

THE FAILURE OF THE INDEPENDENCE PRINCIPLE IS PREDICTED BY CONTINUUM DIFFUSION THEORY

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Abstract

The reasons for the failure of the "independence principle" in an ensemble of potassium selective, cholinergic, channels are examined. A modern very general, but nonetheless simple formulation of thermodynamic continuum membrane diffusion theory is used to analyze the data for this purpose. This theory shows that the independence principle actually depends on the applicability of three physical assumptions. They are: that no coupling between ionic flows exists; that the intracellular concentration of the permeant species is invariant in the face of its extracellular manipulation; and that the permeability does not change as a function of the extramembrane concentration of the permeant ion. The first of these constraints is met under the conditions of these experiments. The second is not, but the error which it produces can be easily corrected. Modern continuum theory predicts that the third will, in general, be invalid. The experimental results confirm this prediction. The miscarriage of the independence principle is thus due to the oversimplified approximations inherent in that principle, and not to flaws imbedded in all continuum theory, which, on the contrary anticipates the failure of the principle. It is shown that a similar conclusion applies also to problems that emerge with the use of both the Goldman-Hodgkin-Katz equations and the Ussing unidirectional flux ratio. Modern continuum theory which is free of the problems afflicting these older approaches, is also shown to be capable of revealing previously inaccessible channel properties. Problems connected with the use of continuum theories for work on systems made up of discrete elements are discussed. They are shown not to be of concern with regard to ionic diffusion through membrane channel systems.

1. Introduction

The "independence principle" and the equations to which it led (Hodgkin and Huxley, 1952) have provided an important tool with which to investigate ionic permeability in membranes. This principle seemed, at first, to be in reasonable agreement with the physical realities of several typical ionic channels. It was, as a result, tacitly assumed to be generally applicable. But more recent work has revealed discrepancies between it and experiment, sometimes even in channel types to which it was originally thought to apply (Hille, 1975a,b; Ulbricht, 1977). It is now clear that

independence does not generally hold. It is important to examine, in detail, the reasons for this failure, because they are undoubtedly related to inaccurate assumptions regarding membrane permeability mechanisms. These assumptions require careful identification.

The independence principle is based on the supposition that "the chance that any individual ion will cross the membrane in a specified interval of time is independent of the other ions which are present" (Hodgkin and Huxley, 1952; p. 467). Hodgkin and Huxley examined the consequences to which this supposition led with regard to the unidirectional fluxes of an ion crossing a membrane. They thus derived a descriptive expression with which one could predict certain aspects of the behaviour of an ensemble of channels that is selectively permeable to only one ionic species. They demonstrated that one should, in particular, be able to anticipate the electrical current through the channel ensemble at any external concentration of the permeant species from the measured current at some other appropriate concentration.

Hodgkin and Huxley's approach to this problem was that of diffusion through a continuum. But ions, in actuality, cross many of the membranes to which the independence principle is applied, through scattered, discrete channels. Most of the membrane surface is then in fact, impermeable. Such membranes are, strictly speaking, not continua. Furthermore, while traversing such channels, the ions will interact with charged regions and energy barriers, both of which are imposed by the intrachannel structure. Continuum diffusion theory makes no direct reference to any of these events. It has therefore often been assumed that such a theory is incapable of accounting for their effects on the diffusion process, and a tendency has consequently developed to ascribe the independence principle's failures to defects resulting from the application of continuum diffusion theory to such "non-continuous" membranes.

This tendency has been buttressed by the emergence of two further problems relating to the use of continuum theory. The first is the frequent nonsuccess of the Goldman-Hodgkin-Katz formulation of this theory (Goldman, 1943; Hodgkin and Katz, 1949) — a formulation whose flux equations also yield the independence expressions. The second involves the inability of the Ussing-Teorell expression for the unidirectional flux ratio (Ussing, 1949; Teorell, 1949) to describe certain ionic channels (Hodgkin and Keynes, 1955; Horowicz, Gage and Eisenberg, 1968; Begenisich and DeWeer, 1979; Hille, 1979). This flux ratio is also a derivative of continuum theory. The unfortunate result has been a trend towards the conclusion that progress in this field now demands that the use of continuum diffusion theory be abandoned (Hille, 1975a,b; 1978; 1979; Ulbricht, 1977). This conclusion, if correct, would have a serious consequence. It would eliminate a very powerful tool from the rather small selection available to us in our attempts to unravel the mechanisms of diffusion through membranes. It is therefore important to determine whether so drastic a conclusion is actually justified.

The assumption that the discrete nature of both the transmembrane ionic channels and the intrachannel environments invalidate a continuum approach is actually not well founded. All diffusion systems including those not in membranes are, in the final analysis, discrete at a molecular level. In gases we have successive molecular

collisions, in solids and liquids we have successive jumps from one stable molecular configuration to another (Frankel, 1946).

Diffusion through such non-membrane systems can, nevertheless, be very well described by continuum theory based on the thermodynamics of irreversible process (see for instance, Katchalsky and Curran, 1965). This success of continuum theory is made possible by the fact that the large number of discrete events of which the diffusion process actually consists are summed and averaged by the process itself, creating, in fact, a continuum. This summation actually furnishes the bridge between the discrete and the continuous, which are interrelated aspects of reality.

In the case of the transmembrane ionic diffusion, a similar mechanism operates. In that case the summation takes place over the ensemble of discrete ionic channels as well as over the discrete sites within these channels. Thus, while the discrete nature of the channels and of the intrachannel events is not denied, a continuum is nevertheless generated which is sufficient for the macroscopic features of thermodynamics to be applicable. Indeed, this thermodynamic description is effective even in the case of single-file diffusion through such channels (Heckmann, 1972). It is, in fact, the sometimes incomplete smoothing, caused by the occasional absence of a sufficient number of elements in the ensemble, that unmasks the fluctuations due to the discrete events which are then measured as noise during certain experiments. One can conclude that the explanation of the failure of the continuum-based independence principle cannot be expected to lie in an inherent inability of continuum theory to deal with these summed and averaged discrete membrane events. There is, on the contrary, every reason to expect that the thermodynamically based continuum diffusion theory should have this capability. The explanation of the cause of the observed difficulties must therefore be sought elsewhere.

The application of continuum diffusion theory to membranes usually begins in a somewhat different manner from that chosen by Hodgkin and Huxley (1952). It begins with the Nernst-Planck equation which is the fundamental thermodynamic expression describing diffusion in an isothermal, isobaric system in which coupling between fluxes is absent (see, for instance, Schwartz 1971b). In spite of the difference between the two approaches, continuum theory based on the Nernst-Planck equation produces a result identical to that of Hodgkin and Huxley, provided that certain additional constraints are invoked. The independence principle therefore is a particular constrained member of a group of expressions that can describe diffusion across membranes under various conditions, and which result from an analysis of this problem based on thermodynamic continuum diffusion theory. Some of these expressions are much more general in their applicability than are those of the independence principle. It is therefore possible that the failure of this principle is due to constraints on its range of validity which make its use with certain membranes a physically unreasonable process. It would consequently seem that investigations into the failures of the independence principle should include, at their start, a comparison of the differences between diffusion as specified by it, on the one hand, and as allowed by the more general continuum theory, on the other.

The question has, until now, unfortunately not been placed in this way. (In comparing their results with parallel work based on a more classical thermodynamic view

of membrane diffusion, Hodgkin and Huxley (1952) recognized that problems might arise due to special assumptions used in their independence derivations. Their note of caution seems, however, to have been either overlooked or quickly forgotten.) Two misconceptions have, instead, emerged. The first is that thermodynamic continuum diffusion theory as applied to membranes can be equated with either the independence or the Goldman-Hodgkin-Katz formulations; that is, that continuum theory is of necessity limited to these two constrained forms. The second is that the Nernst-Planck equation encompasses all of the possibilities inherent in a continuum approach (Hille, 1979). These misconceptions lead to the false notion that with these formulations all of the useful aspects of continuum diffusion theory have been exhausted. In fact the independence and Goldman-Hodgkin-Katz formulations, as well as the Ussing-Teorell unidirectional flux ratio are, in different ways, quite special cases of a much more general theory. They are actually applicable only to a very restricted subset of the membrane diffusion phenomena which general continuum diffusion theory can accurately describe. The evidence for this conclusion in the cases of both the unidirectional flux ratio and the Goldman-Hodgkin-Katz equations will be reviewed at this point; the evidence in the case of the independence principle is the subject of the remainder of this paper.

The Ussing-Teorell unidirectional flux ratio is dependent on the use of isotopic tracers for its measurement, and on the Nernst-Planck equation for its derivation. (Although it was originally obtained by Ussing (1949) and Teorell (1949) from the Nernst-Planck equation, a more general version of the unidirectional flux-ratio has been derived from extended forms of that equation (see, for instance, Hoshiko and Lindley, 1964; Kedem and Essig, 1965; Schwartz, 1971a). The extended forms take into account the non-ideality of solutions, pressure differences across the membrane, as well as three dimensional membrane inhomogeneities. But this in no way alters the above argument.) Its applicability in any given case therefore hinges on the validity of an assumption that is buried in both the Nernst-Planck equation and its extended forms, and is especially significant during such measurements: that coupling between isotopic fluxes is absent (Hoshiko and Lindley, 1964; Kedem and Essig, 1965; Schwartz, 1971a,b). If that assumption fails in some particular membrane so that these fluxes actually interact, continuum diffusion theory predicts flux ratios that differ from those of the Ussing-Teorell theory (Kedem and Essig, 1965). But the analysis of this case requires the tools provided by the thermodynamics of irreversible processes, and is of necessity more general than that which follows from either the Nernst-Planck equation or from its above mentioned extended forms. The "abnormal" ratios that the general continuum theory predicts for this case include precisely the sort of abnormal ratio that has been observed in several channels, and which has been cited as evidence for "single-file" diffusion (Kedem and Essig, 1965; Essig, 1966). Continuum theory of sufficient generality therefore includes the case of single-file diffusion as an isotopic coupling effect. The Ussing-Teorell unidirectional flux-ratio equation thus fails in this case because it has been pushed beyond the limits of its applicability, and not because of any flaw in the continuum theory. In no way can one justify the sometimes drawn conclusion (Hille, 1979) that this failure implies that the

basic tenets of "free diffusion" have been violated in the membrane under investigation.

The Goldman-Hodgkin-Katz equations require the electrical field in the membrane to be constant, the ionic mobility in the channels not to be a function of position as the membrane is crossed, and the phase-boundary potentials at the two membrane-solution interfaces to be equal and opposite. (The first two of these constraints are necessary to the derivation of the Goldman-Hodgkin-Katz flux equation, but can be relaxed for the zero-current membrane potential (Schwartz, 1971b,c).) Continuum diffusion theory need not, in general, include such constraints. When the analysis is freed of them, this theory yields simple expressions that do not suffer from the weaknesses inherent in the Goldman-Hodgkin-Katz formulation (Schwartz, 1971b,c; Schwartz and Kado, 1977a,b). In fact, contrary to the Goldman-Hodgkin-Katz procedure of assuming the nature of the intramembrane physical environment, these new expressions allow the investigator to use experiments to determine what macroscopic, averaged, physical features actually do or do not exist within the membrane, thereby yielding physical information that was previously unattainable because it had been "swept under the rug" (Schwartz and Kado, 1977a,b). These new expressions have been used for the analysis of a K^+ selective, cholinergic channel in an *Aplysia* neuron. This channel's instantaneous conductance is voltage dependent (Marty and Ascher, 1978; Lacerda and Schwartz, in preparation) in a manner that cannot be accurately described by the Goldman-Hodgkin-Katz theory (Ginsborg and Kado, 1975; Schwartz and Kado, 1977a,b). (In spite of some earlier confusion on this score, it is now clear that both the Ginsborg and Kado, and Schwartz and Kado measurements actually involved the near-instantaneous conductance measured following a delay of only 1 (one) second in a channel system characterized by a long relaxation constant — typically 2.5 secs at -45mV and 15°C (Marty and Ascher, 1978; Lacerda and Schwartz, in preparation).) Analysis with the newer, less constrained expressions has demonstrated that a constant field does not generally exist in this channel, and that its two phase-boundary potentials are not equal and opposite (Schwartz and Kado, 1977a,b). Therefore the inability of the Goldman-Hodgkin-Katz theory to accurately describe diffusion in this channel is due not to the failure of continuum diffusion theory but rather to the inapplicability of the Goldman-Hodgkin-Katz constraints (Schwartz and Kado, 1977a,b).

The applicability of both the unidirectional flux-ratio, and the Goldman-Hodgkin-Katz formulations of continuum theory is consequently very restricted. They are quite specialized and cannot be expected to yield physically correct results except when the appropriate physical constraints really exist. The frequent failure of these "diffusion theories" to yield results that correspond to experiment should, therefore, be no great surprise. One certainly cannot conclude from their difficulties that continuum theory has run its gamut. But, to complete the picture, the sources of failure of the independence principle must also be determined. That question is addressed in this paper.

The difference between the diffusion process as constrained by the independence principle, and as allowed by general continuum theory will be explored. It will be

demonstrated that the independence relations actually require for their validity among other things that the membrane permeability not be a function of the extramembrane concentration of the permeant species. But the general continuum theory predicts that permeability will, on the contrary, usually be dependent on this concentration. Examination of the same K^+ channel in *Aplysia* that was previously used to explore the failure of the Goldman-Hodgkin-Katz equations demonstrates that the experimental results confirm this theoretical prediction. An approach based on independence must therefore miscarry. A preliminary report of this work has appeared (Schwartz, 1978).

2. Methods

The data for this paper were collected during a series of experiments for which the methods used have already been described in detail (Schwartz and Kado, 1977a,b).

Summarizing briefly, individual cells in the medial group of either the right or left pleural ganglia of *Aplysia californica* (Kehoe, 1972b) were voltage clamped. The somatic membrane of these cells contains cholinergic, potassium selective, channels (Kehoe, 1972a,b; Schwartz and Kado, 1977a,b) which were selectively activated by the iontophoresis of carbamylcholine from a sufficiently distant point (Ascher and Kehoe, 1975). Iontophoresing current was held constant throughout the various portions of each experiment.

The membrane currents required to clamp the membrane at a series of voltages were determined, first in the absence and then in the presence of carbamylcholine. In both cases these currents were measured at the end of one second pulses. The current-voltage (I-V) characteristics due to the effects of the drug on the ensemble of channels was then obtained by plotting the change in the membrane clamping current produced by the carbachol, against the membrane potential. This I-V characteristic reflects the near instantaneous channel properties (Marty and Ascher, 1978; and Lacerda, 1985) (see also the previous discussion of this question in the present paper). This procedure for producing a carbachol I-V characteristic was then repeated with the same cell and at the same iontophoresing current, but at a different external potassium concentration. Changes in potassium concentration always involved an equimolar exchange for sodium, so that the total external ionic concentration always remained constant.

The standard bathing medium was artificial seawater formulated as follows: NaCl, 480 mM; KCl, 10 mM; $CaCl_2$, 10 mM; $MgCl_2$, 50 mM; pH was held at 7.7 at 25°C by adding Tris-Cl, 10 mM, except that in some of Lacerda's (1985) later work Hepes Buffers were used.

3. Results

I-V characteristics were determined for scores of cells at 5 mM, 10 mM and 20 mM external K^+ concentrations by Schwartz and Kado (1977a,b) and further by Lacerda

(1985) who repeated the work at these concentrations, but also extended it to 7.5 mM and 50 mM external K^+ . However, the results at 10 mM and 20 mM K (Figure 1) have proven to be representative of the entire family of results. They will consequently be used as the basis of further discussion. If the behaviour of this ensemble of channels could be accurately described by the independence principle, one would be able to use the independence equations to predict the I-V characteristic at 20 mM K from that at 10 mM K. Theory and experiment can therefore be compared.

I have modified Hodgkin and Huxley's (1952) independence equation in two minor ways to make this comparison easier. The equation has, firstly, been made to conform to the modern convention regarding membrane polarity: so that the potential is referenced to the outside. The equation has, secondly, been recast in terms of activities instead of concentrations. It will thus conform more simply to modern expressions derived from more general diffusion theory. It then yields:

$$(1) \quad \frac{\Delta I_2}{\Delta I_1} = \frac{\frac{a_2(s_1)}{a_1(s_1)} \exp\left\{-\frac{F}{RT}(E-E_{01})\right\} - 1}{\exp\left\{-\frac{F}{RT}(E-E_{01})\right\} - 1}$$

as the current ratio predicted by independence. ΔI is the change in the clamping current produced by the carbachol, E is the membrane potential, E_0 is reversal potential, a is potassium activity, R , F , and T have their usual meanings. The subscripts 1 and 2 refer to the 10 mM and 20 mM cases, respectively, except that the bracketed (s_1) means the solution on side 1, which is taken to be the outside of the cell.

Comparison of the 20 mM I-V characteristic predicted by this equation (Figure 1, Independence Principle, Original Form) with that obtained from experiment shows the match to be rather poor. This channel does not, therefore, obey the independence principle.

4. Discussion

A. What Assumptions Does the Independence Principle Really Contain?

The core of the independence principle would, at first glance, appear to be the requirement that ion flows be independent, that is, that they not be coupled. This conclusion is fostered by the principle's name. Appearances can, however, be deceiving; the derivation of the independence equations directly from the more general continuum theory, reveals that more is actually demanded. Our analysis begins with the Nernst-Planck equation, which contains within itself the assertion that coupling between fluxes is absent. Although this equation therefore already includes the statement that fluxes of different ions are, in this sense, independent, this by itself will prove to be insufficient to yield the independence expressions.

The Nernst-Planck equation can be modified to take intra-channel activity coefficients into account. (Except as a temporal average one cannot sensibly discuss thermodynamic variables like concentration, activity, activity coefficient, mobility,

Figure 1(a)

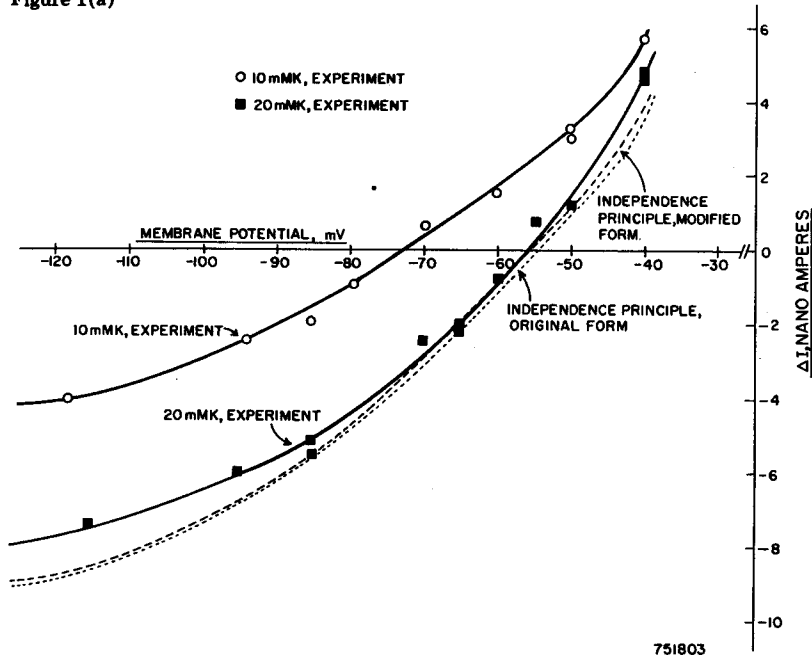
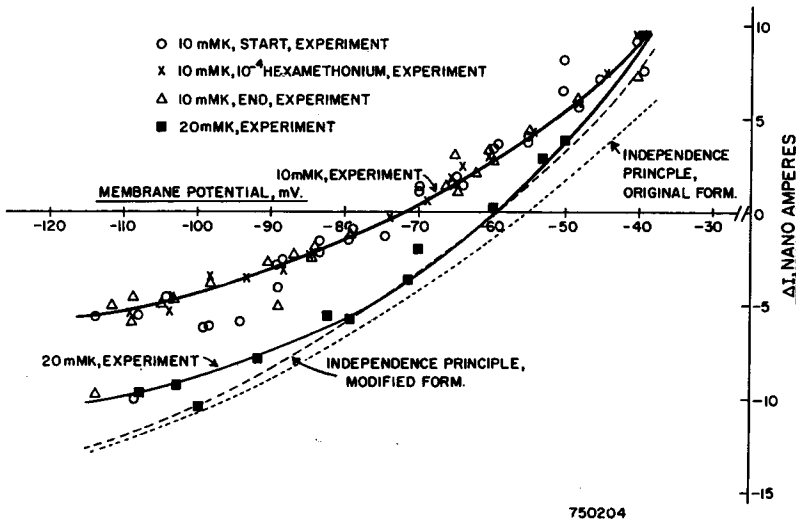


Figure 1(b)



etc., inside a single channel of atomic dimensions. These intrachannel properties must be otherwise regarded as averaged over an ensemble of channels.) Integration across the membrane under steady-state conditions then yields the expression,

$$(2) \quad I = A \frac{RTF}{Q'} \left[a(s_2) \exp \left\{ \frac{FE}{RT} \right\} - a(s_1) \right],$$

for a univalent cation (Schwartz, 1971b,c; Schwartz and Kado, 1977a,b). Here

$$(3) \quad Q' \equiv \frac{1}{\beta} \int_0^\delta \frac{\gamma}{\omega} \exp \left\{ \frac{F}{RT} (\phi - \phi(m_1)) \right\} dx,$$

where the membrane thickness is denoted by δ . The activity coefficient γ , the mobility ω , as well as the electrical potential ϕ , may all be functions of x , the trans-membrane coordinate. The total area that the sum of all of the active channels provide for diffusion is A , (s_2) means the solution on side 2 — which is taken to be the inside of the cell, (m_1) refers to a point just inside the membrane on side 1, and

$$(4) \quad \beta \equiv \frac{a(m_1)}{a(s_1)}$$

is the partition coefficient on side 1. Electrical current through the ensemble of channels is given by I , and the ensemble's permeability to the cation is (ART/Q') .

The derivation of equations 2 and 3 is quite general in that it has no need for the assumptions required to derive either the independence or Goldman-Hodgkin-Katz equations. (One point should be noted. These equations have been derived on the assumption that partition, double-layer, and image-force effects can be accounted for as discrete steps at each of the two membrane-solution interfaces. Equilibrium is assumed to prevail across these interfaces so that the regime is membrane-diffusion limited. A set of equations of the same form can, however, be shown to apply in the

Fig. 1. Current-voltage relationships for the ensemble of carbamylcholine activated potassium channels. The results for two different cells are shown, marked (a) and (b). Outward currents are positive, and the membrane potential is referred to the extracellular medium. All curves were drawn by eye. Experimentally determined points are shown for both 10 mM and 20 mM extracellular K^+ . In addition, calculated curves are presented for the 20 mM case. Those marked "Independence Principle, Original Form" were determined from the 10 mM results according to Equation 1. They correspond to the original, Hodgkin and Huxley (1952) expression. Those labelled "Independence Principle, Modified Form" were calculated with Equation 2C. It has been assumed that the activity coefficient for K^+ is the same in both the intra- and extracellular solutions, in calculating these curves. For cell (b), some 10 mM points were obtained with 10^{-4} M hexamethonium present. This agent blocks the excitatory response of other neurons in these ganglia, but has no effect on the K^+ , inhibitory, response of this channel (Kehoe, 1972b). In addition, for cell (b), the 10 mM curve was determined twice: once at the start of the experiment, and again following the 20 mM exposure. Both sets of results are presented. Comparison of the 10 mM and 20 mM in plots shows that they yield conductances that are quite different at the same membrane voltage. This in turn implies permeabilities that differ similarly (Schwartz and Kado, 1977a,b). The ratios of these differing permeabilities are shown in Fig. 2.

more realistic case that these effects are actually distributed across a thin membrane, provided that an equilibrium region still exists near each surface. Electroneutrality in the membrane is not required in either case (Appendix D.) This definition of permeability is therefore also quite general. Indeed, it has been shown that this permeability is the permeability of which the Goldman-Hodgkin-Katz permeability is a special constrained case (Schwartz and Kado, 1977a,b).

The general flux equation (Equation 2) will, when it is appropriately constrained, yield the independence equation (Equation 1). Our present task is to identify the necessary constraints. Applied to an analysis of the experiment in this paper, I in equation 2 becomes ΔI , the change in the clamping current due to the application of carbachol, so that for present purposes the generalized analog of equation 1 is,

$$(5) \quad \frac{\Delta I_2}{\Delta I_1} = \frac{\left[\frac{A}{Q'} \right]_2}{\left[\frac{A}{Q'} \right]_1} \left[\frac{a_2(s_2) \exp \left\{ \frac{FE}{RT} \right\} - a_2(s_1)}{a_1(s_2) \exp \left\{ \frac{FE}{RT} \right\} - a_1(s_1)} \right]$$

(The general flux equation has been derived from an extended form of the Nernst-Planck equation which retains the usual constraint on the use of that expression: that there be no coupled flows between diffusing species. Since we are here discussing a channel that, under the conditions of these experiments, is permeated by only one ion type (Kehoe, 1972a; Schwartz and Kado 1977a,b), and no tracers for it are used. It is clear that only one ionic flux can possibly be present. It follows that coupling between flows is absent because such coupling demands the presence of at least two ionic fluxes. The limitations of the Nernst-Planck equation in this regard cannot, therefore play any role in this case.)

In addition to their statement regarding the independence of ionic movements, Hodgkin and Huxley (1952) also made the simplifying assumption that the intracellular concentration of the permeant species does not change when its extracellular concentration is altered. It is easy to show (Appendix A), that, in that case, the general equation (5) reduces to the independence relationship (Equation 1) if:

$$(6A) \quad \left[\frac{A}{Q'} \right]_1 = \left[\frac{A}{Q'} \right]_2,$$

that is, if the permeability is not a function of the extracellular concentration of the permeant species. Indeed it is, in retrospect, easy to see how the Hodgkin and Huxley derivation also implied this condition. It is a consequence of their requirement that the proportionality factors relating unidirectional transmembrane fluxes and extramembrane concentrations be constants depending only "on the condition of the membrane and on the potential difference across it" (Hodgkin and Huxley, 1952, p. 467). This is demonstrated in Appendix B.

There are thus three physical constraints essential to the derivation of the independence relationship: lack of coupling between fluxes, constancy of the internal

concentrations of the permeant species, and constancy of permeability in the face of external concentration changes.

B. Are These Constraints Met by the Membrane?

This ensemble of channels has been demonstrated to be permeable only to potassium under the conditions prevailing during these experiments (Kehoe, 1972a; Schwartz and Kado, 1977a,b). Coupling between fluxes cannot therefore play any role in this case (see also the earlier discussion). The first constraint is therefore met.

Experiment indicates that small net potassium movements into or out of the cell occur in response to alterations in the extracellular concentration of this ion (Ginsborg and Kado, 1975; Ascher, Kunze, and Neild, 1976; Schwartz and Kado, 1977a,b). The second constraint is therefore not met. Experiments have also demonstrated that (A/Q') is not independent of either the extracellular potassium concentration or the membrane potential (Figures 2 and 3, and Schwartz and Kado, 1977a,b). The third constraint thus also fails to apply.

The separate effects of the breakdown of these two constraints can be examined to determine their relative importance. One can isolate the effect of small variations in the intracellular potassium concentration by abandoning the assumption regarding its constancy while, at the same time, clinging to the assumption that (A/Q') does not depend on concentration. Equation 5 then becomes (Appendix C):

$$(2C) \quad \frac{\Delta I_2}{\Delta I_1} = \frac{a_2(s_1)}{a_1(s_1)} \left[\frac{\exp \left\{ -\frac{F}{RT} (E - E_{02}) \right\} - 1}{\exp \left\{ -\frac{F}{RT} (E - E_{01}) \right\} - 1} \right]$$

The result of predicting ΔI_2 with this expression can be seen in Fig. 1 (Independence Principle, Modified Form). Experiment and theory must of necessity correspond at two points: at reversal, where a fit has been forced by Equation 2C; and at -66.8 mV, where (A/Q') in fact does not change with concentration. (According to this generalized diffusion theory (Schwartz and Kado, 1977a,b) this membrane potential is equal to the sum of the phase-boundary potentials at the membrane-solution interfaces. In that set of experiments Schwartz and Kado reported that sum to be -65.4 mV for the same channel. The small difference between that and the present value reflects variations from cell to cell in a somewhat different experimental series, and is within the range of experimental error.) Experiment and theory, as a result, now compare quite well between and in the neighbourhood of these two points: between reversal and -70 mV for the experiment of Figure 1a, and between reversal and -78 mV for the experiment of Figure 1b. But outside of these regions the theoretical fit remains poor. Indeed, the major effect produced by this correction to the independence expression for changing intracellular concentrations, is to shift the reversal potential from an improper value to its proper experimentally determined value. One must conclude that although the incorrect assumption of an invariant intracellular potassium concentration contributes some problems to the independence principle, it clearly does not bear the primary responsibility for its failure. That

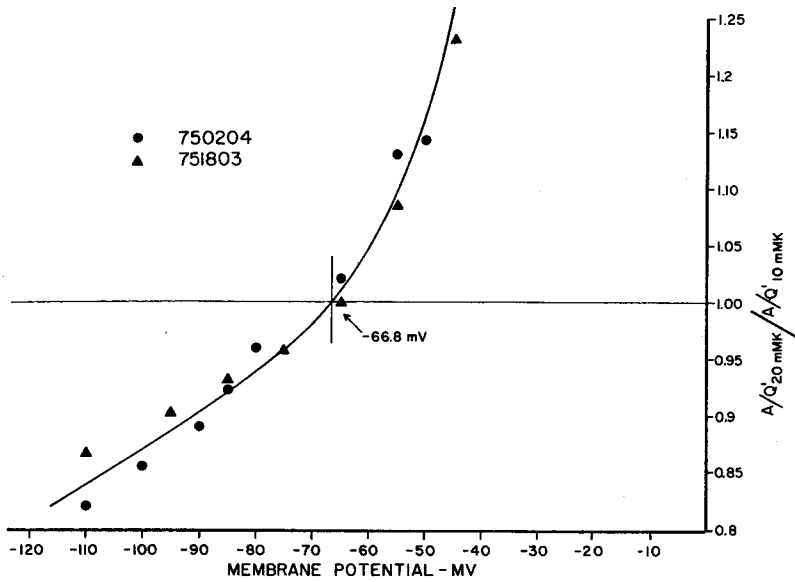


Fig. 2. The ratio of A/Q' measured in 20 mM extracellular potassium and at the indicated membrane potential to A/Q' measured in 10 mM extracellular K at the same membrane potential, plotted against membrane potential. A/Q' is calculated from the raw data for each external potassium concentration, and at each membrane potential according to the relationship:

$$(6) \quad \frac{A}{Q'} = \frac{G}{F^2 a(s_1)} \left[\frac{\frac{F}{RT} [E - E_0]}{\exp \left\{ \frac{F}{RT} [E - E_0] \right\} - 1} \right]$$

From Schwartz and Kado (1977a,b; Equation 4). As discussed in that paper, to which the reader should refer, for clarity, G , in this expression, which is the familiar chord conductance, is determined from the appropriate I-V curve. This figure demonstrates that A/Q' is a function of both membrane potential and external potassium concentration. It is seen to be concentration independent only at -68.8 mV. This happens because it is at this membrane potential that the electrical field inside the membrane becomes constant, Schwartz and Kado (1977a,b), Lacerda (1985). If A/Q' had been concentration independent at all potentials, this curve would have been coincident with a horizontal line drawn for a ratio of 1 (one). If A/Q' were concentration but, not membrane potential, dependent, the curve would have been coincident with some horizontal line other than that for a ratio of 1 (one). Since neither of these constraints are met by the data, the above conclusion regarding the nature of the function A/Q' follows.

Figure 3(a)

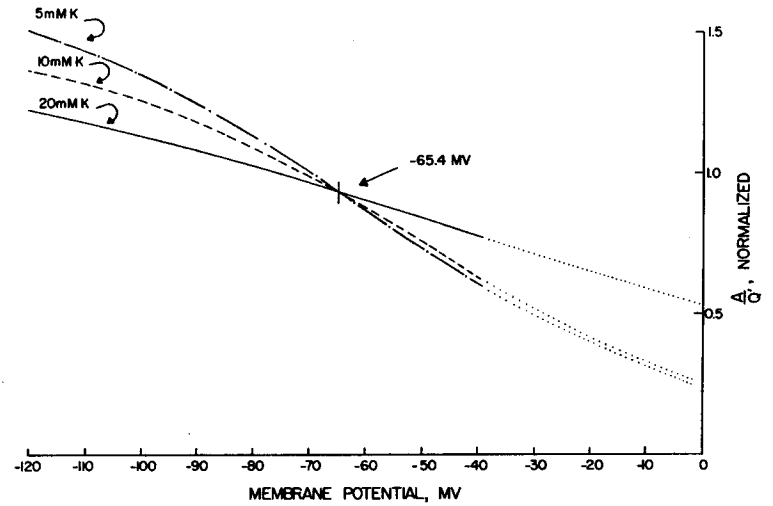


Figure 3(b)

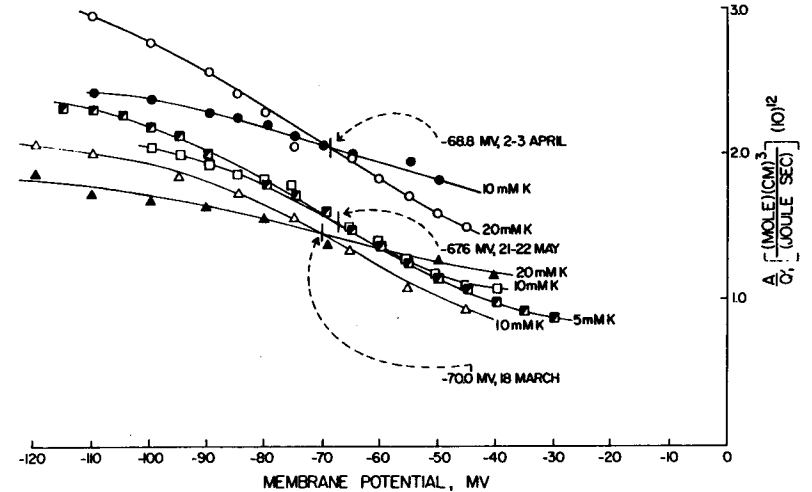


Fig. 3. Permeability as a function of membrane potential. (a) An average of the normalized A/Q' curves at three different external concentrations. Normalization has made it possible to pool data taken with several different cells. Lines were drawn by eye. (b) Three different experiments for which the data were not normalized. Lines were again drawn by eye. Units of A/Q' are (moles \times cm³/Joule seconds) $\times 10^{12}$. This figure was taken from Schwartz and Kado (1977a,b).

role must therefore belong to the collapse of the third constraint: that (A/Q') be invariant with external potassium concentration.

C. Can Continuum Diffusion Theory Account for (A/Q') 's Concentration Dependence?

Continuum theory predicts that Q' will, in general, be a function of the electrical potential in the channels' interiors (Equation 3). Permeability therefore certainly depends on the membrane potential, and experiments have confirmed this prediction (Figures 2 and 3, and Schwartz and Kado, 1977a,b). However, this dependence on potential does not involve just the voltage difference across the exterior of the membrane. It actually depends on the detailed structure of the electrical potential within the membrane (see Equation 3). Thus, even if a membrane is maintained at some constant transmembrane voltage, its permeability is expected to vary in case anything is done which alters the averaged intrachannel profile of the electrical potential. This potential profile is, in turn, directly dependent on the configuration of charges inside the channels since the relationship between charge density and potential is specified by Poisson's equation (see, for instance, Panofsky and Phillips, 1955). The details of the intrachannel charge configuration will, itself, generally be affected by changes in the extra-membrane concentrations of the permeant species, since some of these ions will be driven into or out of the channels depending upon whether their concentration was increased or decreased. Indeed, in the channel under investigation in this paper, the observed conductances indicate that the intrachannel potassium concentration increased when the external potassium concentration was raised (Schwartz and Kado, 1977a, Fig. 3 and Eq. 3A). The extramembrane concentration of the permeant species can, therefore, alter intrachannel conditions in such a way as to change the electrical profile and with it the membrane's permeability. Continuum theory therefore predicts that the permeability should be concentration dependent precisely because it is potential dependent.

The relationship between intrachannel charge density and permeability in the potassium channels that is the subject of this paper has been explored. A preliminary report has been made (Schwartz, 1981). But, the results will be presented in detail in a subsequent paper (Schwartz, in preparation). I will however, summarize a few of its pertinent aspects here to make the above argument more complete. The observed permeabilities (see Figs 2 and 3, and Schwartz and Kado, 1977a,b) are completely and consistently accounted for by a system in which each charged intrachannel site is associated with a counterion, but in which a net bound channel charge results from polarization effects produced by the membrane potential itself, acting on a channel system that is inhomogeneous in that its dielectric constant varies as the membrane is traversed.

The polarization charges produced are quite small. For example, if a channel is taken to have the dimensions of a "pore" with a radius of 4\AA in a 70\AA thick membrane, an average channel will develop a net positive charge whose magnitude is $0.32 (10)^{-3}$ electronic charges when the membrane is held at 100 mV inside negative, and the external solution is 10 mM in potassium. In 20 mM potassium this increased to a positive charge of $0.48 (10)^{-3}$ electronic charges per channel. The concentration

dependence of this channel's permeability thus appears to be the result of an increased polarizability in the presence of a higher intrachannel potassium concentration. Q' might also owe its concentration dependence, in part, to variations in β , γ , or ω . The evidence of these experiments suggests, however, that this is not true for this particular channel under these experimental conditions since these parameters then appear to remain constant in spite of changes in the concentration of the external potassium.

Spatial variations of the standard chemical potential μ_0 , of the permeant ion in the membrane's interior that result from image force effects, have been implicated in the voltage dependence of lipid bilayer conductances (Neumcke and Lauger, 1969; Lauger and Neumcke, 1973). This follows from the fact that a changing μ_0 , in effect, modified the intrachannel electrical potential. But, since μ_0 is not a function of concentration, this effect cannot be involved in the permeability's concentration dependence. In these experiments, then, the concentration dependence seems entirely due to ϕ . But theory does indicate that ω or γ or both, are implicated in the concentration effects produced when a second ion, such as rubidium or cesium, blocks the permeation of the first (Schwartz, unpublished).

5. Conclusion

I have demonstrated that the independence principle miscarries in this potassium selective channel ensemble because the ensemble's permeability is concentration dependent. This failure reflects an inadequacy in the principle. But it does not involve any problems imbedded in more general continuum membrane diffusion theory, which as a matter of fact, predicts that this difficulty will arise when the independence principle and its equations are used. Indeed, despite the discrete nature of transmembrane ionic diffusion, which asserts itself both at the single channel level and at the level of single sites within these channels, the ensemble of channels involved in the macroscopic diffusion process can be treated as a continuum. There is, therefore, no *a priori* reason to expect continuum theories to be inapplicable to such systems.

What sorts of information can one expect to obtain in this way? The theory is, after all, based on thermodynamics, which does not deal with events at the level of single atoms or molecules. It deals instead with macroscopic systems; with the averaged-in-space-or-time properties of a collection of many molecules — an ensemble. It is accurate to say that the primary objective of work in this field is to develop an understanding of the mechanisms of permeability and selectivity and of their control at a molecular level. There has also been some fear, based on the above description of thermodynamically founded diffusion theory, that this sort of theory is fundamentally unable to yield such molecular-level insights. This fear seems, however, to be unfounded. It is well known from studies of the photoelectric effect and of spectra by quantum mechanics that events at the molecular and atomic levels produce macroscopic effects which a complete theory is required to explain. Consequently, the observed macroscopic properties of an ensemble of diffusion channels must contain clues to the microscopic structures of these channels.

Furthermore, the thermodynamically based theories provide us with much needed strength because they can be used to gain more insight into macroscopic channel parameters without the need for prior information about the molecular level structure of the diffusion channels.

Work with continuum theory is therefore not dependent on the at present uncertain and non unique models that attempt to picture the channel's interior. But it can yield the macroscopic diffusion parameters that reflect microscopic events in that interior. It should, therefore, prove useful in determining the underlying molecular mechanisms, in particular when it is used to provide a more realistic basis for modeling and kinetic approaches.

The following channel properties have already been demonstrated to be deducible from data — consisting in the main of I-V plots — through the use of this new, generalized, form of continuum theory: the sum of the phase-boundary potentials at the two membrane-solution interfaces (the sum of these potentials, as opposed to the individual potentials, is as thermodynamically accessible as the membrane potential itself (Bockris and Reddy, 1970)), the permeability as a function of voltage and concentration, a permeability parameter that is related to the Goldman-Hodgkin-Katz permeability but does not suffer from the internal contradictions that affect the Goldman-Hodgkin-Katz permeability and is characteristic of the channel ensemble and independent of concentration and voltage (Schwartz and Kado, 1977a,b), and an estimate of the averaged intrachannel charge density (Schwartz, 1981). Further investigation and application of this new body of theory promises to yield additional information of a sort that was previously thought to be unavailable. It, for example, predicts the occurrence of concentration but not voltage dependent permeability ratios when two ions simultaneously permeate a common set of channels (Schwartz, 1979; a more detailed discussion is in preparation). Such permeability ratios have been observed (Cahalan and Begenisich, 1976; Eisenman, Sandblom, and Neher, 1976). But, because they had no place in either the Goldman-Hodgkin-Katz or the independence theories, their existence had until now been erroneously regarded as additional evidence for the incorrect conclusion that all continuum theory is unworkable.

The continued fruitfulness and power of thermodynamically derived membrane diffusion theory seems, in summary, to be established. The use of its modern, more general, form needs to be expanded both to determine what additional information it can reveal, and to fix, by experiment, its own limits of applicability.

Appendix A

If the intracellular concentration of the permeant species does not change

$$(1A) \quad a_2(s_2) = a_1(s_2) \equiv a(s_2),$$

Multiplying both the numerator and denominator of Equation (5) by:

$$\frac{\exp\left\{-\frac{FE}{RT}\right\}}{a(s_2)}.$$

$$(2A) \quad \frac{\Delta I_2}{\Delta I_1} = \frac{\left[\frac{A}{Q'}\right]_2}{\left[\frac{A}{Q'}\right]_1} \left[\frac{1 - \frac{a_2(s_1)}{a(s_2)} \exp\left\{-\frac{FE}{RT}\right\}}{1 - \frac{a_1(s_1)}{a(s_2)} \exp\left\{-\frac{FE}{RT}\right\}} \right].$$

It follows that:

$$(3A) \quad \frac{\Delta I_2}{\Delta I_1} = \frac{\left[\frac{A}{Q'}\right]_2}{\left[\frac{A}{Q'}\right]_1} \left[\frac{1 - \frac{a_2(s_1)}{a_1(s_1)} \frac{a_1(s_1)}{a(s_2)} \exp\left\{-\frac{FE}{RT}\right\}}{1 - \frac{a_1(s_1)}{a(s_2)} \exp\left\{-\frac{FE}{RT}\right\}} \right].$$

Since

$$(4A) \quad E_0 = \frac{RT}{F} \ln \frac{a(s_1)}{a(s_2)},$$

$$(5A) \quad \frac{\Delta I_2}{\Delta I_1} = \frac{\left[\frac{A}{Q'}\right]_2}{\left[\frac{A}{Q'}\right]_1} \left[\frac{\frac{a_2(s_1)}{a_1(s_1)} \exp\left\{-\frac{F}{RT}(E - E_{01})\right\} - 1}{\exp\left\{-\frac{F}{RT}(E - E_{01})\right\} - 1} \right].$$

This expression reduces to the independence relation (Equation 1) if, and only if

$$(6A) \quad \left[\frac{A}{Q'}\right]_1 = \left[\frac{A}{Q'}\right]_2.$$

Appendix B

The inward and outward unidirectional fluxes given by Equation 2 are

$$(1B) \quad I_{IN} = A \frac{RTF}{Q'} a(s_1),$$

and

$$(2B) \quad I_{OUT} = A \frac{RTF}{Q'} a(s_2) \exp \left\{ \frac{FE}{RT} \right\},$$

respectively.

But Hodgkin and Huxley's (1952, Equations 7 and 8) derivation required that

$$(3B) \quad I_{IN} = k_1 c(s_1)$$

and

$$(4B) \quad I_{OUT} = k_2 c(s_2),$$

where c is concentration, and k_1 and k_2 are required to be constants depending only "on the condition of the membrane and the potential difference across it". Eliminating I_{IN} between Equations 1B and 3B, and I_{OUT} between Equations 2B and 4B yields

$$(5B) \quad k_1 = A \frac{RTF}{Q'} \gamma(s_1)$$

and

$$(6B) \quad k_2 = A \frac{RTF}{Q'} \exp \left\{ \frac{FE}{RT} \right\} \gamma(s_2).$$

It follows that to maintain the constraints on k_1 and k_2 for a given membrane, bathed in solutions of constant ionic strength, Q' is allowed to be a function only of E , but not of $c(s_1)$ nor of $c(s_2)$.

Appendix C

If $(A/Q')_1$ and $(A/Q')_2$ are equal, Equation 5 yields

$$(1C) \quad \frac{\Delta I_2}{\Delta I_1} = \frac{a_2(s_1)}{a_1(s_1)} \left[\frac{\frac{a_2(s_2)}{a_2(s_1)} \exp \left\{ \frac{FE}{RT} \right\} - 1}{\frac{a_1(s_2)}{a_1(s_1)} \exp \left\{ \frac{FE}{RT} \right\} - 1} \right]$$

after a minor algebraic rearrangement. Utilizing Equation 4A to introduce measurable reversal potentials in place of unknown intracellular concentrations, this becomes

$$(2C) \quad \frac{\Delta I_2}{\Delta I_1} = \frac{a_2(s_1)}{a_1(s_1)} \left[\frac{\exp \left\{ \frac{F}{RT} (E - E_{02}) \right\} - 1}{\exp \left\{ \frac{F}{RT} (E - E_{01}) \right\} - 1} \right].$$

Appendix D

The diffusion equations used in the rest of this paper are here re-examined to determine their applicability in the case in which the boundary effects at the two membrane-solution interfaces are not assumed to occur in discontinuous jumps. They are, instead, treated as a continuous transition from the bulk solution into the membrane on the outside (side 1) and a similar transition from the membrane into the solution on the other side: the inside of the cell (side 2). The usual assumption that the entire process is diffusion limited is, however, retained in this continuous treatment.

The relationship,

$$(1D) \quad j_i = -\omega_i c_i \frac{\partial \bar{\mu}_i}{\partial x}$$

where j_i is flux density and $\bar{\mu}_i$ is the electrochemical potential still describes diffusion through these channels.

Neumcke and Lauger (1969) have demonstrated that boundary effects due to image forces can be accounted for in a continuous manner by allowing μ_i^0 (the standard chemical potential to vary continuously in space as the interface is crossed. I shall adopt this approach.

Thus,

$$(2D) \quad \bar{\mu}_i = \mu_i^0 + RT \ln a_i + Z_i F \phi,$$

and

$$(3D) \quad \frac{\partial \bar{\mu}_i}{\partial x} = \frac{\partial \mu_i^0}{\partial x} + RT \frac{\partial \ln a_i}{\partial x} + Z_i F \frac{\partial \phi}{\partial x},$$

so that

$$(4D) \quad j_i = -\frac{\omega_i}{\gamma_i} a_i \left[\frac{\partial \mu_i^0}{\partial x} + RT \frac{\partial \ln a_i}{\partial x} + Z_i F \frac{\partial \phi}{\partial x} \right].$$

is the modified Nernst-Planck equation that applies in this case. Its similarity to the standard form of this equation can be made clearer if one defines a new variable:

$$(5D) \quad \xi_i = \frac{\mu_i^0}{Z_i F} + \phi$$

so that

$$(6D) \quad \mu_i^0 = Z_i F [\xi_i - \phi]$$

substituting into equation 4D:

$$(7D) \quad j_i = -\frac{\omega_i}{\gamma_i} a_i \left[Z_i F \frac{\partial \xi_i}{\partial x} + RT \frac{\partial \ln a_i}{\partial x} \right]$$

which, with three minor new features is *formally* identical to the classical Nernst-Planck equation. (See equation 2-24, Schwartz, 1971b.) The first of the new features is that ω_i of the classical equation has been replaced by $\frac{\omega_i}{\gamma_i}$. The second is that a_i has replaced the c_i of the classical equation; thus, activities have been taken into account. The third new feature is that ξ_i has replaced the ϕ of the classical equation. It follows that both activities and continuous variation of μ_i^0 can be taken into account without altering the form of the Nernst-Planck equation. It is also clear that if equation 4D is integrated across the membrane thickness in a manner identical to the way in which the classical Nernst-Planck equation was treated by Schwartz (1971b). The analogous new result must have the same form as Schwartz's equations 2-137 and 2-138, excluding the terms in those equations caused by active transport.

The new equations can easily be shown to be:

$$(8D) \quad Q_i = \int_{(s_1)}^{(s_2)} \left(\frac{\gamma}{\omega} \right)_i \exp \left\{ \frac{Z_i F}{RT} [\xi_i - \xi_i(s_1)] \right\} dx$$

and,

$$(9D) \quad j_i = -\frac{RT}{Q_i} \left[a_i(s_2) \exp \left\{ \frac{Z_i F}{RT} [\xi_i(s_2) - \xi_i(s_1)] \right\} - a_i(s_1) \right]$$

where the symbols (s_1) and (s_2) indicate those points in the bulk solutions on side (1) and side (2), respectively, at which stirring leaves off and diffusion begins.

I shall make the usual reasonable assumption that, at each interface there is a region between the membrane's interior and the immediately adjacent bulk solution in which exchange is so rapid that these regions yield an equilibrium between the membrane region and the bulk solution on the side of the interface facing that solution, as well as between the other interfacial surface and the membrane interior. The edge of the equilibrium zone inside the membrane on side (1) is, then, denoted by (m_1) , and that on side (2) by (m_2) , while 0 and δ denote the actual edges of the membrane itself. This usage does not, in any manner, conflict with the notation used earlier in this paper.

We then have the following sequence of boundaries: On side (1);

- (s_1) equilibrium zone on the solution side
- (0) edge of the membrane
- (m_1) equilibrium zone on the membrane side;

On side (2);

- (m_2) equilibrium zone on the membrane side
- (δ) edge of the membrane
- (s_2) equilibrium zone on the solution side.

The membrane's thickness is thus given by δ .

Equation 8D can, therefore be rewritten as:

$$(10D) \quad Q'_i = \int_{(s_1)}^0 \left(\frac{\gamma}{\omega} \right)_i \exp \left\{ \frac{Z_i F}{RT} [\xi_i - \xi_i(s_1)] \right\} dx + \\ + \int_0^{\delta} \left(\frac{\gamma}{\omega} \right)_i \exp \left\{ \frac{Z_i F}{RT} [\xi_i - \xi_i(s_1)] \right\} dx + \\ + \int_{\delta}^{(s_2)} \left(\frac{\gamma}{\omega} \right)_i \exp \left\{ \frac{Z_i F}{RT} [\xi_i - \xi_i(s_1)] \right\} dx.$$

In the equilibrium regions at the two interfaces, the rapidity of exchange leading to equilibrium implies that $\omega_i \rightarrow \infty$. As a consequence of this the first and last integrals in equation 10D are small enough to be negligible, and;

$$(11D) \quad Q'_i \cong \int_0^{\delta} \left(\frac{\gamma_i}{\omega_i} \right) \exp \left\{ \frac{Z_i F}{RT} [\xi_i - \xi_i(s_1)] \right\} dx.$$

The physical message conveyed by this result is that, in this diffusion limited regime, the main contribution to permeability is made by conditions in the membrane's interior where diffusion rules.

The equilibrium prevailing in the phase-boundary region on side (1) implies that:

$$(12D) \quad \bar{\mu}_i(m_1) = \bar{\mu}_i(s_1)$$

so that:

$$(13D) \quad \xi_i - \xi_i(s_1) = \xi_i - \xi_i(m_1) - \frac{RT}{Z_i F} \ln \beta$$

where, from Schwartz and Kado (1977a) equation 2:

$$(14D) \quad \beta \equiv \frac{a(m_1)}{a(s_1)}$$

from equation 5D we obtain;

$$(15D) \quad \xi_i - \xi_i(m_1) = \frac{1}{Z_i F} [\mu_i^0 - \mu_i^0(m_1)] + [\phi - \phi(m_1)]$$

Leumcke and Lauger (1969) demonstrated that, deep in the membrane's interior μ_i^0 varies very little. This taken with the fact that, for the reasons discussed in the course of deriving equation 11D from 10D, the integral in 11D is non-zero only in the inner

regions of the membrane leads to the conclusion that in the case of this integrand:

$$(16D) \quad \frac{Z_i F}{RT} [\xi_i - \xi_i(s_1)] \cong \frac{Z_i F}{RT} [\phi - \phi(m_1)] - \ln \beta.$$

and;

$$(17D) \quad \exp \left\{ \frac{Z_i F}{RT} [\xi_i - \xi_i(s_1)] \right\} \cong \frac{1}{\beta} \exp \left\{ \frac{Z_i F}{RT} [\phi - \phi(m_1)] \right\}.$$

Thus:

$$(18D) \quad Q'_i \cong \frac{1}{\beta} \int_0^{\delta} \left(\frac{\gamma_i}{\omega_i} \right) \exp \left\{ \frac{Z_i F}{RT} [\phi - \phi(m_1)] \right\} dx.$$

Comparison of equation 18D and (3) shows that Q'_i derived for the case in which boundary phenomena are depicted in a continuous manner is identical to that obtained when they are treated as discrete jumps.

We now turn our attention to the remaining portion of the flux equation; equation 9D.

We note first that equation 5D tells us that:

$$(19D) \quad \xi_i(s_2) - \xi_i(s_1) = \frac{1}{Z_i F} [\mu_i^0(s_2) - \mu_i^0(s_1)] + [\phi(s_2) - \phi(s_1)].$$

On physical grounds, the standard chemical potentials in the two bulk solutions will be essentially the same under physiological conditions. Thus:

$$(20D) \quad \xi_i(s_2) - \xi_i(s_1) = \phi(s_2) - \phi(s_1).$$

Substituting into equation 9D:

$$(21D) \quad j_i = - \frac{RT}{Q'_i} \left[a_i(s_2) \exp \left\{ \frac{Z_i F}{RT} [\phi(s_2) - \phi(s_1)] \right\} - a_i(s_1) \right].$$

From equation 1, Schwartz and Kado (1977a,b) we have that:

$$(22D) \quad E \equiv \phi(s_2) - \phi(s_1).$$

Substituting into equation 21D:

$$(23D) \quad j_i = - \frac{RT}{Q'_i} \left[a_i(s_2) \exp \left\{ \frac{Z_i F E}{RT} \right\} - a_i(s_1) \right].$$

To complete this picture we shall convert this flux density, j_i , into a current, I_i . For a univalent cation the current density, i_i , is given by Schwartz (1971a) equation 2-168,

$$(24D) \quad i_i = -Fj_i,$$

Converting the current density, i_i into a measurable current, I_i , requires multiplication by the total area available for the diffusion of the i^{th} species, A_i .

For a univalent cation we then have:

$$(25D) \quad I_i = A_i i_i = -FA_i j_i,$$

Substituting into equation 23D:

$$(26D) \quad I_i = \frac{A_i RTF}{Q_i} \left[a_i(s_2) \exp \left\{ \frac{Z_i FE}{R} \right\} - a_i(s_1) \right]$$

Comparison of equation 26D with equation 2 shows that I_i calculated in this continuous manner at the boundaries is identical to that predicted by theory when the same interfacial events were treated as discrete jumps. It therefore follows that the validity of the theory presented in this paper is independent of whether phase boundary phenomena are actually discrete or continuous.

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