Membrane Structure and Ion Permeation

Study of ion exchange membrane structure and function is relevant to analysis of biological ion permeation.

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This article is concerned with the relationship between membrane structure and membrane permeation by ions. With the increasingly diverse types of ion-exchange membranes available through recent progress in chemistry [for example, solid (*1*) and liquid (*2–4*), organic (*5, 6*) and inorganic (*7, 8*), porous (*9*) and nonporous (*10*)], the effects of membrane structure on membrane properties are becoming better understood (*11, 12*). Such understanding is relevant to the development of physical explanations for the bioelectric and ion-transport properties of living membranes, the subject of our primary interest. Despite the increasingly broad (*13*) and detailed (*14*) descriptions of biological ionic permeability and membrane structure (*15, 16*) that have been given, the physical mechanisms by which ions cross such membranes are not known (*13*) and are the subject of diverse speculations. For example, it has been postulated that ion permeation utilizes one or more of the following mechanisms: pores lined by fixed charges (*17, 18*), lipid-soluble carrier molecules (*19*), diffusion in a homogeneous dielectric medium having rate-limiting "gating mechanisms" at its surface (*20*), and translocation of vesicles (*21*).

A variety of membranes can be constructed whose structure is well defined and whose permeation mechanisms and particular parameters can be varied in a controlled manner. By correlating structure and ion permeation in such membranes it should be possible to develop experimental criteria for determining the structure, and hence the mechanism, of ion permeation through an unknown membrane. In an attempt to develop these criteria we have examined such variables as the presence or absence of ion-exchange sites, their fixation or freedom of motion, their degree of dissociation, and the extent to which their chemical properties depend on external forces. In order to present clearly a number of important concepts we emphasize certain recent results, at some sacrifice to completeness of coverage of the subject. The reader is therefore referred elsewhere (*1, 5, 11*) for excellent reviews.

We start by assuming that ion permeation across all membranes, whether nonliving or living, is governed by the same laws, and we postulate that the characteristics of a particular membrane are direct consequences of the type of ion-transport mechanism within the membrane and of the boundary conditions at the membrane-solution interfaces. As far as the interior of any homogeneous membrane is concerned, only four mechanisms of ion transport can be distinguished—namely, ion migration involving (i) a collision mechanism (essentially frictional), (ii) a jump mechanism (from site to site), (iii) a carrier mechanism, and (iv) a solvent drag mechanism. Ions may distribute at the boundary according to a Donnan equilibrium (*18, 22, 23*), according to more specific ion-exchange equilibria (*72, 24, 25*), or according to partition coefficients (*26, 27*). The boundaries may also contribute to the membrane properties through the presence of unstirred layers (*1*), high-energy barriers (*28*), or particular macromolecular structures (*20*).

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A Classification of Membranes According to Structure

A membrane can be categorized (see Fig. 1) according to whether it contains sites for ion exchange or is site-free, and, if it is an ion exchanger, according to whether its sites are fixed or mobile and whether the sites and their counter-ions are associated or dissociated. We restrict this discussion to membranes whose properties may be considered to be homogeneous in the plane of the membrane, and we do not deal explicitly with the complexities which result from either mozaic membranes, in which local eddy currents occur (*29*), or series membranes, in which space-charge regions exist analogous to those at a *p-n* semiconductor junction (*30*).

Site-free membranes. The simplest type of membrane is the site-free membrane, by which term we mean only that the number of ionic charges *confined* to the membrane (and therefore capable of functioning as ion-exchange sites) is negligible. In contrast to the ion-exchange membranes considered below, all charged species in the site-free membrane are free to cross the membrane-solution interfaces. Membranes of this type can be formed of any uncharged material that will form a phase which is immiscible with the solution phases, hereafter considered to be aqueous. Such a membrane can be a solid (such as paraffin or quartz) or a liquid (such as benzene or silicone oil).

Although selective ion permeabilities have often been proposed for site-free membranes, these have usually been assumed to result from the presence of small numbers of particular "carrier" species (*14*), or from ion-specific barriers at the boundaries (*20*). However, site-free membranes can also be selectively permeable to particular ions as a result of a lower friction or of a more favorable energetic environment for one species than for another. For example, in a typical insulator such as quartz the activation energies for migration of monovalent cations are substantially lower than the activation energies of anions or more highly charged cationic species (*31*) because ionic migration takes place in an environment of fixed (electronegative) oxygens. A solid quartz insulator therefore behaves not only like a system semipermeable for monovalent cations, but also like one in which the mobilities of the different cations are quite different from each other (*32*) due to the balance of attrac-

tive and repulsive energies. A selective permeability can also exist in a liquid. For example, it has been found in nitrobenzene that cesium is a much more permeant species than fluoride and that iodide is a much more permeant species than lithium (*33*). These observations are understandable if we realize that various ionic species will be partitioned in accordance with their solvation energies in water and in the nonaqueous membrane phase. Thus, when electrostatic interactions dominate the solvation energy, those species having the larger charge and the smaller size (and hence higher hydration energies) tend to be excluded from solvents having dielectric constants lower than that of water.

From these considerations it seems reasonable to suppose that the properties of site-free membranes are similar to those of ion-exchange membranes and that there are no properties unique to site-free media. It should also be noted that the process of adding small numbers of sites to a neutral solid or liquid involves passage through systems of ionic semiconductors.

Solid ion exchangers. If one adds dissociable groups to a solid insulator (for example, if one adds Na_2O to SiO_2), the insulator becomes a fixed-site ion-exchange membrane whose conduction of electricity is electrolytic. The usual ion exchanger can be regarded as consisting of interpenetrating networks of ion-exchange matrix and aqueous channels or "pores." The sites of the ion exchanger matrix are its dissociable groups, which are fixed and therefore cannot cross the membrane-solution interfaces. The permeant species are the counter-ions, which neutralize the charge of the sites, and the co-ions, whose sign is the same as that of the sites. Recognizing that the matrix might be an inorganic mineral ion exchanger (for example, a zeolite or glass) as well as the more usual organic resin phase, one can describe all solid ion exchangers in terms of a spectrum in which the diameter of the aqueous pores is the principal variable. We consider only the extremes of this spectrum here. At one extreme, where the pores are sufficiently wide, we can neglect specific interactions between the ions in the pore and the ion-exchange sites lining the walls. The membrane properties are then determined by the movements of ions in a thermodynamically homogeneous phase which is an aqueous solution of normal bulk properties except for the constraint on the concentrations of counter-ions and co-ions implied by the charged sites on the walls of the pore (*18, 34*). At the other extreme, where the pore is so small (for example, less than about 10 angstroms in diameter) that there is no longer any liquid water [but only "bound" water (see *35*)], a situation is approached in which only site-to-site migration occurs, essentially as would occur in the anhydrous matrix. The extreme limit of this case occurs when water is totally excluded from the exchanger and the "pores" are just the interstices of the anhydrous matrix.

Because the counter-ions and sites in wide-pore solid exchangers can be regarded as completely dissociated, while in narrow-pore solid exchangers they may be thought of as associated, we have classified wide-pore solid exchangers in Fig. 1 as FD (fixed-dissociated) systems and narrow-pore solid exchangers as FA (fixed associated) systems, to emphasize the analogy to the dissociated and associated states to be discussed for liquid exchangers.

Solid ion exchangers may be regarded as models for the pores lined by fixed charges postulated to exist across biological membranes (*17, 18*).

Liquid ion exchangers. If one dissolves, in a site-free oil membrane, a water-insoluble organic ion, one obtains a liquid ion-exchange membrane having mobile sites. The sites are the charged groups on the organic molecules, and with them will be associated counter-ions which are free to exchange with ions of the same sign in the aqueous phases. Note that, although the sites are confined to the oil phase, they are free to move within that phase. The situation in liquid exchangers is simpler than that in solid exchangers in that the membrane can be regarded as a single phase; but two new properties are encountered which complicate any analysis. First, the sites, not being rigidly anchored to the matrix, can to some extent cross the membrane-solution interface and participate in the boundary reactions, so that an ion-exchange equilibrium will only approximately represent the boundary conditions, the "sites" redistributing between the aqueous solutions and the membrane according to their partition coefficients. The partition coefficients, in turn, may depend on solution concentrations; for example, the sodium salt of the typical cation exchanger di-2-ethylhexyl phosphoric acid is insoluble in concentrated salt solutions but is quite soluble in dilute aqueous solutions (*36*). Second, in response to external forces, since the sites are no longer fixed in space, their concentrations will rearrange within the boundaries of the membrane, and this rearrangement will lead to important changes in permeation properties (*18, 37*).

In addition, the possible occurrence of associated sites and counter-ions, with formation of diffusible neutral molecular species (or higher-order aggregates), introduces the possibility that diffusion fluxes, which are much larger

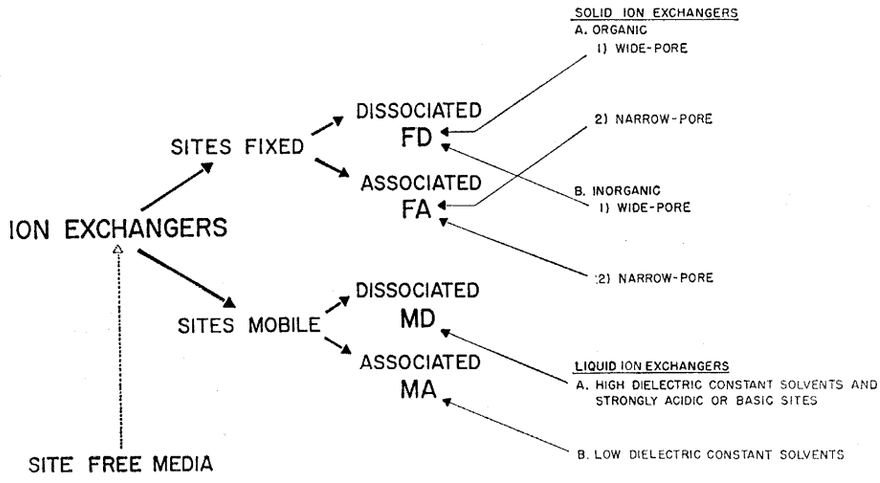

Fig. 1. A classification of membranes according to their structure. *Solid ion exchangers: A-1,* wide-pore loosely cross-linked resins (*1*) and "wet" collodion (*85*) (see also *5, 9, 18, 22, 23, 34, 44, 45, 62, 86, 87*); *A-2,* narrow-pore tightly cross-linked resins (*1, 87*), dried collodion (*24, 88*), and oil-impregnated millipore filters (*6, 72*); *B-1,* wide-pore loosely compacted minerals (*7, 8, 89, 90*) and sectioned minerals (*91, 92*); *B-2,* narrow-pore glass electrodes (*59*), oil-impregnated zeolites (*8*), and highly compacted minerals (*7, 90*) (see also *10, 31, 46, 55, 56, 59, 60, 70, 75*). *Liquid ion exchangers: A,* high dielectric constant solvents and strongly acidic or basic sites (*26*; see also *3, 28, 37, 57, 58, 93*); *B,* low dielectric constant solvents (*94*; see also *2, 4, 38, 74*).

than fluxes expected from measurements of electrical resistance, will occur, as well as coupled flows of counter-ions.

Liquid ion-exchange membranes can be made by using any of a number of water-immiscible solvents having dielectric constants covering a wide range of magnitudes. The trapped ion-exchange species must partition strongly in favor of the membrane phase (*38*). The usual anionic liquid exchangers are primary, secondary, and tertiary amines, while the usual cationic exchangers are alkyl phosphoric acids (*1, 2, 38*).

Liquid ion exchangers may be regarded as models for lipid-soluble carriers postulated to exist in biological membranes (*19*). The biological analogs of the solvent and the trapped species seem likely to be the unsaturated hydrocarbon tails of the membrane phospholipids and the monomeric fatty acids (or phosphorylated monoglycerides), respectively.

Theoretical Approaches

In general, three different approaches have been used in characterizing ion permeation: the Nernst-Planck flux equations and extensions thereof; the theory of irreversible thermodynamics; and the transition (rate-process) theory. All these approaches are based on the postulate that thermodynamic equilibrium exists locally (*39; 40*, p. 60). The first approach was formulated by Nernst (*41*) and Planck (*42*), who derived the flux equation in a differential form. When the local thermodynamic properties of the membrane are constant or are known functions of position within the membrane, this formulation leads to a set of differential equations which can be solved for any given set of boundary conditions (*43*). With this method it has been possible to predict adequately the steady-state properties of various membranes (*9, 18, 44–46*) as well as many aspects of their kinetic behavior (*13, 47*). The limitation of this approach is the requirement for detailed knowledge about the structure and thermodynamic properties of the membrane.

The theory of irreversible thermodynamics (*48, 49*) provides a means of avoiding this limitation and permits one to make a phenomenological description of the membrane, regarding it either as a continuous phase or as a surface of discontinuity (see *1*, p. 342) and interrelating such properties as ionic fluxes, water transport, and electric potentials without specifying the ion permeation mechanism (*50*). In its usual (linear) form the theory of irreversible thermodynamics also postulates the existence of linear relationships between fluxes and forces, and the system is then entirely determined by the nature of the coefficient matrix. Assuming microscopic reversibility, Onsager (*51*) has shown that this coefficient matrix is symmetric, a finding which diminishes the number of independent measurements needed to characterize the transport phenomena. Although this method describes the magnitude and coupling between various transport processes and can be used to obtain criteria for stability (*48*) as well as for active transport (*52*), it cannot be used to describe kinetics or the details of such steady-state properties as the current-voltage relationship or to distinguish among various types of transport mechanisms.

The third theoretical approach to membrane transport is the transition theory (*53*). According to this theory, the transport of an ion or molecule occurs by a series of discrete jumps where each ion or molecule has to cross an energy barrier between adjoining equilibrium positions. To this physical picture, Eyring *et al.* (*54*) and Ciani (*55*) have applied a statistical mechanical theory of rate processes and obtained results which are in good agreement with those obtained from the kinetic and phenomenological approaches. The transition theory, in principle, offers a wider range of application than the kinetic approach but requires an even more detailed knowledge of membrane structure for quantitative conclusions.

Distinguishing Features of Ion Permeation in Various Systems

Let us now turn to what is known about ion permeation in the systems outlined above and examine how this depends on structure. It is usual to characterize ion permeation by such measurable properties of the membrane as current-voltage (*I-V*) relationships, ionic fluxes, and membrane potentials. Many times it is also useful to measure the concentration profiles within the membrane (*18, 56–58*), as well as the partition coefficients by equilibrium methods (*6, 27*).

Solid membranes (fixed-dissociated and fixed-associated systems). Ion permeation in a solid, fixed-site membrane may be considered to be under the influence of such factors as (i) the available pore area of the membrane and (ii) the tortuosity of the pores—factors which are purely geometrical. In addition, specific interactions with the pore walls occur which manifest themselves in (iii) ion-site and (iv) ion-matrix interactions. Solvent drag (v) can also occur within the pore, and the possibility (vi) must also be considered that the chemical properties of the exchanger might alter as a function of ion exchange. For example, the degree of hydration of crystalline ion exchangers and their unit cell dimensions are functions of the counter-ions (*60*), which implies that chemical properties will depend on the counter-ion mole fractions in those systems in which mobilities and standard chemical potentials are strong functions of hydration or unit cell size (*59, 61, 62*).

Fixed-dissociated membranes. In FD membranes with a pore diameter sufficiently large that the majority of permeant ions are moving in what amounts to a free aqueous solution, only factors (i), (ii), and (v) are important. For those membranes in which solvent drag (v) is negligible, the properties of the membranes have been deduced by considering that the pores contain an aqueous solution of counter-ions and co-ions, whose concentrations are determined by Donnan equilibria with the external solutions (*18, 44, 45*). Here the current-voltage relationship is determined by the free-solution mobilities and by the external compositions. The *I-V* properties are also a function of the fixed charge density (*18, 23, 44*) in the FD systems, which imperfectly exclude co-ions, as opposed to the FA systems, which highly exclude co-ions, and in which the rectification properties are independent of the fixed charge density (*46, 63*).

Perhaps the most distinguishing feature of FD systems is the possibility of solvent drag (factor v) in these. The fixed charges on the pore walls create electric double layers, giving rise in the presence of an applied field to electroosmotic water flow and to such related phenomena as anomalous osmosis and ion sorting (*64*). Another interesting effect involving electroosmosis was first observed by Teorell (*65*)—namely, the existence of regions of negative resistance in the current-voltage relationship (Fig. 2) and the possibility of self-sustained oscillations. These effects result from a coupling between pres-

sure, electric forces, and osmotic forces and offer an interesting model for biological "all-or-none" responses [that is, excitability phenomena (see *66*)].

Fixed-associated membranes. When one considers increasingly narrower pores, factors iii and iv become of greater importance. In particular, specific interactions between the movable species and the sites (as well as the matrix) enter in such a way as to alter the mobilities and standard chemical potentials from the values characteristic of aqueous solutions. Finally, when the pore diameter is decreased sufficiently, we reach a limit (an FA system) in which there is no free solution (all water being "bound"), and factor (v) can be neglected, while the purely geometrical factors (i and ii) are no longer appropriate. At this limit, it is probably most convenient to regard the exchanger as a single thermodynamic phase from which co-ions are completely excluded and in which the standard chemical potentials and mobilities of ions are very different from those characteristic of an aqueous solution. Now the Donnan boundary conditions must be replaced by boundary conditions characteristic of an ion-exchange equilibrium in which specificity differences among counter-ions bearing the same charge (for example, Na^+ and K^+) become apparent (*25*; see *67*).

The FD and FA systems also differ in the magnitudes and characteristics of the mobilities and activation energies of the permeant species. Thus, the mobilities and activation energies for diffusion in FD systems have the values characteristic of diffusion in an aqueous solution and are therefore relatively insensitive to the swelling or shrinking of the ion-exchanger matrix or of the degree of ion exchange. In contrast, the mobilities in FA systems are much smaller than those in aqueous solutions, while the activation energies are much higher (*59, 61, 68*). Moreover, whether mobilities and activation energies in FA systems are constant or are dependent on mole fraction is a function of the mechanism of ion migration. For example, if a defect mechanism (*40*) [for example, a simple vacancy or, more probably, a Frenkel vacancy pair (*69*)] should be involved in diffusion in FA systems, the mobilities will be dependent on mole fraction, since the concentration of vacancies is a function of the degree of exchange. On the other hand, if diffusion does not involve a defect mechanism, mobilities should be constants independent of the degree of exchange, provided that the chemical properties of the exchanger remain constant.

We can clarify the foregoing points by citing some experimental results. In FD systems, Mackay and Meares (*9*) have recently shown a substantial agreement between the observed properties of an organic ion-exchange membrane interposed between NaCl solutions and properties theoretically expected when the mobilities were assumed to be constants having the values characteristic of diffusion in aqueous solution. Doremus (*56, 70*) has shown, for silver-sodium diffusion in an FA system (dry glass), perfect agreement with the expectations of the Nernst-Planck flux equations, with constant mobilities of silver and sodium.

On the other hand, a mole-fraction-dependence of mobilities has been found in an FA system (a thin hydrated glass membrane) by Eisenman and Sandblom (*71*). This is illustrated in Fig. 3, where the observed electrical resistance of a membrane interposed between identical solutions of varying Na^+-K^+ composition is plotted as a function of the mole fraction of potassium (X_K) in solution. The dashed curve illustrates the resistance to be expected if mobilities were constant, and the observed maximum in resistance indicates that mobilities are mole-fraction-dependent. Despite this complexity, the steady-state membrane potentials under zero-current conditions are described precisely in terms of the mole fraction of Na^+ and K^+ in solution by a generalized Nernst equation:

$$V_o = \frac{RT}{F} \ln \frac{X'_{Na} + (P_K/P_{Na})X'_K}{X''_{Na} + (P_K/P_{Na})X''_K} \quad (1)$$

where $P_K/P_{Na} = 8.5$ corresponds to the K^+: Na^+ permeability ratio and the superscripts refer to the solutions on the two sides of the membrane. From the data of Fig. 3 and Eq. 1 it is possible to calculate two sets of conductances, $G_K(X_K)$ and $G_{Na}(X_{Na})$, and then to solve explicitly for the current-voltage relationship under various solution conditions through use of Eqs. 2 and 3 (*63*):

$$\frac{I_K}{I_{Na}} = \frac{P_K}{P_{Na}} \cdot \frac{X''_K \exp\left[\frac{FV}{RT}\right] - X'_K}{X''_{Na} \exp\left[\frac{FV}{RT}\right] - X'_{Na}}, \quad (2)$$

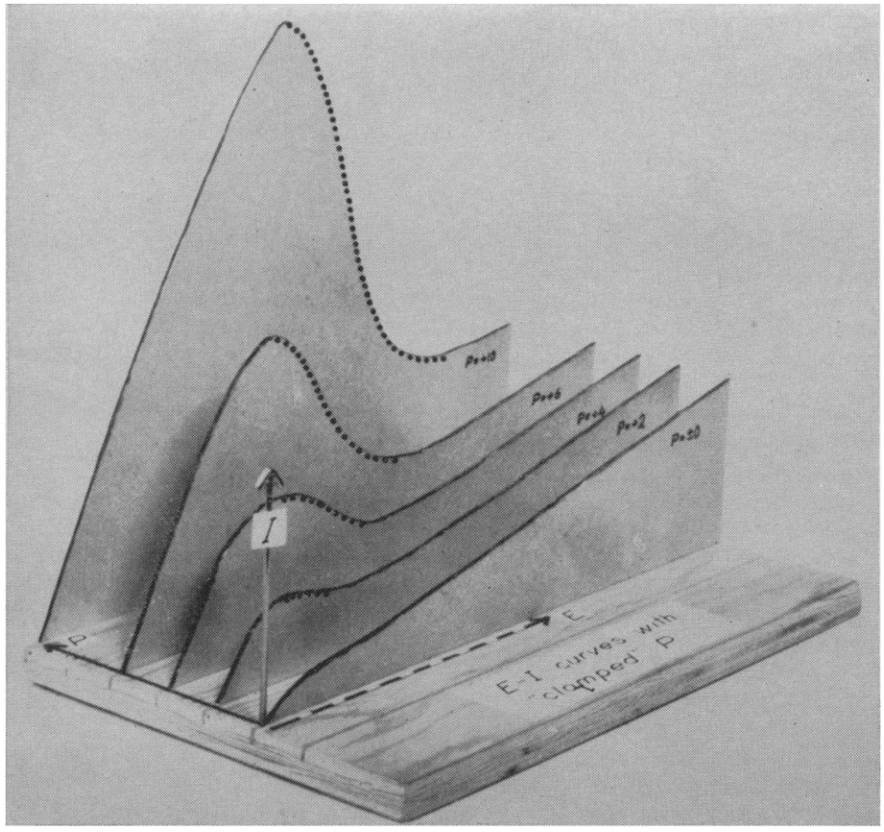

Fig. 2. The phase space diagram of the relations between the three state variables (pressure, voltage, and current, labeled *P*, *E*, and *I* here, respectively) of the electrohydraulic analog. Dotted lines indicate negative resistance. [Reproduced with permission of the *Biophysical Journal* after Fig. 16 of Teorell (*65*)]

$$I = \frac{RT}{F} \cdot \frac{\left(X''_K + \frac{P_{Na}}{P_K} X''_{Na}\right) \exp\left[\frac{FV}{RT}\right] - \left(X'_K + \frac{P_{Na}}{P_K} X'_{Na}\right)}{\exp\left[\frac{FV}{RT}\right] - 1} \cdot$$

$$\cdot \int_{X'_K}^{X''_K} \frac{G_K(X_K)\, dX_K}{X_K \left[X_K - \frac{X''_K \exp\left[\frac{FV}{RT}\right] - X'_K}{\exp\left[\frac{FV}{RT}\right] - 1}\right]} \qquad (3)$$

where T is the absolute temperature, R is the gas constant, F is the Faraday constant, I_{Na} and I_K are the currents carried by Na^+ and K^+, and the total current $I = I_{Na} + I_K$. This method is not based on any assumptions about the thermodynamic properties of the membrane other than the assumption that they are functions only of the local mole fractions at every point in the membrane. Using Eqs. 2 and 3, we can calculate the individual currents and the current-voltage relationship without solving for the internal concentration profiles. Typical observed and computed *I-V* relationships are illustrated in Fig. 4. It should be noted that the complex behavior of these relationships, as well as the resistance maximum in Fig. 3, can be attributed to the existence of a Frenkel defect mechanism of migration in hydrated glass or, alternatively, to a dependence of the chemical properties of the glass on the degree of exchange. Similar observations have recently been made by Ilani (*72*) who also suggests a Frenkel defect mechanism for oil-filled fixed-site membranes. It should also be noted that, in a system having mole-fraction-dependent mobilities, neither measurements of fluxes nor *I-V* relationships contain enough information for calculating separately the mobility ratios and the ion-exchange equilibrium constant (*63*). This is in contrast to the situation where mobilities are independent of mole fraction—a situation for which it has been stated that the *I-V* relationship can be used to measure separately the ion-exchange equilibrium constant and the individual ionic mobilities (*46*).

Another important distinction between FD and FA systems appears because of factor vi. Although wide-pore (FD) systems are likely to have constant chemical properties regardless of counter-ion profiles (since the mobilities and standard chemical potentials of the ions in the pore fluid should be uninfluenced by these profiles), such is not the expectation for narrow-pore (FA) systems. In the latter case, differences in partial molal volumes may lead to pressure gradients within the membrane or to volume changes (associated with alterations of the degree of hydration) as a function of the concentration of a particular counter-ion species at a given point. Since mobilities (*59*, chapter 5; *61*) and standard chemical potentials (*24, 73*) are likely to be very sensitive to the extent of hydration, a strong dependence of mobilities (and also of equilibrium constants) on the concentration profiles of the counter-ions is to be expected except under very particular circumstances.

The kinds of complexities which can result in FA systems when such effects come into play are illustrated in Fig. 5 by the current-voltage characteristics of the above-described glass membrane when it separates NaCl and CsCl solutions (*33*). For these ions, pronounced hysteresis phenomena are observed. The solid curve of Fig. 5 indicates the characteristic voltage-current relationship observed in one membrane "state," while the dashed curve is observed in another "state." The resistance of the membrane when filled with Na^+ (third quadrant) may be seen to differ by a factor of more than 7 in the two states. Repeated transitions between these two states can be made by appropriate applied electric currents and by alterations of the composition of the external solutions.

It is clear that an FA membrane can exhibit a metastability of its electric resistance characteristics—a finding not unexpected in the light of the known metastability of the equilibrium ion-exchange properties of aluminosilicate minerals (*62*). Since metastability properties appear to be intrinsic to the biological excitation process (see *66*), their existence in a simple FA system without any specialized chemical structure is of some biological interest. Indeed, it is not out of place to speculate that metastability characteristics may be general features of simple ion-exchange membranes and do not require highly specialized structures for their existence.

Liquid membranes (completely dissociated and associated systems). A mobile-site membrane may be regarded as one which differs from a fixed-site membrane only through the rearrangeability of its sites. We have calculated the steady-state properties of such a system, assuming complete co-ion exclusion, after deducing the concentra-

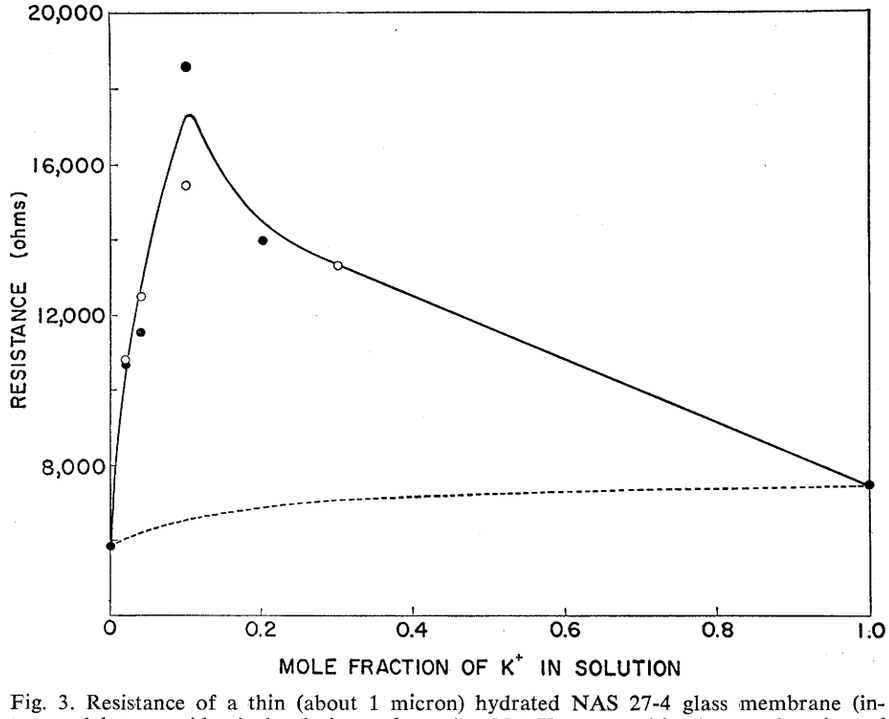

Fig. 3. Resistance of a thin (about 1 micron) hydrated NAS 27-4 glass membrane (interposed between identical solutions of varying Na^+-K^+ compositions) as a function of the mole fraction of K^+ in solution (*71*). The dots and open circles refer to two series of measurements of the resistance, which was found to be time-independent and voltage-independent under these experimental conditions.

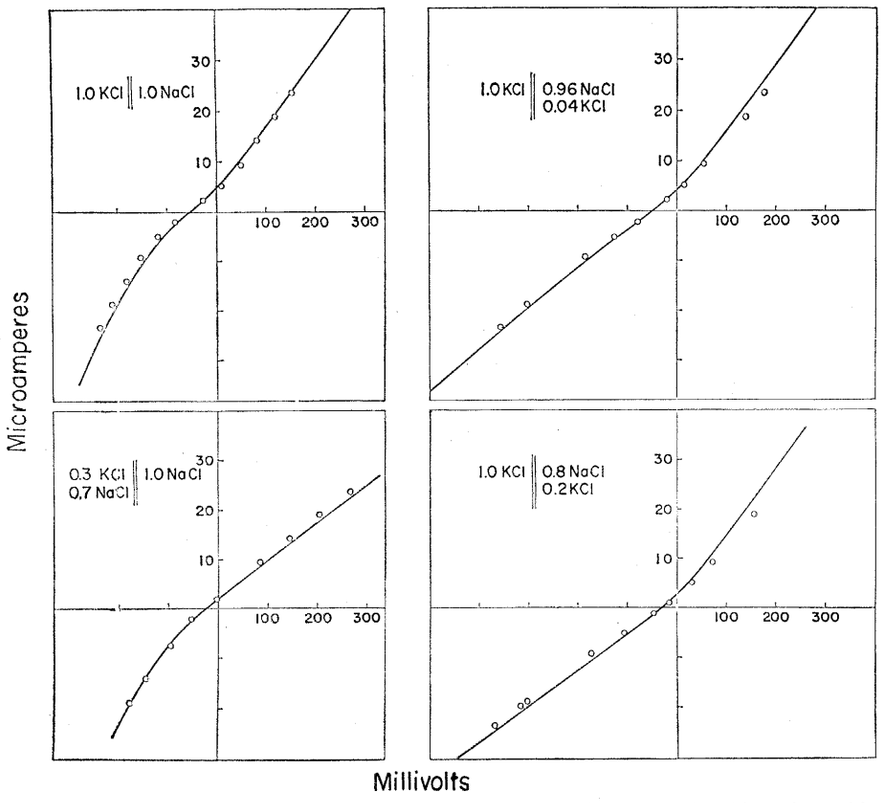

Fig. 4. Computed and observed steady-state current-voltage relationship of a thin (about 1 micron) hydrated NAS 27-4 glass membrane when interposed between solutions having the indicated compositions (*71*). The continuous curves are computed from the data of Fig. 3 and Eqs. 1–3; the open circles are the experimentally measured values. Positive current flows from left to right of the indicated systems. (Solution conditions on the two sides of the membrane are given in the inserts of the subfigures.)

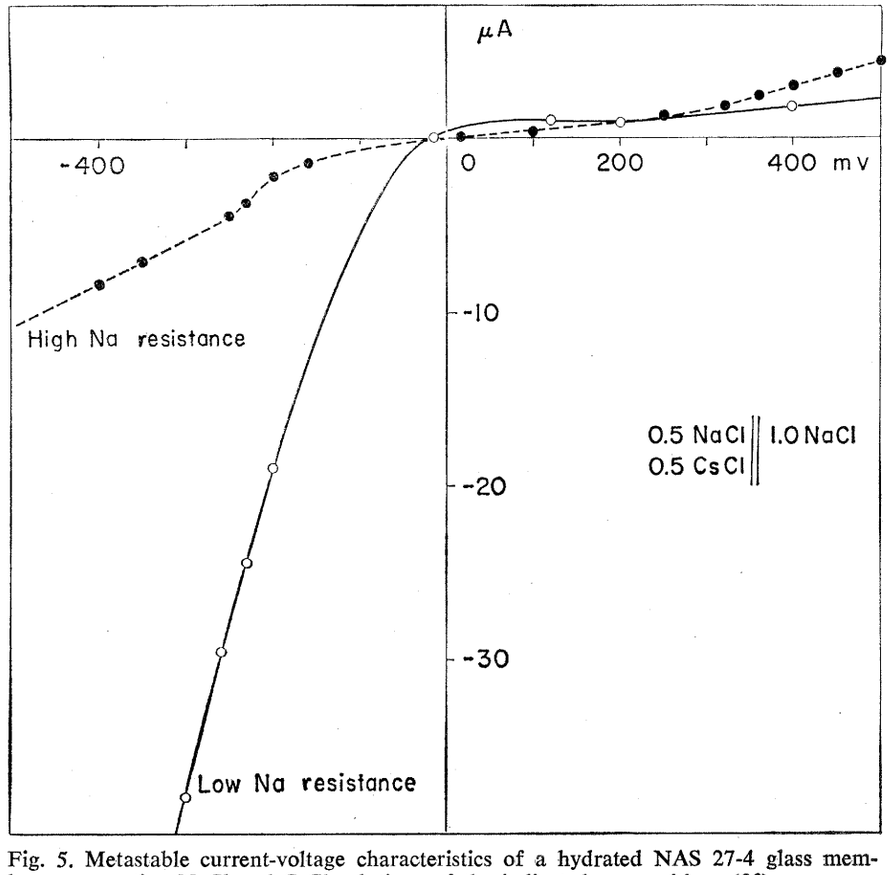

Fig. 5. Metastable current-voltage characteristics of a hydrated NAS 27-4 glass membrane separating NaCl and CsCl solutions of the indicated compositions (*33*).

tion profile of the sites as a function of external conditions for the completely dissociated (MD) case (*37*) and also taking into account the effects of association (*74*). In the latter (MA) case it has been assumed that the predominant effect of association is to produce associated neutral ion pairs whose mobility is constant within the membrane and whose concentrations are related to the concentrations of the different dissociated species through the law of mass action. From such calculations it can be shown that the steady-state current-voltage characteristics will be significantly different in fixed-site and mobile-site membranes: fixed-site systems exhibit a current-voltage relationship in which finite limiting *conductances* are approached in the limits of high positive and negative applied fields, while mobile-site systems are characterized by finite limiting *currents*. Further differences may be found in V_0, the membrane potential in the absence of electric current, which is generally described by Eq. 1 for fixed-site membranes but not for mobile-site membranes except in certain special cases (*75*).

The steady-state *I-V* relationships of mobile-site systems with one counter-ion and having varying degrees of dissociation are illustrated in Figs. 6 and 7. Figure 6 represents the particularly simple case where the mobility of the undissociated species (u_{12}) is assumed to be equal to the mean mobility of the dissociated species ($\bar{u}_{12}$):

$$\bar{u}_{12} = \frac{2u_1u_2}{u_1 + u_2}, \qquad (4)$$

where the subscript 1 refers to the counter-ion and the subscript 2 refers to the mobile site. Alpha, the fraction of dissociated species (Fig. 6), is related to K, the dissociation constant, and C, the total concentration, through the relationship

$$\frac{\alpha^2 C}{1 - \alpha} = K. \qquad (5)$$

In this case ($u_{12} = \bar{u}_{12}$) the limiting current (indicated by the horizontal asymptote at the right) is independent of K and is directly proportional to C.

For comparison, the dashed straight line of Fig. 6 gives the *I-V* relationship expected (both instantaneously and in the steady state) for a fixed-site membrane permeable only to species 1, while the dotted line gives the instantaneous *I-V* relationship for the zero field steady-state (that is, at the origin) for the completely dissociated ($\alpha = 1$) case of a mobile-site membrane.

More generally, the steady-state *I-V* relationship in MA systems also depends on the mobility of the undissociated species, as is illustrated in Fig. 7 for the case in which the mobility of the undissociated species is three times the mean mobility of the dissociated species (see *76*). Figure 7 illustrates that the magnitude of the limiting current is a function of the degree of dissociation, the mobility of the undissociated species ($\bar{u}_{12}$), and the mean mobility of the dissociated species (u_{12}). For the limits of complete dissociation ($\alpha \rightarrow 1$) and of strong association ($\alpha \rightarrow 0$), the limiting current densities are given, respectively, by

$$I(\alpha \rightarrow 1) = \frac{4RTFC}{d} u_1 \qquad (6)$$

and

$$I(\alpha \rightarrow 0) = \frac{4RTFC}{d} u_1 \cdot \frac{u_{12}}{\bar{u}_{12}}, \qquad (7)$$

where d is the thickness of the membrane (see *77*).

It may be seen from Eqs. 6 and 7 and from Figs. 6 and 7 that all mobile-site membranes are characterized in the steady state by the existence of a finite limiting current (indicated by the short horizontal bars at the right in Figs. 6 and 7), regardless of their degree of dissociation. This is because the existence of a saturating current depends on the reduction of site and counter-ion concentrations at one end of the membrane to zero; but, as the concentration is reduced to zero, dissociation becomes essentially complete, regardless of the value of K in Eq. 5.

Another effect of association—namely, its effect on the steady-state conductances at zero current—is also illustrated in Fig. 7. Whereas in FD, FA, and MD membranes, the current at steady state is carried only by the counter-ions, association permits the sites to contribute to the steady-state electric current within an MA membrane [the sites move as free ions in one direction and as neutral species combined with counter-ions in the other direction, thus acting as "carriers" (*19*; see *78*)]. If the mobility (u_{12}) of the neutral species is larger than the mean mobility ($\bar{u}_{12}$) of the ions, the steady-state resistance will initially decrease as the association increases (in Fig. 7, compare $\alpha = 0.5$ with $\alpha = 1$), but, as the association becomes still stronger, the sites will be "consumed" and the resistance will thereafter increase (in Fig. 7, compare $\alpha = 1$ with $\alpha = 0.25$ and $\alpha = 0.125$).

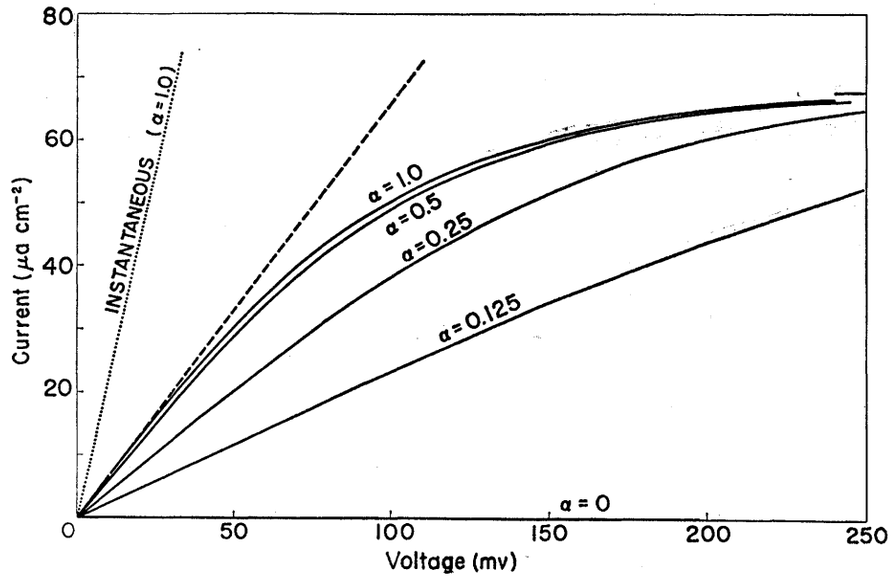

Fig. 6. Theoretical current-voltage relationships for mobile-site membranes having the indicated degrees of dissociation for the case $u_{12} = \bar{u}_{12}$. For these curves, C has been held constant at 10^{-3} equivalent per liter, and the change in α represents the effects of a change only in K through Eq. 5. The values of the parameters used in drawing these curves are given in *76*.

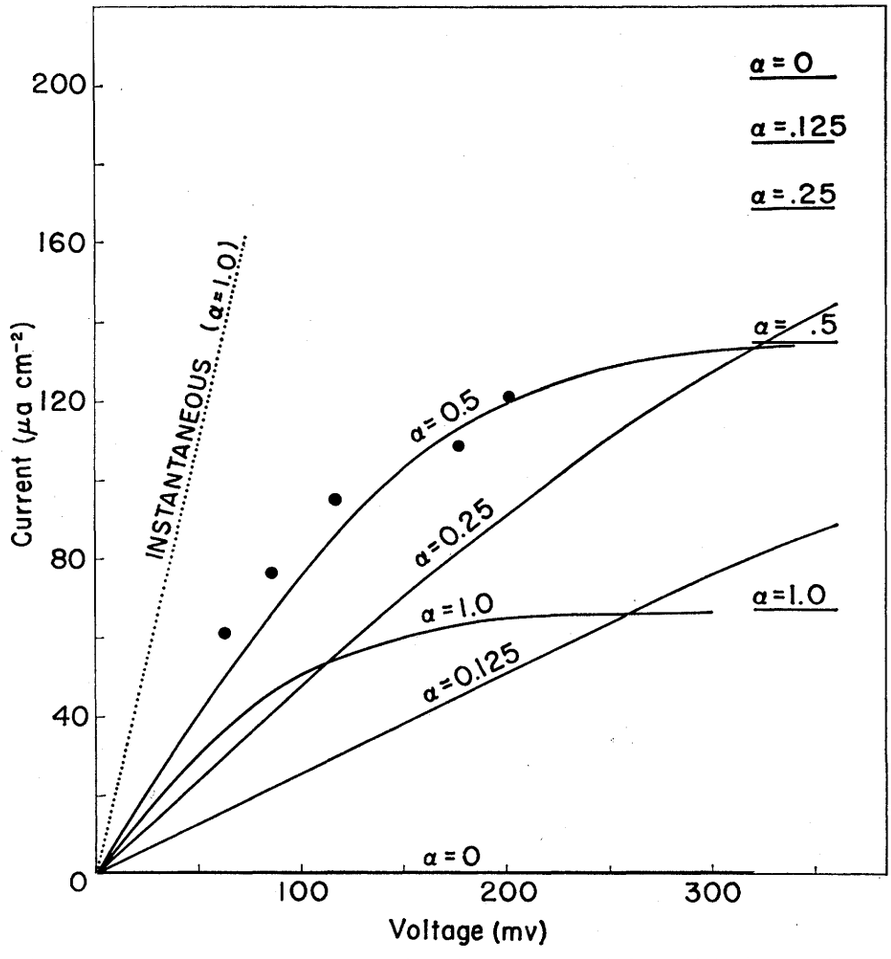

Fig. 7. Theoretical current-voltage relationships (*74*) (curves) for mobile-site membranes having the indicated degrees of dissociation for the case $u_{12} = 3\bar{u}_{12}$, corresponding to the measured values for HCl in isopropanol (see *76*). C has been held constant at 10^{-3} equivalent per liter. Note the initial decrease in resistance and subsequent increase as α is varied from 1.0 to 0 by varying K at constant C. Also note that the limiting current at high positive voltage increases monotonically as α is varied from 1.0 to 0. For comparison, the dots give the values experimentally measured (*58*) for $\alpha = 0.53$.

The carrier property of MA membranes becomes increasingly pronounced as association increases. The neutral species which are not acted on by electric forces will produce sources and sinks and act as a "reservoir" for the ions. Therefore, no significant skewing of the concentration profiles will occur until the field is very high and the fluxes are large enough to overcome the capacity of the "reservoir." Consequently, the *I-V* relationship be comes increasingly linear with increasing association, as may be seen in Figs. 6 and 7. Because of this, the decision (from the *I-V* relationship) concerning whether or not an unknown membrane is utilizing a fixed-site or a mobile-site permeation mechanism becomes increasingly difficult as the system becomes less and less dissociated.

The distinction between fixed- and mobile-site mechanisms can also be made by comparing instantaneous and steady-state *I-V* relationships. Thus the dotted lines in Figs. 6 and 7 give the instantaneous *I-V* characteristic at zero field for the MD ($\alpha = 1$) case. It may be seen that the slope of the instantaneous *I-V* relationship differs from the slope of the steady-state *I-V* relationship in the limit of zero field because, in the instantaneous relationship, the sites also carry a portion of the electric current. For the same reason, the instantaneous *I-V* relationship in the steady state for any applied field differs from the chord conductance (that is, does not pass through the origin) not only when $\alpha = 1$ (*57*), but also when dissociation is incomplete (*74*). In contrast to this, a fixed-site membrane permeable solely to species 1 is characterized by the same *I-V* relationship instantaneously and in the steady state (see dashed line in Fig. 6).

Perhaps the clearest distinction between fixed-site and mobile-site membranes lies in the marked discrepancy between the diffusion constants for the membrane as measured electrically and the constants expected from diffusion studies in the absence of electric current. This is most easily illustrated by comparing the diffusion constant measured electrically (D_{el}) with that measured by steady-state tracer diffusion (D_{diff}). Here we deduce from the theory (*74*) that the ratio of D_{diff} to D_{el} [measured from the instantaneous (or high-frequency alternating-current) resistance] is given by

$$\frac{D_{diff}}{D_{el}} = \frac{u_1}{u_1 + u_2} + \frac{u_{12}}{u_1 + u_2} \cdot \frac{1 - \alpha}{\alpha}, \quad (8)$$

from which it may be seen that, in the limit of complete dissociation (where $\alpha \rightarrow 1$), the ratio is given by the transference number of the counter-ion $[u_1/(u_1 + u_2)]$. On the other hand, as α approaches 0, the ratio D_{diff}/D_{el} approaches

$$\frac{u_{12}}{u_1 + u_2} \cdot \frac{1}{\alpha}, \quad (9)$$

so that the discrepancy between the diffusion constant measured electrically and that measured by tracer diffusion is inversely proportional to α. This is a classical example of what is called "exchange diffusion" in the biological literature (*79*; see also *19*). This effect may be thought of as a consequence of the fact that association leads to a transport of the counter-ion in an electrically neutral form due to combination with the oppositely charged mobile site.

In summary we can therefore state that increasing association makes a mobile-site membrane progressively more difficult to distinguish from a fixed-site membrane on the basis of its purely electrical properties, but progressively easier to distinguish on the basis of a comparison of electrical and flux properties.

Effects of co-ions. It should be emphasized that in distinguishing between fixed-site and mobile-site membranes it is essential to have co-ion exclusion. In the presence of co-ions, for example, a mobile-site membrane will not have saturating currents, and a fixed-site membrane will not have a time-independent V_0 obeying Eq. 1 (*75*). The general effect of co-ions will therefore be to obscure the consequences of different ion-permeation mechanisms.

Effects of thickness. Before concluding this description we should mention that, if an ion-exchange membrane is made sufficiently thin (*80*), several additional effects may appear. In the first place, the condition of macroscopic electroneutrality will apply only above a given minimum concentration of sites for a particular thickness; for, if a membrane is made sufficiently thin, the space charge will no longer be negligible in terms of the total concentrations of charged species within the membrane. Second, although the properties in the steady state are independent of thickness (except for appropriate scaling parameters), the electric field intensity for a given potential difference across the membrane can become enormous as the membrane becomes progressively thinner. For thin membranes, the assumptions that mobilities and chemical properties are independent of applied fields become questionable, since the applied field itself can be sufficiently strong to produce significant dissociation field effects (*81*) or even a breakdown of the applicability of the macroscopic flux equations (*55*). However, for the usual thicknesses and potential differences, such as those of biological membranes, if at least five jumps are interposed in an ion-migration mechanism, the macroscopic flux equations have been found to be good to within 5 percent (*55*).

The thickness of the membrane also determines the time constant for diffusion, which is directly proportional to the square of the membrane thickness as long as the transport processes are membrane-controlled (as opposed to boundary-controlled). Since such processes as establishment of space charge and mechanical equilibrium are independent of the thickness, the kinetic behavior (which depends on the ratios of various time constants) will vary greatly with thickness in certain ranges (*13*).

Structure and Ion Permeation in Biological Membranes

A number of characteristics of biological membranes may serve as bridges to the physicochemical description given above. From the structural point of view, biological membranes appear to be thin (50 to 100 angstroms thick) structures composed of a bi-layer of lipid, which has polar head groups oriented toward the aqueous phases and which is covered by adherent layers of protein or polysaccharide, or both (*16*). The hydrocarbon region of this structure is likely to be more or less liquid, in view of the unsaturation of a number of the fatty acids thought to be present (*82*). If biological membranes are continuous lamellar structures, as electron micrographs seem to indicate, ion permeation is likely to occur either through a site-free interior or by means of an MA mechanism utilizing lipid-soluble polar molecules as the mobile carriers (an MD mechanism seems unlikely in view of the low dielectric constant in the lipid interior). However, it has been suggested that the biological membrane has a micellar substructure with transverse pores lined with the polar head groups (*17*). In this case the permeation mechanism might be either on a fixed-dissociated (FD) or a fixed-associated (FA) basis.

Functionally, the sequences of cation

selectivity observed in biological systems, as well as their magnitudes, are consistent with those known to exist in narrow-pore fixed-site systems (*35, 83*), but they may also be consistent with those in mobile-site systems, for which the selectivity properties are at present insufficiently well characterized for comparison. The large values for the temperature coefficients of ionic mobilities and ionic concentrations in biological membranes (*13*) as well as the low mobilities [of the order of 10^{-8} and 10^{-9} centimeter per second per volt per centimeter for Na^+ and K^+, respectively (*13*)] are inconsistent with an FD system but perfectly consistent with the possibility of an FA, MA, or MD system.

A number of serious discrepancies have been observed in the electrical properties and fluxes of biological membranes. The case of "exchange diffusion" (*19, 79*), in which the fluxes are larger than those expected on the basis of the membrane's electrical conductance, have already been mentioned (see Eq. 8). Such a discrepancy is expected in MA membranes but not in MD or simple fixed-site membranes. On the other hand, an opposite type of discrepancy has been noted in plant cells, where it has been found that the electrical conductance is nearly ten times that expected from flux measurements (*84*), an observation which could be the result of a defect mechanism for ion migration in an FA system.

Cole (*13*) has recently pointed out that biological membranes have more complex behavior than can be accounted for by simple electrodiffusion processes; but, as we have seen (and this is perhaps our most general conclusion), while idealized models have proved very powerful for analyzing the properties of a variety of physicochemical membranes, structurally simple ion-exchange membranes of the FA and MA type can have more complex properties than such models have suggested. This means that considerably more must be learned about the details of ion-permeation mechanism in many ion-exchange membranes before all the properties of these membranes can be defined. In partial compensation for this, the properties of such membranes are sufficiently complex to offer models for such biological phenomena as excitability (*95*).

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measured to be 3.8 × 10^{-7} mole cm^{-3}), u_{Cl}, u_H, and u_{HCl} have been measured to be 1.06 × 10^{-9}, 2.48 × 10^{-9}, and 4.5 × 10^{-9} mole cm^2 sec^{-1} $joule^{-1}$. In drawing Fig. 6, u_{HCl} was assumed to have the value measured for $\bar{u}_{HCl}$ (1.48 × 10^{-9} mole cm^2 sec^{-1} $joule^{-1}$) while in drawing Fig. 7 the measured value was used.
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Innovation in Undergraduate Teaching

Programed instruction, despite great promise, gains acceptance slowly in undergraduate teaching.

Everard M. Williams

Since World War II, there have been numerous developments in teaching at all levels of education. The most apparent changes are in class size. Lecture classes became increasingly larger in an attempt to alleviate the escalation of instructional costs and, possibly, to free faculty members for research activities. Today, in many institutions, lecturers address a group in one lecture room, while the lecture is relayed by closed-circuit television to other classes in other classrooms. In some of these institutions, the lectures at one hour are recorded on video tapes for reproduction at other hours. These tapes can be replayed as needed. The recorded video tape is of major value because it relieves various scheduling problems such as those which arise because not all students in a course are available at a particular hour, a professor cannot teach two or more different courses at the same hour, or because insufficient classroom space may be available. The term "reproduction" is used here to indicate the general procedure of teaching enlarged student groups within a university by electronic means. When the student group to whom the reproduction is available is enlarged to include students in classes at one or more universities, the term "distribution" is used.

Reproduction and distribution require no restructuring of educational tactics. A basic known instructional technique, such as the lecture or experimental demonstration, is extended by reproduction and distribution to serve more students than could be served by the original lecture itself. The economy inherent in servicing large numbers may at the same time support a more polished and expensive lecture performance or a more elaborate demonstration experiment than would be economically feasible for a small group of students. Although some educators believe that reproduction and distribution adversely affect student motivation, most do not believe that these innovations have a deleterious effect on the educational process. Indeed, extensive test data (*1*) show that the size of a student group in a single class has no effect upon learning.

Elements of Programed Instruction

The "programed" instructional methods are being used extensively at the university level, although probably less widely in science and engineering than in some other fields. A complete programed instructional instrument is made up of the program itself and a device to administer it to the student. When the device is a mechanical construct, it is termed a "teaching machine." The function of a teaching machine is to ensure that the student precisely follows a program without skipping material. If a machine is not used, the administrative control is exercised by a machine-analog arrangement of the program in paper form, such as a book with special page sequencing, or file cards. These latter forms require the cooperation of the student in adhering to the programed plan.

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