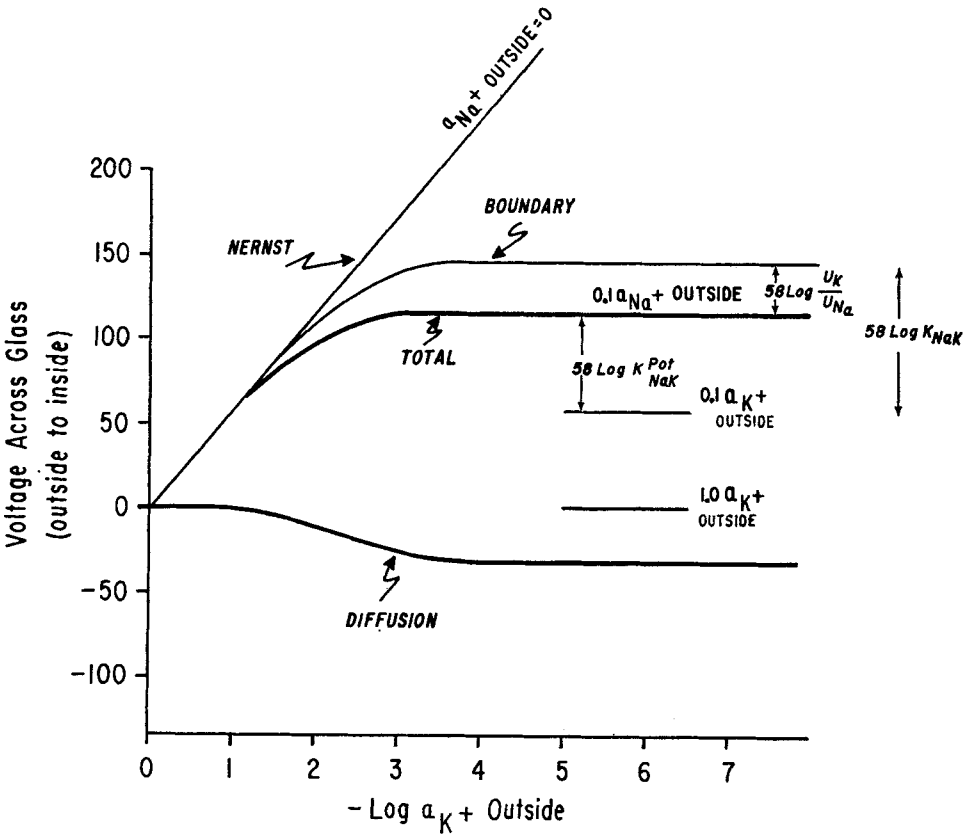

FIGURE 3. Diagram of the relative contributions of diffusion and phase boundary potentials to the total potential of a K^+-selective glass electrode (Eisenman, 1965). This figure summarizes the various contributions to the total potential in a typical NAS 27-4 (type I) glass electrode filled with a solution of 1.0 N K^+ activity when dipped in solutions having a constant 0.1 N Na^+ activity and variable K^+ activity. The voltage (in millivolts) is plotted as a function of the negative logarithm of the outside K^+ activity. The observed potential in this situation follows the heavy curve labeled "total". The curves labeled "diffusion" and "boundary" give the diffusion potential and the sum of the boundary potentials, respectively, under these conditions. When the outside solution contains no Na^+, the Nernst potential is observed. Notice that the boundary potential differs from the total potential by the value of the diffusion potential. The two horizontal lines labeled "0.1 a_{K^+} outside" and "1.0 a_{K^+} outside" represent the values of potential which would be observed in pure K^+ solutions of these activities. Interrelationships among the values of potential are given by the arrows. The parameter "K^{pot}_{NaK}" is identical with P_K/P_{Na}. "K_{NaK}" corresponds to K.

complex behavior indicating that only a thin surface layer is hydrated, with the result that the electrode properties are a mixture of hydrated surface and "dry" bulk glass properties. For this reason we will restrict our considerations here to K^+-selective electrodes.

It has been found that both the mobility ratio and the ion exchange equilibrium constant contribute to the permeability ratio of K^+ to Na^+ in hydrated glass with the peculiarity that the more permeable ion (K^+) is the less mobile. Typical values of permeability ratio, mobility ratio, and ion exchange equilibrium constant for the two batches of K^+-selective glass electrodes are given below:

	P_K/P_{Na}	u_K/u_{Na}	K_{NaK} (calculated)
Type I glass	10.3±0.1	0.30±0.09	34±10
Type II glass	8.5±0.3	0.15±0.05	55±17

The relative contributions of the diffusion and phase boundary potentials to the total potential of a K^+ electrode are illustrated diagrammatically in Fig. 3 using the parameters from type I glass.

In addition it has been found that hydration of glass increases the mobility of Na^+ by about 4 orders of magnitude over its value in dry glass. The largest value observed for the self-diffusion constant of sodium ion in hydrated glass has been 10^{-10} cm^2 sec.$^{-1}$ which implies an electrical mobility in hydrated glass of the order of 4×10^{-8} cm/sec. per volt/cm. The mobility of Na^+ in a hydrated glass can therefore be greater than that (10^{-9} cm/sec. per volt/cm) calculated for the resting squid axon by Cole (1964), but it is still some 5 orders of magnitude lower than that in aqueous solution.

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