PERMEABILITY, PHASE-BOUNDARY POTENTIAL, AND CONDUCTANCE IN A CHOLINERGIC CHANNEL WITHOUT CONSTANT FIELD

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ABSTRACT A potassium-selective, chemically excitable channel, whose characteristics cannot be accurately described by constant-field theory, is studied by a new approach based on diffusion theory but with no need for the classical assumptions of constant field, homogeneous membrane, and equal phase-boundary potentials at both interfaces. Permeability is defined, free of these constraints, and the Goldman coefficient is demonstrated to be a special case useful only when the constraints apply. Permeability can be evaluated directly from current-voltage data, and it is found not to be a parameter in this channel, but rather a function of both the voltage and the concentration of the permeant ion. However, it becomes concentration-independent when the membrane voltage is equal to the sum of the phase-boundary potentials. That sum can therefore be determined from these data, and it is −65 mV in this channel. The permeability at that potential is a channel parameter, and equal to 8.66×10^{-6} cm/s for this channel. A constant field is shown not to exist in this channel and the Goldman coefficient not to be a parameter but a function of potential and concentration. Although errors introduced into this coefficient by nonconstant field and unequal surface potentials partially cancel each other, the coefficient is nevertheless not a correct measure of permeability.

INTRODUCTION

Investigations of ionic permeability in biological membranes generally start from the premises provided by the Goldman-Hodgkin-Katz formulation of diffusion theory (Goldman, 1943; Hodgkin and Katz, 1949). Expressions derived based on this approach have proven extremely useful; however, contradictions between them and the results of experiment have been noted in several permeability systems (Jaffe, 1974; Hille, 1975; Ginsborg and Kado, 1975; Cahalan and Begenisich, 1976). Ginsborg and Kado (1975), in particular, have reported differences between rectification in a potassium-selective channel as predicted by this theory and as actually observed. The channel is cholinergic and occurs in the somatic membrane of the medial cells of *Aplysia californica* (Kehoe, 1972*a*, *b*). The reversal potential for potassium is sufficiently negative (−70 to −80 mV) in these cells to allow the membrane to be clamped to a wide range of potentials without initiating an action potential, and the channel is selectively

activated under certain conditions of agonist application (Ascher and Kehoe, 1975; Ginsborg and Kado, 1975). These features, coupled with its single-ion selectivity, make this channel most convenient for investigating problems relating to ionic permeability in general, and for determining the source of this contradiction between theory and experiment in particular. That problem, however, is clearly not confined to this specific channel.

The passive flow of ions through a biological membrane is usually described as diffusion through a regime in contact with aqueous media at both of its faces. This approach yields descriptive differential equations that, to be useful, must be integrated across the thickness of the membrane. But the integrands generally contain unknown functions of potential or concentration. Integration is therefore impossible without several somewhat arbitrary assumptions as to the nature of these functions. The most widely used set of such assumptions are due to Goldman (1943), and Hodgkin and Katz (1949), who supposed that the electrical field in the membrane is constant, that the membrane regions through which diffusion occurs are macroscopically homogeneous so that mobilities may be taken to be constant in space,[1] and that the phase-boundary potentials at the two membrane-solution interfaces are equal. The source of conflict between theory and experiment may thus lie either in inadequacies connected with these simplifying assumptions or, more basically, in the diffusion approach itself. The question thus merits investigation.

The resolution of this problem requires an approach still based on diffusion, but without the suppositions necessary to the Goldman-Hodgkin-Katz theory. One of us had previously developed such an approach (Schwartz, 1971*a*, *b*), and we have extended it for the purposes of this paper. An integral expression for the permeability had been obtained. We demonstrate that this integral can, for a single-ion channel, be evaluated directly from experimentally determined conductances without the assumption of either a constant field or a homogeneous membrane, and neither the phase-boundary potentials nor the partition of ions need be taken to be identical at the two membrane-solution interfaces. The analysis then yields three channel properties: (*a*) its permeability as a function of membrane potential, (*b*) the sum of the two phase-boundary potentials, and (*c*) a permeability factor that is a constant, characteristic of the channel. The values of both the phase-boundary potentials and the permeability factor are consistent with estimates obtained by other methods. The Goldman coefficient is, by contrast, shown not to describe the permeability of this channel. These findings seem to confirm the continued applicability of diffusion theory as well as the usefulness of this new, more physically exact solution of this problem.

Preliminary reports of this work were presented to the ICN-UCLA 1976 Winter Conference on Neurobiology (Schwartz and Kado, 1977), and at the meetings of the Biophysical Society (Schwartz and Kado, 1976).

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[1]These first two constraints have been shown to be more stringent than required for the expression for the zero-current potential, but are still necessary for the flux equations (see, for instance, Schwartz, 1971*a*, *b*).

METHODS

Either the right or left pleural ganglion of *Aplysia californica* (obtained from Pacific Bio-Marine Laboratories, Inc., Venice, Calif.) was isolated, connective tissue was dissected away so that the cell bodies were entirely exposed, and the ganglion was firmly pinned to the transparent Sylgard bottom (Dow Corning Corp., Midland, Mich.) of a chamber by utilizing connective tissue remaining on the nerves. The ganglion was positioned so that single cells in the medial group (Kehoe, 1972*b*) could be impaled with two electrodes, one to measure voltage and the other to pass current.

Carbamylcholine was applied to the somatic membrane of the impaled cell by iontophoresis from a point sufficiently distant that only the potassium-selective response was observed (Ascher and Kehoe, 1975), and located in a region of rapid solution flow so that the drug could be quickly washed away. The iontophoresing electrode system was composed of one double-barreled electrode, with both barrels filled with 0.1 M carbamylcholine and then electrically connected in parallel, and a second pipette with a larger tip to provide a return electrical path. The paralleled, double-barreled electrode was found to minimize tip blockage and variations in drug application during the long iontophoresing times used in this study. Drug was generally applied until the membrane's response began to decrease. The iontophoresing current was held constant by either a large series resistance or an active constant-current source. It was necessary to isolate this current source very carefully with regard to both resistance and capacitance to avoid artifactual transients in the voltage-clamp current-measuring circuit.

A fast voltage-clamp composed of operational amplifiers with a high-voltage output stage (Kado, 1971) was used. The bath was grounded through an agar-seawater salt bridge connected to a current-to-voltage converter. Total resistance to ground was 800 Ω, sufficiently low that at the currents encountered (up to 100 nA), the error in voltage measurement was negligible. Ginsborg and Kado (1975) have demonstrated that this method of voltage-clamping holds the entire region of the cell membrane reached by the carbachol at the clamping potential.

All electrodes were pulled with a DeFonbrune microforge so as to yield resistances between 5 and 20 MΩ, depending on their purpose. Finer-tipped electrodes were used for measuring voltage, and were filled with 3 M KCl. Coarser-tipped electrodes were used to pass current, and were filled with 0.6 M K_2SO_4. The iontophoresing electrodes have already been described.

Artificial seawater, formulated as follows, was used as the standard bathing medium: NaCl, 480 mM; KCl, 10 mM; $CaCl_2$, 10 mM; $MgCl_2$, 50 mM, and pH was held at 7.7 at 25°C by adding Tris-Cl, 10 mM. Alterations of potassium concentration always involved an equimolar exchange for sodium. Bath temperature was held between 12 and 18°C by a Peltier cell under the chamber. A constant-flow, gravity-feed system changed the chamber contents approximately once per minute.

RESULTS

Current-Voltage Characteristics

The steady-state currents required to hold the membrane at a series of voltages were determined, first in the absence and then in the presence of the drug (Ginsborg and Kado, 1975). Steady-state was generally reached within 1 s at a given voltage. Each cell was exposed to at least two concentrations of external potassium.

The *I-V* characteristic of the drug-activated channel was then obtained by plotting the change in the clamping current produced by the drug against the membrane po-

tential. *I-V* plots for three concentrations of external potassium typical of this channel are shown in Fig. 1.

These current-voltage characteristics are nonlinear and, as was pointed out by Ginsborg and Kado (1975), have a curvature opposite to that in the neuromuscular junction for iontophoretically applied carbamylcholine or acetylcholine (Magleby and Stevens, 1972; Dionne and Stevens, 1974). In the neuromuscular junction the nonlinear *I-V* plots have been attributed to voltage-dependent parameters in the receptor-agonist channel gating mechanisms. We shall demonstrate that the conductance characteristics of this *Aplysia* channel may, by contrast, be due to shifting intrachannel concentration profiles.

Channel Selectivity

Channel reversal potential (E_0) was determined after prolonged exposure to various external potassium concentrations. The pooled results of all experiments followed the Nernst equation (Fig. 2). In a few individual experiments the change of potential with

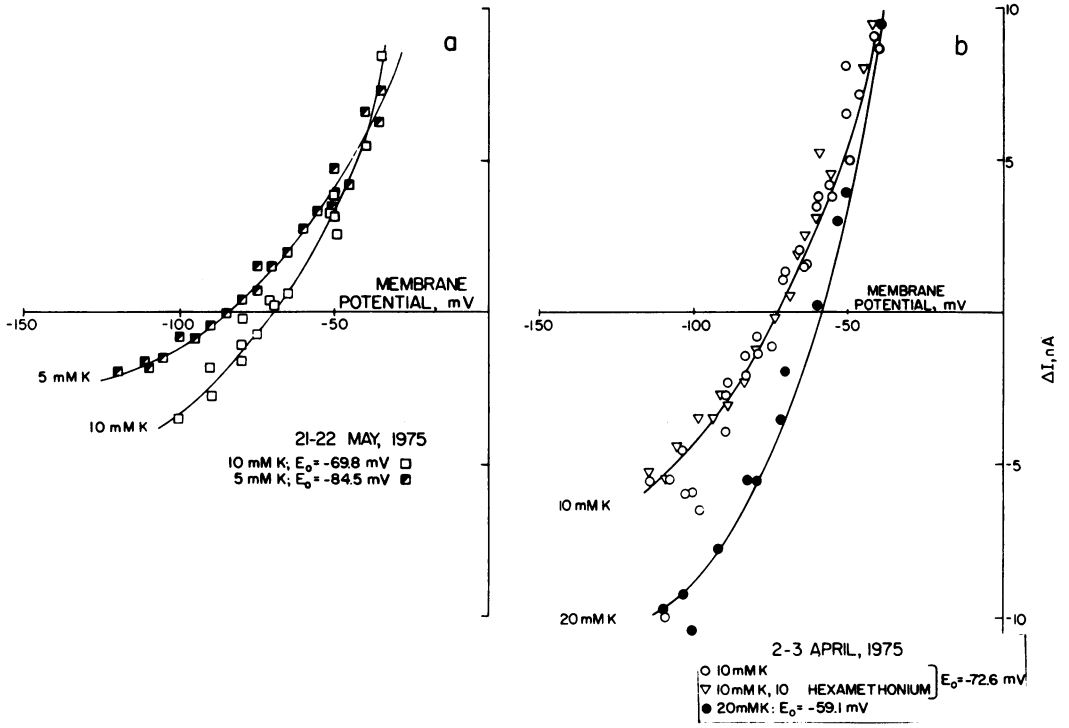


FIGURE 1 Current-voltage relationships for the carbachol-activated K channel. ΔI is the change in current under voltage-clamp after drug application. Outward currents are taken as positive, and potential is referenced to the bath. Curves were drawn by eye. (*a*) 5 and 10 mM external potassium. (*b*) 10 and 20 mM external potassium. Some 10-mM points were obtained in the presence of 10^{-4} hexamethonium, an agent known to block the excitatory response in these neurons, but with no effect on the inhibitory response of this channel (Kehoe, 1972*b*).

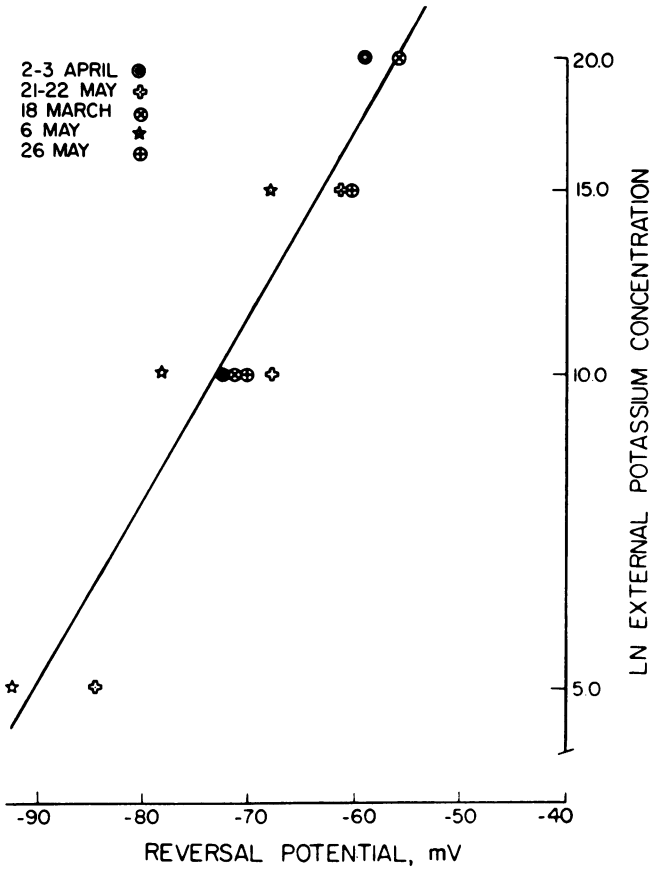


FIGURE 2 Channel reversal potential as a function of the logarithm of external K concentration. The line was drawn at the slope of F/RT, with $T = 15°C$, an approximate average temperature for these experiments.

concentration was less than that predicted by theory (Fig. 2). This was probably caused by potassium leakage into the cell when external K was higher than that in seawater, and out of the cell when it was lower (Ginsborg and Kado, 1975; Ascher et al., 1976; Ascher, personal communication). Our results are thus in agreement with Kehoe's: this channel is highly selective for potassium.

Calculating from E_0 with an assumed intracellular activity coefficient of 0.68 (Baker et al. 1962), we obtain an average K activity of 126.8 mM inside the cell. Kehoe (1972*a*) calculated 167.3 mM for these cells, and Russell and Brown (1972) have reported 165.3 mM in the related giant cell of the abdominal ganglion.

ANALYSIS AND DISCUSSION

Channel Conductance

Some information regarding the diffusion process in this channel can be obtained by examining its chord conductance. Paired values of current and voltage were read

from the *I-V* curves, thereby smoothing the raw data, and conductance (the term is reserved for chord conductance in this paper) was calculated as $I/(E - E_0)$. Analysis required the comparison of conductances calculated for different cells. This, however, was impossible without normalization, because one could not determine how many channels or, stated otherwise, how much membrane area was activated by the applied drug. For a constant iontophoretic current, this area varied with iontophoresing-electrode tip characteristics, distance from the cell of the drug injection point, ganglion geometry, and the rate and pattern of flow of the perfusing solution. With great care these parameters could be maintained constant within a single experiment; but generally not between separate experiments with different cells. We have normalized all conductances for any one cell with a single normalization factor: the chord conductance of that cell at its reversal potential in seawater. All experiments were thus placed on an equal footing, and their results could be combined.

Normalized conductances obtained at 5, 10, and 20 mM external K were plotted as functions of membrane potential (Fig. 3*a*). Two features stand out. First, conductance is a strong function of external K concentration, increasing with rising concentration. Second, at all concentrations the conductance increases nonlinearly, and with no evidence of saturation, as the membrane is depolarized.

The voltage dependence of the conductance may be due to either of two mechanisms. The conductance of an ensemble of channels of this sort would in general be expected on theoretical grounds to depend on the intrachannel concentration of the diffusing ion (Finkelstein and Mauro, 1963, and Eq. 3A). According to the first mechanism, the intrachannel concentration may be altered by manipulating the conditions external to the membrane. In that case, intrachannel concentration would be expected to be sensitive to changes in the concentration of the diffusing ion exterior to the membrane on either of its sides as well as to $E - E_0$, the force driving these ions into and through the membrane. For a given external concentration, this mechanism would react to changes in either E or E_0. According to the second mechanism, however, the conductance is controlled not by intrachannel concentration shifts but by gating processes affected by changes in the electrical field inside the channel. In that case the voltage dependence of the conductance would be sensitive to E, which reflects the electrical field, but not to E_0. Thus their different properties with regard to E_0 may provide information as to which of these mechanisms is actually operative.

Reversal potentials differed somewhat from cell to cell in our experiments, even when at the same external potassium concentration, providing us with a way to examine dependence on E_0. If the mechanism of conductance control depends primarily on intrachannel concentration shifts, it follows that the normalized conductances measured with different cells should correlate better with each other when viewed as functions of $E - E_0$ than as functions of E. In the case of gating, however, this change of independent variable would not be expected to matter.

In actuality, this correlation is considerably better with $E - E_0$ than with E (Fig. 3*a* and *b*). This suggests that diffusion-controlled intrachannel concentration

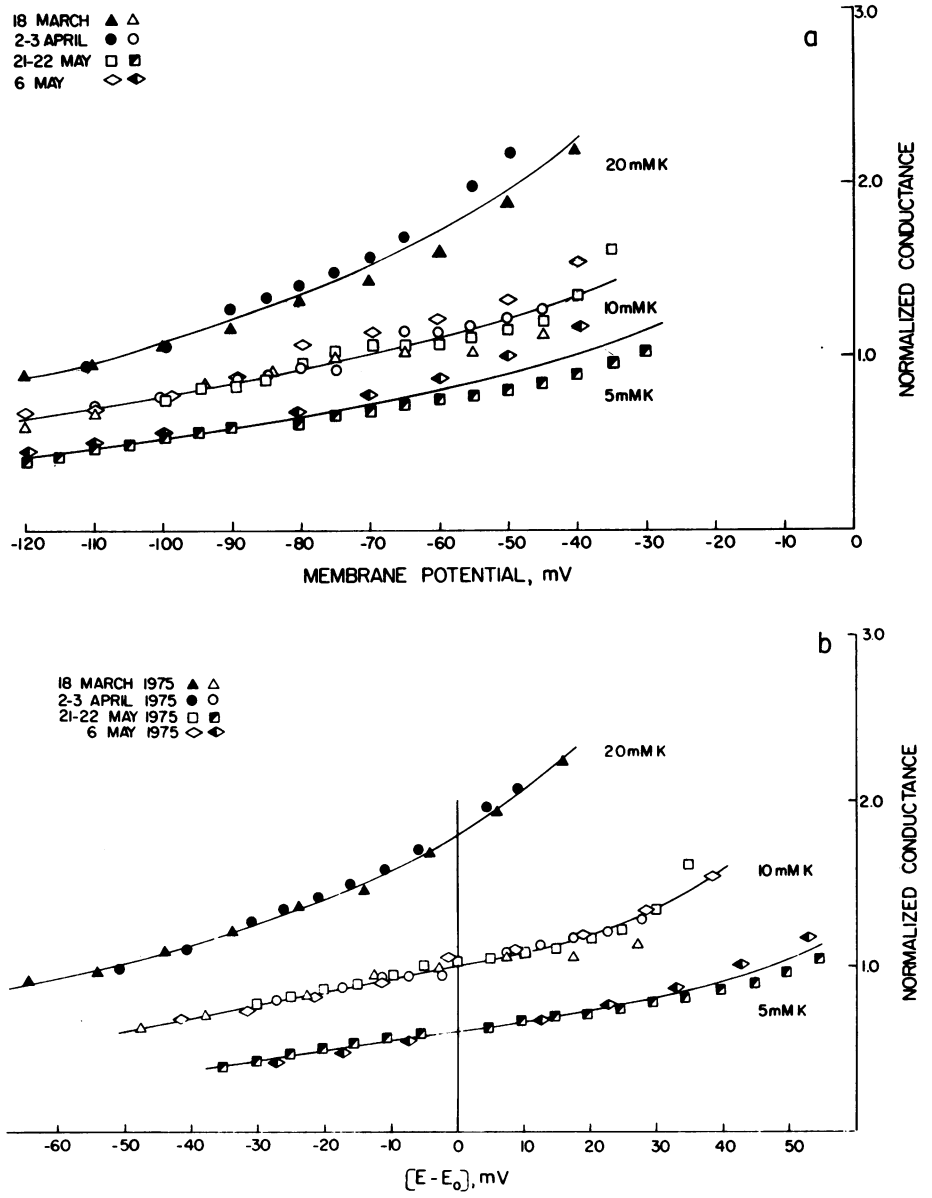


FIGURE 3 Normalized channel chord conductance (*a*) as a function of membrane potential, and (*b*) as a function of the departure of the membrane potential from the reversal potential. Data have been pooled from several experiments, and lines drawn by eye.

shifts, and not gating, produce the voltage dependence of this conductance. Its strong dependence on external K concentration is also consistent with this sort of mechanism.

Additional, though less direct, evidence along these lines exists. The relationship between conductance and external concentration is linear when the membrane is held at E_0, even though E_0 itself of course also varies with concentration (Fig. 4). Furthermore, this relationship displays opposite curvature about the E_0 line for any other pair of voltages, $E_0 + V$ and $E_0 - V$. It is therefore clear that some simple connection between conductance, external potassium concentration, and the net diffusive driving force, $E - E_0$, must exist, again implying an important role for diffusion in the control of this conductance. Even so, further investigation is required because one cannot yet rule out the presence of some gating effects alongside of these diffusion phenomena. But, completely apart from the problem of this conductance's voltage dependence and independently of its resolution, we shall demonstrate that the diffusion equation describes other aspects of the channel's behavior quite well.

Channel Permeability

The two forces that produce simple ionic diffusion, an electrical gradient and an activity gradient, give rise to two ways of defining permeability. The definition will differ depending upon which force is chosen as reference (Appendix A). Terminology is such that only when the activity gradient is chosen does one speak of permeability; when the electrical gradient is chosen one speaks instead of conductance. But, terminology notwithstanding, they both remain "permeabilities" and must therefore bear a close relationship to each other. One might then expect the determination of one to present no more difficulty than the other. In spite of this, conductance has been evaluated straightforwardly and relatively easily, but permeability only with various ap-

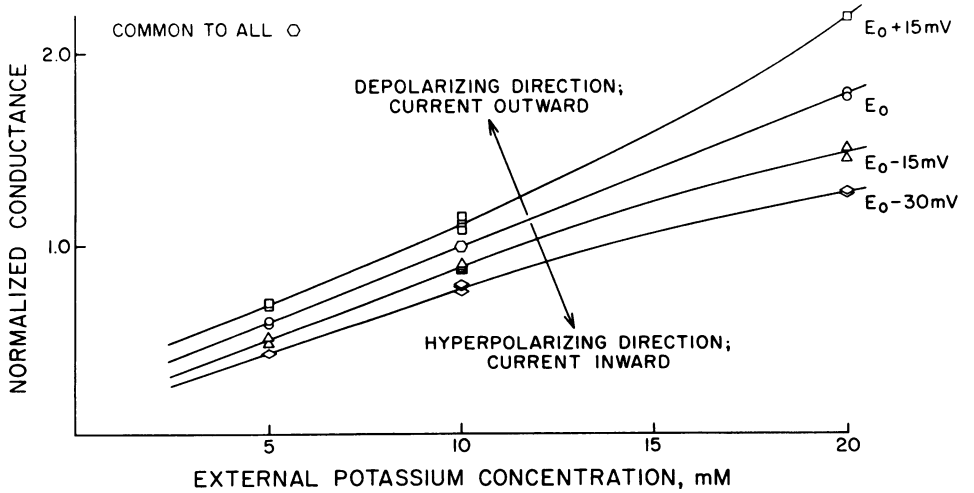


FIGURE 4 Normalized conductances at several fixed values of $E - E_0$, as functions of external potassium concentration. This figure contains the pooled data of all experiments.

proximations and difficulties. In this section we show that just as conductance can be easily determined from electrical measurements even though the mathematical integration involved in its definition (Eq. 3A) cannot in general be performed, so also can the permeability be determined in spite of a similar problem.

A general expression relating the steady-state current due to a univalent cation to the driving force has been derived for the case in which the concentration gradient is reference, thereby defining a generalized permeability (Schwartz, 1971*a*, *b*). When that expression is modified to deal with measurable quantities in the solution instead of unmeasurable ones interior to the membrane (Appendix B), and with activities instead of with concentrations, we obtain

$$I = (ARTF/Q')[a(s2)e^{(F/RT)E} - a(s1)],$$

where

$$Q' = \frac{1}{\beta} \int_0^{\delta} \left(\frac{\gamma}{\omega}\right) e^{(F/RT)[\varphi - \varphi(m1)]}\, dx, \tag{1}$$

and

$$E \equiv \varphi(s2) - \varphi(s1)$$

Integration is across the membrane, whose thickness is δ. The extracellular and intracellular compartments are denoted by 1 and 2 respectively. The letter m indicates a point just inside the membrane bordering on the appropriate compartment, while the letter s similarly indicates points in the bulk external solutions. The activity coefficient γ, as well as the mobility ω, may be functions of x, a is activity, φ is electrical potential, A is activated area, and

$$\beta \equiv a(m1)/a(s1) \tag{2}$$

is the equivalent of a partition coefficient. R, T, and F have their usual meanings.

The quantity, ART/Q' is the permeability.[2] As we indicated earlier, the integration necessary to determine Q' from first principles cannot be performed because the integrand is unknown. If, to solve this problem, constant field, equal phase-boundary potentials, and a homogeneous membrane are assumed, Eq. 1 yields a Goldman flux equation, and the permeability is given by a Goldman coefficient (Appendix C). The Goldman-Hodgkin-Katz equations are therefore a special case of Eq. 1, and the Goldman coefficient is a special case of the more general permeability, ART/Q'.

ART/Q' can, however, be determined without an integration and, hence, without assuming the nature of the integrand. If I is eliminated between Eq. 1 and

$$I = G(E - E_0) \tag{3}$$

and $a(s2)$ is taken into account with the help of Eq. 6D, we obtain

$$\frac{A}{Q'} = \left[\frac{G}{F^2 a(s1)}\right]\left[\frac{(F/RT)(E - E_0)}{e^{(F/RT)(E - E_0)} - 1}\right], \tag{4}$$

[2] Kimizuka and Koketsu (1964) have defined a related permeability.

a relationship between A/Q' and the chord conductance, G, in which all other parameters are known—a relationship that defines the connection between these two classes of permeability.

We have calculated A/Q' according to Eq. 4, and have plotted the normalized results as a function of membrane potential (Fig. 5).[3] A/Q' is, as expected (Eq. 1), voltage-dependent. It increases with hyperpolarization in an S-shaped manner, and approaches zero for large depolarizations (Eq. 4). Its voltage dependence, however, is itself a function of external potassium concentration, being steeper for lower concentrations. Permeability is thus not a channel parameter but, rather, a complex function of both potential and concentration and dependent on conditions inside the channel. Indeed, even in the case of constant G, permeability cannot be constant.

Phase-Boundary Potentials

Determining the Potentials. There are, however, two channel parameters that can be determined from the permeability by a somewhat different approach to the diffusion equation. We describe the first in this section, and the second under *Permeability at the Potential* η.

An alternate expression for A/Q' is (Appendix D)

$$A/Q' = A(\overline{\omega/\gamma})(\beta/\delta)[1 - f], \tag{5}$$

where

$$f = \frac{e^{(F/RT)(E-E_0)} - e^{(F/RT)(\eta-E_0)} - [\overline{a(m)}/a(m1)](F/RT)(E-\eta)}{e^{(F/RT)(E-E_0)} - 1},$$

$(\overline{\omega/\gamma})$ and $\overline{a(m)}$ are averages inside the membrane, and η is the sum of the two phase-boundary potentials so that

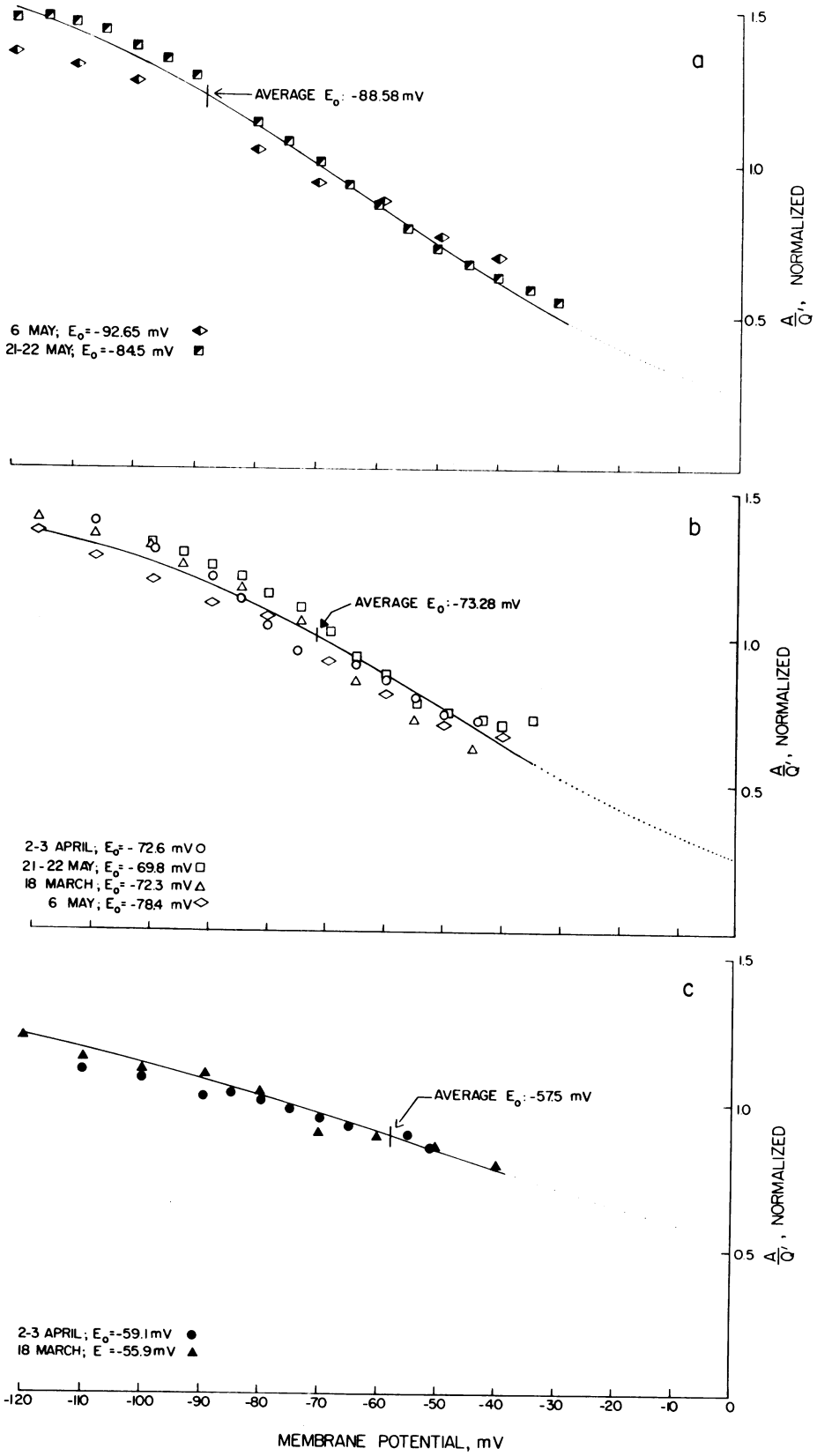
$$\eta = E - \Delta\varphi, \tag{6}$$

where $\Delta\varphi$ is the potential difference across the membrane interior (Eq. 2C).

When cast into this form, the diffusion equation centers attention on conditions prevailing when the membrane is held at $E = \eta$. At this voltage, $\Delta\varphi = 0$ (Eq. 6). In the absence of a net electrical driving force, the permeability is expected to behave in a relatively simple, Fickian, manner. In addition, the derivation that yields Eq. 5 also predicts a constant, zero, field to exist in the channel at this potential (Appendix D). The function f has the important property that it is zero when $E = \eta$; it consequently

[3] Alternatively, the first of Eqs. 1 and Eq. 6D can be combined, and paired values of I and E substituted to obtain A/Q' directly, without first calculating the conductance.

FIGURE 5 Normalized permeability as a function of membrane potential. Points were calculated from conductance data as described in the text. Lines were calculated as described in Appendix E. The normalization factor for each cell was A/Q' at reversal in seawater. (*a*) 5 mM external potassium, (*b*) 10 mM external potassium, and (*c*) 20 mM external potassium.


a
AVERAGE E_o: -88.58 mV
6 MAY; E_o = -92.65 mV
21-22 MAY; E_o = -84.5 mV
b
AVERAGE E_o: -73.28 mV
2-3 APRIL; E_o = -72.6 mV
21-22 MAY; E_o = -69.8 mV
18 MARCH; E_o = -72.3 mV
6 MAY; E_o = -78.4 mV
c
AVERAGE E_o: -57.5 mV
2-3 APRIL; E_o = -59.1 mV
18 MARCH; E_o = -55.9 mV
1.5
1.0
0.5
$\frac{A}{Q'}$, NORMALIZED
-120 -110 -100 -90 -80 -70 -60 -50 -40 -30 -20 -10 0
MEMBRANE POTENTIAL, mV

measures the deviation of channel permeability from its value at that voltage. Indeed, at $E = \eta$,

$$[A/Q']_\eta = A_\eta(\overline{\omega/\gamma})(\beta/\delta). \tag{7}$$

It is reasonable to suppose $(\overline{\omega/\gamma})(\beta/\delta)$ to be a parameter, characteristic of the channel and independent of concentration and voltage. A is a function of applied drug, and there is no reason to believe it to be dependent on potassium concentration. Therefore A/Q' at $E = \eta$ should be concentration-independent (Eq. 7), and graphs of A/Q' at different potassium concentrations should all intersect at that voltage. We should stress that A/Q' need not be voltage-independent for this conclusion to hold; only concentration independence is required.

An overlay of Figs. 5*a*, *b*, and *c* shows such an intersection to occur between −60 and −70 mV. To smoothe the data and simplify further calculation, a function of the form suggested by Eq. 5 was fitted to the data at all three concentrations (Appendix E), yielding the solid lines in Figs. 5*a*, *b*, and *c*. A single graph of these fitted curves shows a common point of intersection at −65.4 mV (Fig. 6*a*).

We have eliminated the possibility that this is an artifact of the normalization by examining nonnormalized results for three cells, each exposed to two concentrations of external potassium (Fig. 6*b*). The two resulting curves for each cell again intersect, and all three intersections lie between −67.6 and −70.0 mV.

This common permeability point can, furthermore, be tested in still another manner, and that is directly from the *I-V* plots and without calculating A/Q'. With Eq. 6D, Eq. 1 may be rewritten for $E - \eta$, and normalized by I_η in seawater. If the subscript, *sw*, denotes seawater, and *c* denotes concentration, we then have

$$\frac{[I_\eta/I_{\eta,sw}][e^{(F/RT)(\eta - E_0)} - 1]_{sw}}{e^{(F/RT)(\eta - E_0)} - 1} = c(s1)/c(s1)_{sw}, \tag{8}$$

provided $(A/Q')_\eta$ is indeed not a function of concentration. Note that here also only concentration independence is required. If the intersection has been properly identified, a graph of the left side of this equation against concentration must yield a straight line with a slope of 0.10, passing through the origin. Except for a negligible difference in the slope, it does (Fig. 7).

We conclude that the sum of the phase-boundary potentials can be directly determined for such a single-ion channel from the common intersection of graphs of A/Q' for different external concentrations of the permeant ion. In this particular channel the phase boundary potential sum is approximately −65 mV. It should be noted that this method of determining these potentials is a direct consequence of diffusion theory; it does not require additional modeling of events at the membrane-solution interfaces, and it should be valid whether voltage-sensitive gating is present or not.

Asymmetric conditions in the channel. That η is not zero has as its consequence that conditions at the inner and outer boundaries of the channel must be asymmetric. This can be more clearly seen if we define, for the membrane boundary facing

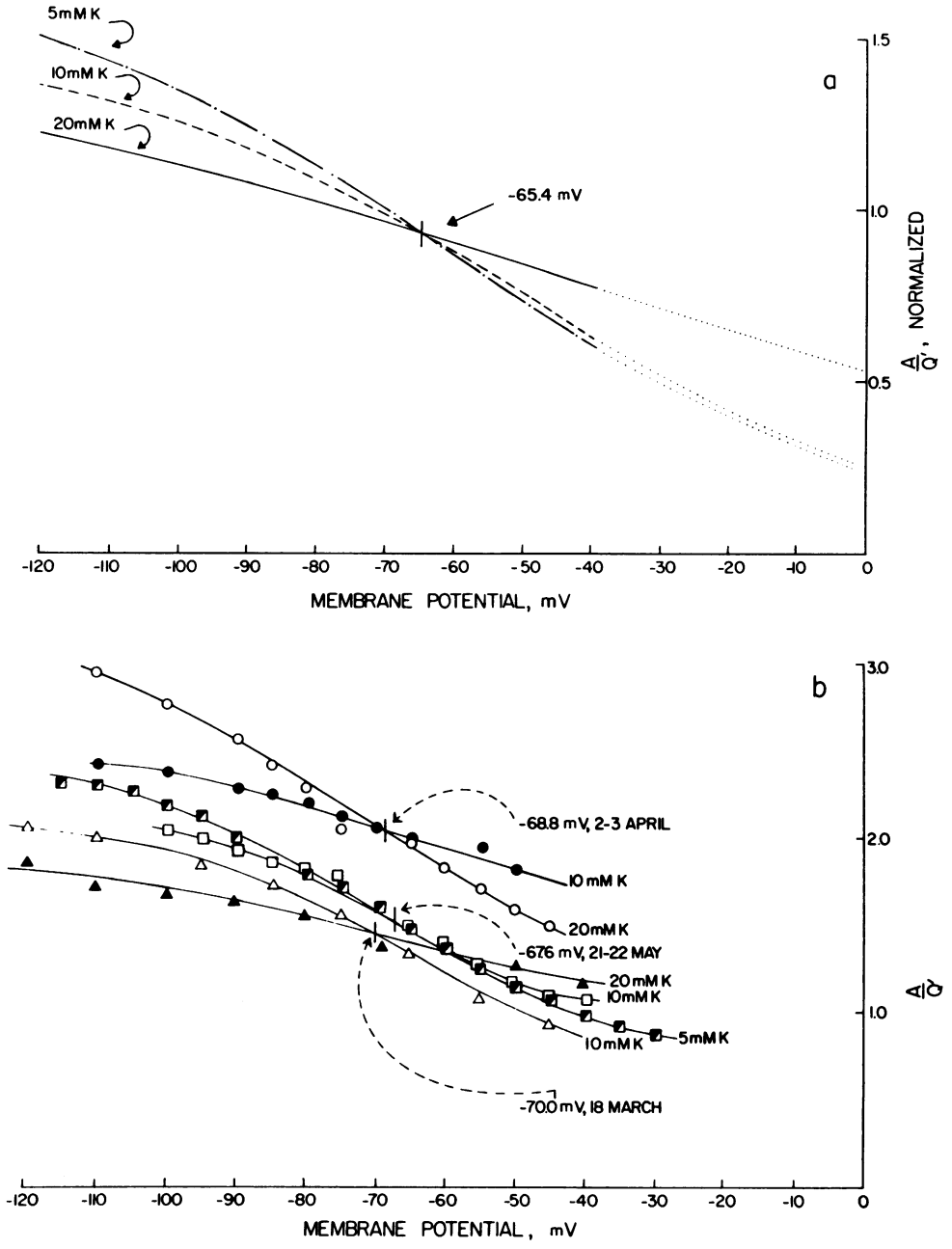


FIGURE 6 Permeability as a function of membrane potential. (*a*) An overlay of the curves in Fig. 5. (*b*) Three different experiments, data not normalized. Lines drawn by eye. A/Q' units are $[(\text{mol} \cdot \text{cm}^3)/(\text{J} \cdot \text{s})] \times 10^{12}$.

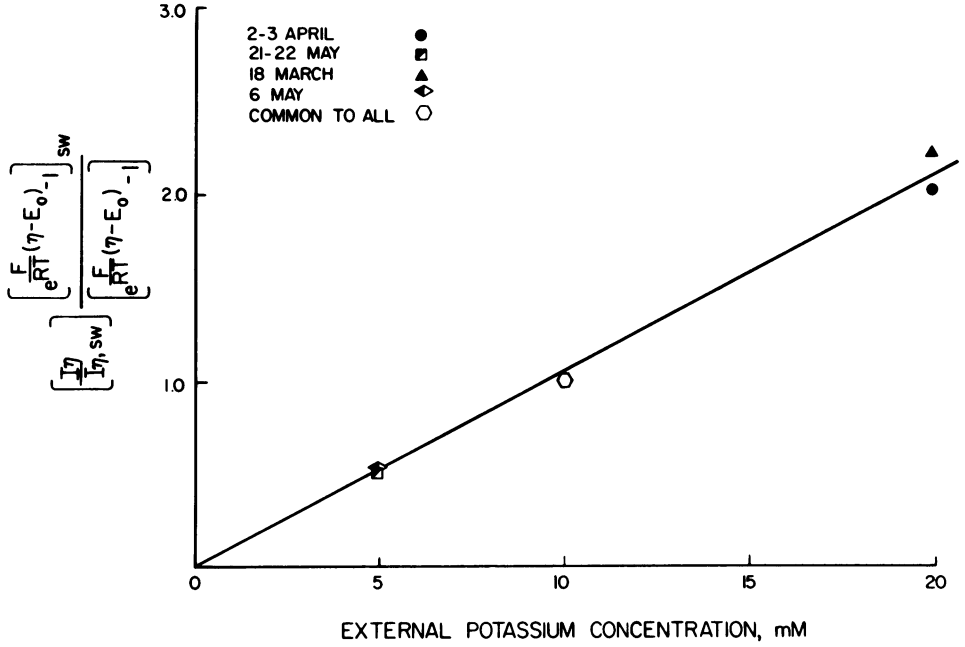


FIGURE 7 Left-hand side of Eq. 8 as a function of external K concentration. Slope is 0.105 liter/mM. I_η was read directly from the *I-V* plots; E_0 was taken as the value found in the corresponding experiment, and η was assumed to be −65.4 mV.

the inside of the cell,

$$\beta' \equiv a(m2)/a(s2), \tag{9}$$

analogous to β at the membrane boundary facing the extracellular medium. It follows from Eqs. 2, 2*A*, 6*D*, and Appendix B, that

$$\beta/\beta' = e^{-(F/RT)\eta}. \tag{10}$$

Calculating for the average temperature of 15°C maintained during these experiments, and for a value of η of −65.4 mV, β/β' is 13.9. Thus, for a given potassium activity in the extra or intracellular solutions, a much higher activity would result in the region just inside the channel but close to the outside of the cell than in a comparable region close to the inside of the cell. Barring large activity coefficient differences, the implication is that of a much higher concentration of fixed anionic charges at the outer than at the inner channel surface.

We can, by our method, determine only the sum of the two phase-boundary potentials. The separate potentials remain unknown. Individual surface potentials and their related surface charges have, however, been estimated for various excitable cells by a quite different method. The voltage dependence of the parameters of membrane excitation can be shifted by altering the ionic composition of the extra or intracellular solutions. Through models, these shifts can be related to the phase-boundary poten-

tial at the appropriate membrane surface (see, for instance, Chandler et al., 1965; Gilbert and Ehrenstein, 1969; Mozhayeva and Naumov, 1970; Brismar, 1973; Drouin and Neumcke, 1974; Hille et al., 1975). Although their method yields information about the individual phase-boundary potentials, it suffers from a lack of uniqueness with regard to the calculated results, due to a surfeit of parameters (Hille et al., 1975). Nevertheless, estimates of 8.3×10^{13} (Gilbert and Ehrenstein, 1969), and 1.4×10^{13} electronic charges/cm^2 (Chandler et al., 1965) have been obtained for the external and internal surfaces of the squid axon, respectively. Thus, in agreement with our conclusion, their very different approach also suggests that a higher charge density exists at the outer than the inner surface.

As a result of the asymmetry of the channel, boundary potentials and activities are quite different from what one would otherwise have assumed (Fig. 8). It is particularly striking that between 10 and 20 mM K, the transchannel interior activity difference reverses; that is, it becomes higher at the outer than at the inner edge, even though the exterior transmembrane activity difference maintains its orientation: inside higher than outside. The potential relationships (Fig. 8*a*) of course also reflect the internal shift in direction. It follows that making the external solution equimolar in K with the internal will not generate symmetrical boundary conditions across the membrane's interior. Actually $\Delta\varphi_0$ will then be 65.4 mV, precisely because E_0 is zero. Symmetrical internal conditions will, in fact, occur at reversal at an external K concentration between 10 and 20 mM, when $E_0 = \eta$.

The boundary potentials at 10 mM external potassium calculated by our method are remarkably similar to those calculated by Hille, et al. (1975) for their model I, using the method of shifting excitability parameters described above. Two points must be noted to make this comparison easier. Firstly, Hille et al. have depicted the electrical double-layer as diffuse and external to the membrane, while we have pictured it as discrete and just inside the membrane; the two pictures are, of course, equivalent. Secondly, β, for which a degree of arbitrariness exists (see legend for Fig. 8), is here rechosen for purposes of comparison to yield a potential of −91 mV just inside the membrane's outer surface, instead of the −114.3 mV used in Fig. 8*a*. The remaining potentials are then recalculated from our data to yield 0, −91, −99, and −73.3 mV, reading from outside to inside. Hille et al. obtained, by comparison, 0, −91, −100, and −75 mV. It is possible that this agreement constitutes an independent confirmation of the parameters assumed in their model I as opposed to model II, although different cell types as well as channels were examined in the two investigations. Indeed, even though they have different physical bases, our method and the method of shifting excitability parameters seem to yield encouragingly similar pictures of membrane surface phenomena.

Permeability at the Potential η

When the membrane potential is held at η, the steady-state channel permeability is equal to $A_\eta RT(\overline{\omega/\gamma})(\beta/\delta)$ (Eq. 7). This quantity is the product of the activated area A, and precisely the permeability factor generally estimated by calculating Goldman

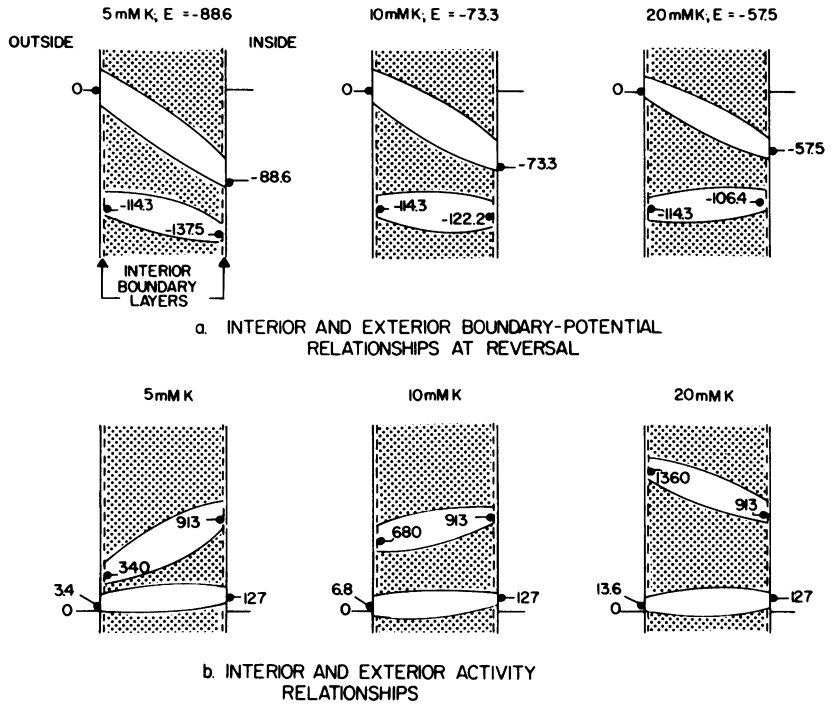


FIGURE 8 The effect of channel asymmetry on potentials and concentrations at the boundaries. Only the ratio β/β' can be determined in these experiments; the individual coefficients remain unknown. β must therefore be fixed at a physiologically reasonable value for purposes of illustration; it was chosen as 100 to construct this figure. The effect of a possible difference between the standard chemical potentials in the bulk external solution and the channel interior has been ignored. Other choices regarding either β or the standard chemical potentials simply cause linear shifts of all potentials relative to the external reference potential, and proportional changes in both interior activities. Neither of these effects are relevant to the point at hand. Drawings are approximately to scale. (*a*) Boundary potentials at reversal. Numbers are in mV. Temperature: 15°C. Passage of current will leave the exterior-interior relationship at each boundary unchanged, but will shift the potentials at one boundary relative to those at the other. (*b*) Boundary activities. Numbers are in mM. The intracellular activity is 127 mM, as determined from reversal potentials in these experiments. The activity coefficient in the extracellular medium was taken as 0.68. These relationships will be undisturbed by current; current can affect only the interior concentrations away from the boundaries.

coefficients. That factor should therefore now be available without the necessity of the usual Goldman-Hodgkin-Katz assumptions. The average value of $A_\eta RT(\overline{\omega/\gamma}) \cdot (\beta/\delta)$ for this channel calculated from the point of intersection on Fig. 6*a* is 5.51×10^{-9} cm^3/s (Table I).

Once η is known, this permeability can also be determined somewhat differently, directly from conductances. At $E = \eta$, Eqs. 4 and 5 yield[4]

[4]Eq. 11 implies a linear activity profile to exist in the (averaged) ensemble of channels at $E = \eta$. This can be shown by integrating Eq. 3A on that assumption. Thus some information about intramembrane concentrations is provided by this new approach.

TABLE I

CHANNEL PERMEABILITY FACTOR

Experiment	From Fig. 6*a*		From Fig. 9	
	Normalizing factor	$A_\eta RT(\overline{\omega/\gamma})(\beta/\delta)$	Normalizing factor	$A_\eta RT(\overline{\omega/\gamma})(\beta/\delta)$
	$[(mol \cdot cm^3)/(J \cdot s)] \times 10^{12}$	$(cm^3/s) \times 10^9$	$mho \times 10^6$	$(cm^3/s) \times 10^9$
18 March	2.21	4.97	0.138	4.84
2–3 April	3.16	7.12	0.197	6.90
6 May	2.11	4.75	0.133	4.65
21–22 May	2.31	5.21	0.144	5.05
Average	—	5.51	—	5.36

The normalized value of $A_\eta(\overline{\omega/\gamma})(\beta/\delta)$ was read from Fig. 6*a* as 0.94. Column 3 was calculated as the product of 0.94, RT at 15°C, and the individual normalizing factors. Column 5 was calculated as the product of the slope of Fig. 9 divided by an activity coefficient of 0.68, RT/F^2 at 15°C, and the individual normalizing factors. Variations between experiments probably mainly reflect variations in activated area.

$$G_\eta\left[\frac{(F/RT)(\eta - E_0)}{e^{(F/RT)(\eta - E_0)} - 1}\right] = [F^2 A_\eta(\overline{\omega/\gamma})(\beta/\delta)\gamma(s1)]c(s1). \qquad (11)$$

A plot of the left side of this equation against external potassium concentration should yield a straight line that passes through the origin, and whose slope is proportional to the permeability. We have made this graph using normalized conductances (Fig. 9),

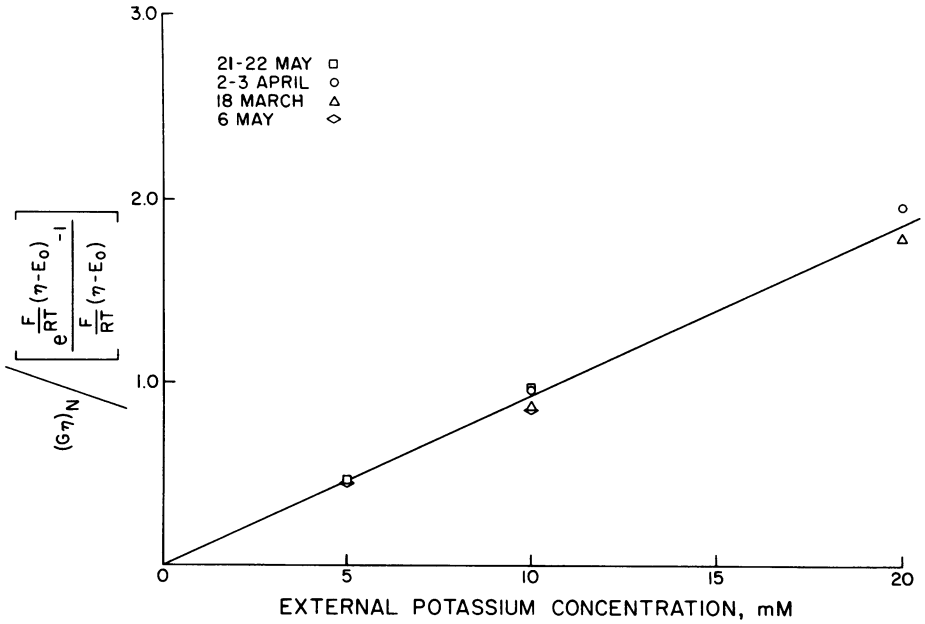


FIGURE 9 Left side of Eq. 11 as a function of external potassium concentration. The subscript N indicates the use of normalized conductances. E_0 was taken as the value found in the corresponding experiment, and η as −65.4 mV. The line was drawn according to a linear regression through the origin with a slope of $9.27 \times 10^4\ mol^{-1}$.

and it is immediately evident that the first two predictions are satisfied. $A_\eta RT(\overline{\omega/\gamma}) \cdot (\beta/\delta)$ calculated from the slope has an average value of 5.36×10^{-9} cm^3/s (Table I) in excellent agreement with the value obtained above from Fig. 6*a*. The two yield an overall average of 5.44×10^{-9} cm^3/s. If one presumes the absence of voltage-sensitive gating, A is a constant that can be taken as the area accessible to the applied agonist. Even so, in a preparation of this sort A is not known; but it may be estimated and the permeability factor may then be deduced. A typical medial cell has an approximate diameter of 200 μm. We have applied the agonist to a spherical cell body in a manner so that only approximately half of the somatic membrane can be readily reached by the drug. An average A can therefore be estimated as 6.28 $(10)^{-4}$ cm^2, and $RT(\overline{\omega/\gamma})(\beta/\delta)$ is 8.66×10^{-6} cm/s. In comparison, Hodgkin and Katz (1949) obtained a Goldman coefficient of 1.8×10^{-6} cm/s for the resting potassium system in squid axon.

Goldman Coefficients

We have shown how channel permeability can be determined from the ordinary data of electrophysiology without resort to certain assumptions about the membrane's interior. It remains possible, however, that the classical Goldman-Hodgkin-Katz approach also works for this channel. In that case a Goldman coefficient would also yield the correct value of the permeability factor. Since we now know the Hodgkin-Katz assumption of a zero sum of phase-boundary potentials to be incorrect, it would then have to follow that the effect of this nonzero sum would have exactly to be counterbalanced by a nonconstant electrical field. Ginsborg and Kado's (1975) observation that the Goldman-Hodgkin-Katz theory predicts a rectification smaller than that actually observed in this channel makes this sort of phenomenon doubtful, but the question should be explored.

To this end, we have first examined the possibility that the electrical field may actually be constant. Assuming constant field and membrane homogeneity to exist in the channel, and allowing η to assume its true value, it follows from Eqs. 6C, 3, and 6D that

$$PA = \frac{G}{Fa(s1)} \left[\frac{E - E_0}{e^{(F/RT)(E-E_0)} - 1}\right]\left[\frac{e^{(F/RT)(E-\eta)} - 1}{F/RT(E - \eta)}\right], \tag{12}$$

where P is the Goldman coefficient under these circumstances (Eq. 7C). In the absence of voltage-sensitive gating, this coefficient shouldn't vary with voltage, nor should it vary with concentration if the above description of the channel is correct.

We have calculated PA according to Eq. 12, and plotted the normalized results as a function of membrane potential (Fig. 10). The coefficient is actually a function of both membrane potential and external K concentration, although its concentration dependence becomes less severe for voltages more hyperpolarized than η. At $E = \eta$, $(PA)_\eta$ is concentration-independent, and it can be shown to equal $(ART/Q')_\eta$. This is a consequence of the existence of a constant field at that potential (Appendix D). However, a constant field appears not to exist at any other membrane potential.

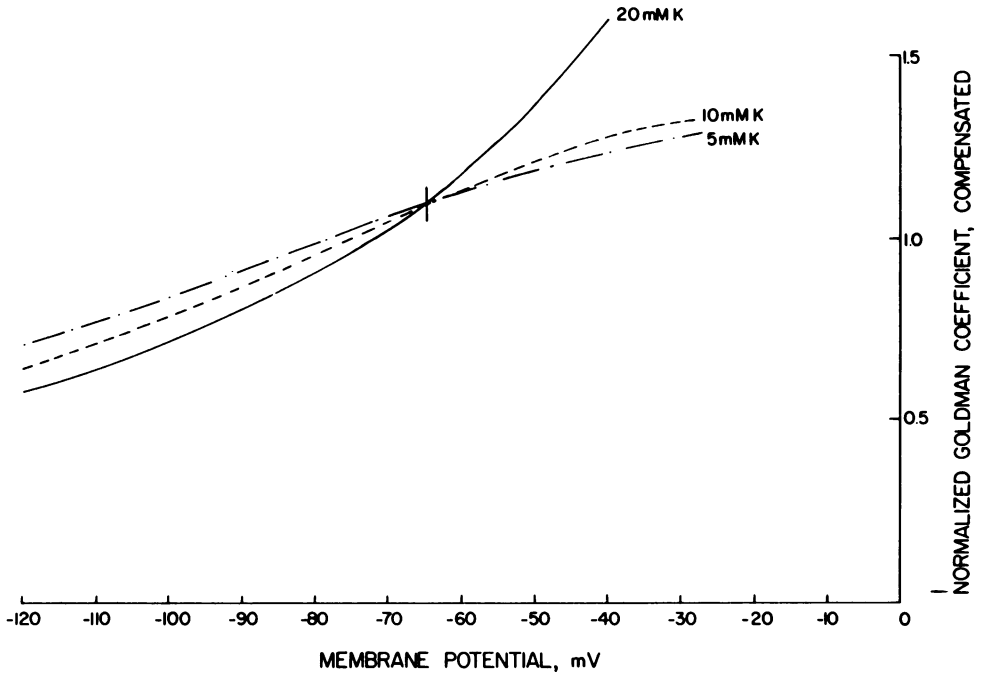


FIGURE 10 Normalized Goldman coefficients as a function of membrane potential for 5, 10, and 20 mM external potassium. These coefficients have been compensated to take into account that $\eta = -65.4$ mV. Calculation was made from the smoothed curves fitted to the normalized plots of A/Q' (Figs. 5 and 6). The normalization factor for each cell was PA at reversal in seawater. The vertical bar indicates the voltage η.

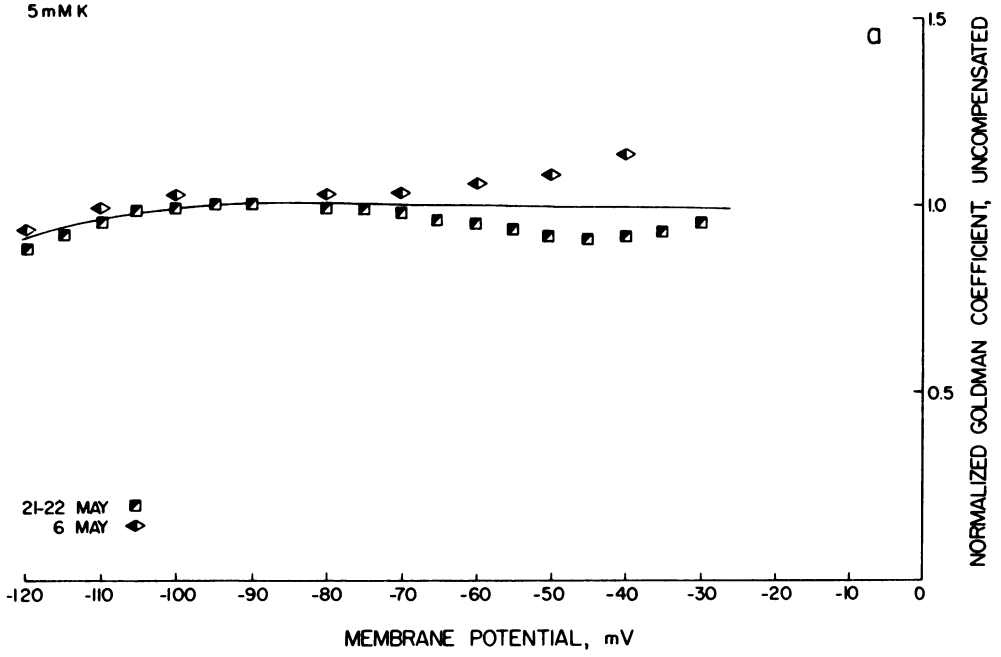
We therefore returned to the possibility that the effects of the nonconstant field and of the nonzero boundary potential sum cancel each other. That possibility may be examined by taking η to be zero in Eq. 12, yielding

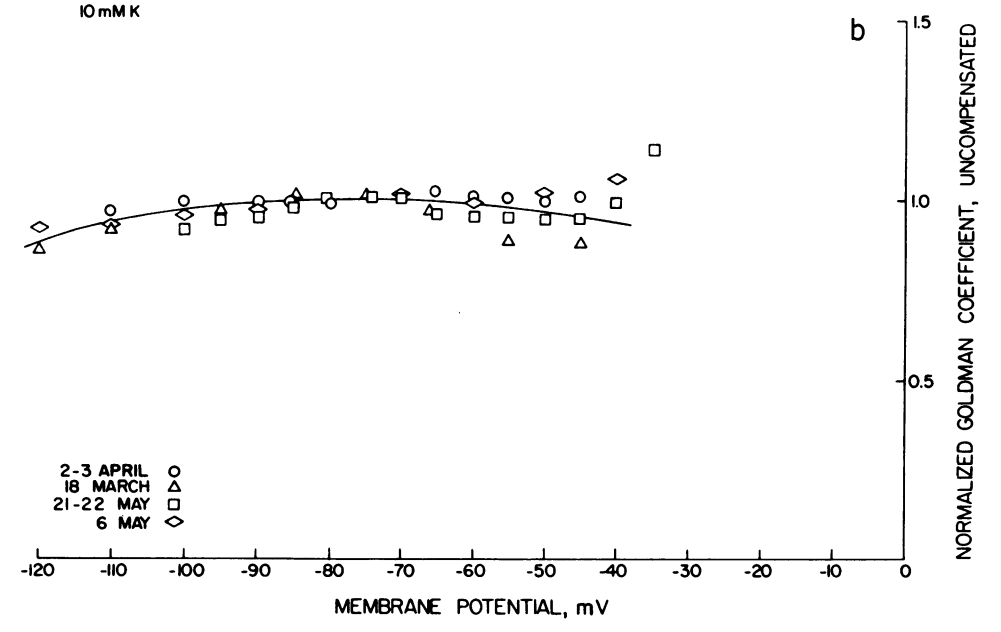
$$PA = \frac{G}{Fa(s1)}\left[\frac{E - E_0}{e^{(F/RT)(E-E_0)} - 1}\right]\left[\frac{e^{(F/RT)E} - 1}{(F/RT)E}\right]. \quad (13)$$

This expression is analogous to the one used by Dodge and Frankenhaeuser (1959) to calculate sodium permeability coefficients. If the effects do indeed cancel, the coefficient should once again not vary with either membrane potential or external K concentration.

We calculated PA according to Eq. 13 and plotted the normalized results (Fig. 11). At 5 mM K the coefficient is constant except for a dip at potentials more hyperpolarized than −90 mV. At 10 mM K it is somewhat more voltage-dependent than at 5 mM K; but comparing these two sets of coefficients shows the effect of the concentration change to be small and within the scatter of the data. At 20 mM K, however, both the voltage and concentration dependencies are pronounced.

It appears, then, that the effects of nonconstant field and nonzero boundary poten-


5 mM K
a
1.5
1.0
0.5
21-22 MAY
6 MAY
-120 -110 -100 -90 -80 -70 -60 -50 -40 -30 -20 -10 0
MEMBRANE POTENTIAL, mV
NORMALIZED GOLDMAN COEFFICIENT, UNCOMPENSATED


10 mM K
b
1.5
1.0
0.5
2-3 APRIL
18 MARCH
21-22 MAY
6 MAY
-120 -110 -100 -90 -80 -70 -60 -50 -40 -30 -20 -10 0
MEMBRANE POTENTIAL, mV
NORMALIZED GOLDMAN COEFFICIENT, UNCOMPENSATED

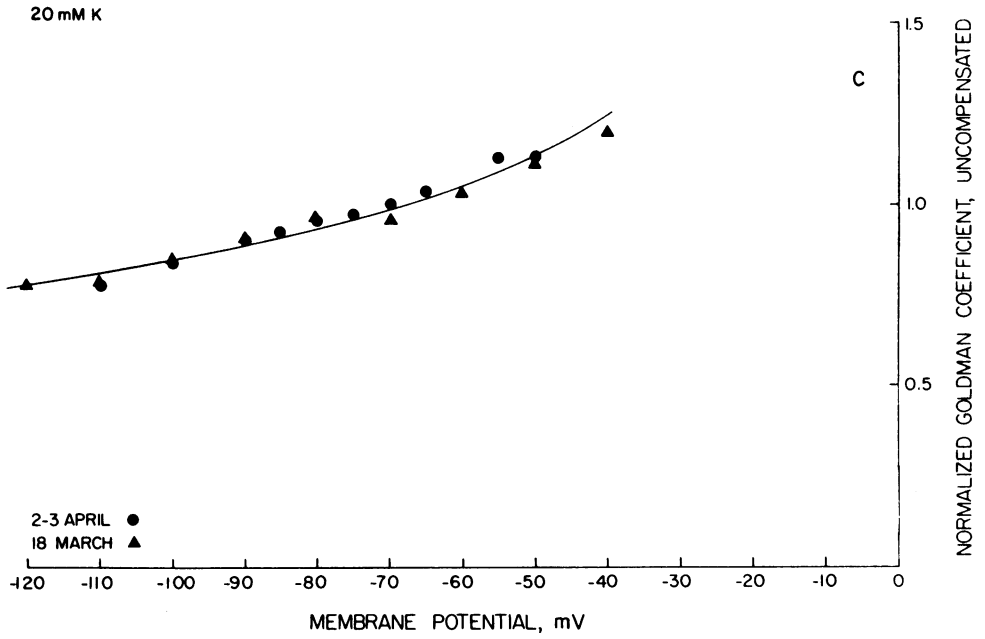


FIGURE 11 Normalized Goldman coefficients as functions of membrane potential, uncompensated in that the phase boundary potentials are assumed equal and opposite, so that their sum is zero. (*a*) 5 mM external potassium, (*b*) 10 mM external potassium, and (*c*) 20 mM external potassium. Normalization was as for Fig. 10.

tial sum do partially cancel each other, and that in the range 5–20 mM K the degree of cancellation decreases as external potassium concentration increases. If the observed rectification is compared with that predicted by the Goldman-Hodgkin-Katz theory, one finds, in agreement with this observation, that the discrepancy between them increases with increasing external K (Table II). In agreement with Ginsborg and Kado (1975), we find the observed rectification to be greater than that predicted by the theory

TABLE II

COMPARISON OF OBSERVED RECTIFICATION WITH THAT PREDICTED BY THE GOLDMAN-HODGKIN-KATZ THEORY

Concentration	Normalized G −40 mV	Normalized G −100 mV	G_{-100}/G_{-40} Observed	G_{-100}/G_{-40} Calculated
mM K				
5	1.02	0.54	0.529	0.529
10	1.42	0.79	0.556	0.594
20	2.31	1.09	0.472	0.673

Rectification is measured as the ratio of the chord conductance at −100 mV to that at −40 mV. The closer the ratio is to unity, the less the rectification. Normalized conductances were read from Fig. 3*b*. The calculated ratios are from Eq. 13, with PA assumed constant.

(Table II). Ginsborg and Kado noted also that at 50 mM external K this disparity appeared to decrease. Our data do not extend to that concentration. In general, permeability cannot be calculated for this channel by determining Goldman coefficients.

APPENDIX A: *Permeability depends on the choice of a reference force*

Electrical Force is Reference

The reference force is stated as a simple gradient. Hence, when the Nernst-Planck equation for the flux density due to a single univalent cation is written as

$$j = -\left(\frac{\omega}{\gamma}\right) aF\left[\frac{RT}{F}\frac{\mathrm{d}}{\mathrm{d}x}\ln a + \frac{\mathrm{d}\varphi}{\mathrm{d}x}\right] \tag{1A}$$

the reference force is electrical, and the driving force due to the activity appears as an additive term. Symbols are as defined for Eq. 1 in the main text. Integration in the steady state then yields a total driving force of $(\Delta\varphi - \Delta\varphi_0)$, where

$$\Delta\varphi_0 = (RT/F)\ln[a(m1)/a(m2)], \tag{2A}$$

and a force-flux proportionality factor, or permeability, of

$$G/F = \frac{A}{\int_0^\delta \frac{1}{F\omega c}\,\mathrm{d}x}, \tag{3A}$$

where G is the chord conductance (Finkelstein and Mauro, 1963). Since the phase-boundary potentials are taken to be invariant with current (Appendix B),

$$\Delta\varphi - \Delta\varphi_0 = E - E_0, \tag{4A}$$

where E includes the phase-boundary potentials (Eq. 1).

Activity Gradient Is Reference

When the Nernst-Planck equation is written as

$$j = -RT\left(\frac{\omega}{\gamma}\right)\left[\frac{\mathrm{d}a}{\mathrm{d}x} + \frac{F}{RT}a\frac{\mathrm{d}\varphi}{\mathrm{d}x}\right], \tag{5A}$$

the activity term appears as a simple gradient with the electrical term more complex, and additive. However, the expression is not useful in this form because the electrical term cannot be integrated to yield a force dependent only on boundary conditions. This difficulty can be circumvented since

$$\frac{\mathrm{d}a}{\mathrm{d}x} + \frac{F}{RT}a\frac{\mathrm{d}\varphi}{\mathrm{d}x} = e^{-(F/RT)\varphi}\frac{\mathrm{d}}{\mathrm{d}x}\left[ae^{(F/RT)\varphi}\right], \tag{6A}$$

so that

$$j = -RT\left(\frac{\omega}{\gamma}\right)e^{-(F/RT)\varphi}\frac{\mathrm{d}}{\mathrm{d}x}\left[ae^{(F/RT)\varphi}\right]. \tag{7A}$$

In this form the driving force $(d/dx)[ae^{(F/RT)\varphi}]$ is the gradient of an electrically modified activity. It returns to its unmodified, Fickian, form when either the diffusing species is uncharged or φ is constant in x. This same modified activity occurs in the derivations by Ussing (1949) and Teorell (1949) of the unidirectional flux ratio. Integration of Eq. 7A and correction for phase-boundary effects yields a permeability of ART/Q' (Eq. 1 and Schwartz, 1971*a*, *b*). In the case of uncharged species this coefficient is analogous to AD, where D is Fick's diffusion coefficient.

APPENDIX B

Assuming local equilibria at the membrane-solution interfaces (Kirkwood, 1954),

$$\beta[a(s2)/a(m2)] = e^{-(F/RT)[E - \Delta\varphi]}, \tag{1B}$$

where $\Delta\varphi$ is the potential difference across the membrane interior (Eq. 2C), and E and β are defined in Eqs. 1 and 2, respectively (see also footnote 4 in Schwartz, 1971*a*). Correction may then be made for phase-boundary effects, to yield Eqs. 1. In accord with the usual practice, the equilibria at the boundaries are assumed undisturbed by current, so that β is a function of neither I nor E (Finkelstein and Mauro, 1963, p. 226).

APPENDIX C

The Case of Constant Field, Homogeneous Membrane, and Equal Phase-Boundary Potentials

When the field is constant and the membrane homogeneous

$$d\varphi/dx = \Delta\varphi/\delta \tag{1C}$$

where

$$\Delta\varphi = \varphi(m2) - \varphi(m1), \tag{2C}$$

and Eqs. 1 yield

$$Q' = \left(\frac{\gamma}{\omega}\right)\left(\frac{1}{\beta}\right)\left(\frac{\delta}{\Delta\varphi}\right)e^{-(F/RT)\varphi(m1)} \int_{\varphi(m1)}^{\varphi(m2)} e^{(F/RT)\varphi}d\varphi. \tag{3C}$$

Integration across the membrane produces

$$Q' = (\gamma/\omega)(RT/F)(1/\beta)(\delta/\Delta\varphi)[e^{(F/RT)\Delta\varphi} - 1]. \tag{4C}$$

But

$$\Delta\varphi = E - \eta \tag{5C}$$

where η is the sum of the two phase-boundary potentials. It follows that

$$I = AFP\left[\frac{(F/RT)(E - \eta)}{e^{(F/RT)(E-\eta)} - 1}\right][a(s2)e^{(F/RT)E} - a(s1)], \tag{6C}$$

where

$$P \equiv RT(\omega/\gamma)(\beta/\delta). \tag{7C}$$

If the phase-boundary potentials are equal but opposite in sign, $\eta = 0$ and

$$I = (AF^2E/RT)P\left[\frac{a(s2)e^{(F/RT)E} - a(s1)}{e^{(F/RT)E} - 1}\right]. \tag{8C}$$

Noting that Goldman, Hodgkin, and Katz worked with concentrations instead of activities, that the mobility used in this paper, ω, is related to theirs, u, by

$$u = F\omega, \tag{9C}$$

Eq. 8C is identical to that derived by them, and P is the Goldman coefficient.

APPENDIX D

The Nernst-Planck equation for current in the channel due to a single cation

$$I = AF(\omega/\gamma)[RT(da/dx) + Fa(d\varphi/dx)], \tag{1D}$$

may be integrated to yield

$$I = AF(\overline{\omega/\gamma})(RT/\delta)[a(m2) - a(m1) + (F/RT)(\Delta\varphi)\overline{a(m)}], \tag{2D}$$

where $\overline{a(m)}$ is an average defined by

$$\overline{a(m)} = \frac{1}{\Delta\varphi}\int_0^\delta a\frac{d\varphi}{dx}\,dx \tag{3D}$$

and

$$(\overline{\omega/\gamma}) = \delta/\int_0^\delta (\gamma/\omega)dx. \tag{4D}$$

These averages result from the use of the mean value theorem of integral calculus. To be valid, all functions associated with these integrals must be continuous within the membrane, and $d\varphi/dx$ must not change sign, although it does not have to be a smooth function. This allows φ to change in a nonlinear, piece-wise continuous fashion, as long as it remains monotonic. The only test we can make of this procedure is a pragmatic one: it seems to work and to yield physically reasonable results consistent with those obtained by other approaches.

Using Eqs. 2, 6, 2A, and 2D we obtain

$$I = AF(\overline{\omega/\gamma})(RT/\delta)\beta a(s1) \cdot \{e^{(F/RT)(\eta - E_0)} - 1 + [\overline{a(m)}/a(m1)](F/RT)(E - \eta)\}. \tag{5D}$$

Since

$$E_0 = (RT/F)\ln[a(s1)/a(s2)], \tag{6D}$$

we can use Eq. 1 to give

$$1/Q' = \left(\frac{\bar{\omega}}{\gamma}\right)\left(\frac{\beta}{\delta}\right)\left[\frac{e^{(E/RT)(\eta - E_0)} - 1 + [\overline{a(m)}a(m1)](F/RT)(E - \eta)}{e^{(F/RT)(E - E_0)} - 1}\right]. \tag{7D}$$

This can be rewritten in the form

$$A/Q' = A(\overline{\omega/\gamma})(\beta/\delta)[1 - f], \tag{8D}$$

where

$$f = \frac{e^{(F/RT)(E-E_0)} - e^{(F/RT)(\eta-E_0)} - [\overline{a(m)}a(m1)](F/RT)(E - \eta)}{e^{(F/RT)(E-E_0)} - 1}. \tag{9D}$$

Note that at $E = \eta$, $\Delta\varphi = 0$ (Eq. 5C). Since $d\varphi/dx$ must not change sign, it follows that a constant zero field exists at this membrane potential.

APPENDIX E

According to Eq. 5, and provided $A(\overline{\omega/\gamma})(\beta/\delta)$ is not a function of membrane potential, the normalized A/Q' data can be fitted with an equation of the form

$$[A/Q']_N = K(1 - f), \tag{1E}$$

where the subscript N denotes normalization, K is a constant given by

$$K = [A(\overline{\omega/\gamma})(\beta/\delta)]_{N,E=\eta} \tag{2E}$$

and f is a function of $E - \eta$ that is zero when $E = \eta$. The advantage in choosing an equation of this form is that its different parts can then, on theoretical grounds, be assigned physical meaning.

At the start, however, η is unknown so that $(A/Q')_N$ must be determined as a function of E, and not of $E - \eta$. An expression of the form

$$[A/Q']_N = K'(1 - f'), \tag{3E}$$

where f' vanishes when $E = 0$ was chosen for this purpose. Then

$$K' = [A/Q']_{N,E=0}. \tag{4E}$$

A choice of

$$f' = \alpha\{\tanh[(F/bRT)(E - \theta)] - k\}, \tag{5E}$$

where k, α, b, and θ are parameters to be chosen empirically to shape the curve, was found to do nicely.

Since we want f' to vanish when $E = 0$,

$$k = -\tanh[(F/bRT)\theta]. \tag{6E}$$

We require, also, that $(A/Q')_N$ vanish for large, positive E so that

$$\alpha(1 - k) = 1, \tag{7E}$$

and

$$[A/Q']_N = \alpha K'\{1 - \tanh[(F/bRT)(E - \theta)]\} \tag{8E}$$

results, with three parameters to be determined.

It was noted, on empirical grounds, that fit was best if f'/E was chosen to have a maximum at E_0. Three relationships follow from this requirement (Schwartz, unpublished notes). They are that

$$K' = [A/Q']_{N,E_0} - m_0 E_0 \tag{9E}$$

where m_0 is the slope of the graph of $(A/Q')_N$ against E, at $E = E_0$; that

$$(F/bRT)E_0 = \frac{1}{2}\frac{K'}{\left[\frac{A}{Q'}\right]_{N,E_0}}\left[1 - e^{(-2F/bRT)E_0}\right]; \tag{10E}$$

and that

$$\tanh[(F/bRT)\theta] = \coth[(F/bRT)E_0] - \frac{(F/bRT)E_0}{\sinh^2[(F/bRT)E_0]} \tag{11E}$$

These relationships provide sufficient information for the remaining parameters to be determined. The procedure is as follows

(*a*) From the known value of E_0, graphically estimate both $(A/Q')_N$ at E_0, and m_0. (*b*) Calculate K' from Eq. 9E. (*c*) Solve Eq. 10E numerically for FE_0/bRT. (*d*) Calculate F/bRT. (*e*) Determine $\tanh(F\theta/bRT)$ from Eq. 11E, and calculate θ. (*f*) Calculate α from Eqs. 6E and 7E. (*g*) Calculate $(A/Q')_N$ as a function of E using Eq. 8E.

After η is determined from the graphs of $(A/Q')_N$ f can be calculated from the relationship

$$f = \frac{\alpha\{\tanh[(F/bRT)(E - \theta)] - \tanh[(F/bRT)(\eta - \theta)]\}}{1 - \alpha\{\tanh[(F/bRT)\theta] + \tanh[(F/bRT)(\eta - \theta)]\}}, \tag{12E}$$

derived from Eqs. 1E, 3E, and 5E.

The successful fits achieved by this procedure constitute additional, though rather indirect, evidence for A being constant with membrane potential.

Calculations were performed on a digital computer.

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