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Ion Flow through Membranes and the Resting Potential of Cells

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Summary. A knowledge of the relationship between ion flow, both passive and active, ionic concentration, and membrane potential is essential to the understanding of cellular function. The problem has been analyzed on the basis of elementary physical and biophysical principles, providing a theoretical model of current flow and resting potential of cells, including those in epithelia. The model assumes that the permeability of the ion channels is not voltage dependent, but applies to gated channels when the gates are open. Two sources of nonlinearity of the current-voltage relationship are included in the analysis: ionic depletion and accumulation at the channels' mouths, and channel saturation at higher concentrations. The predictions of the model have been quantitative, validated by comparison with experiment, which has been limited to the only two cases in which adequate data was found. Application of the theory to the scala media of the mammalian cochlea has explained the source of its high positive potential and provided estimates of the Na^+ and K^+ permeabilities of the membranes of its marginal cells. This analysis provides a theoretically sound alternative to the widely used Goldman equation, the limited validity of which was emphasized by Goldman (D.E. Goldman, 1943, *J. Gen. Physiol.* **27**:37–60), as well as its derivatives, including the Goldman-Hodgkin-Katz equation for resting potentials.

Key Words ions · channels · conductance · concentration · nonlinearities · resting potential · epithelia

Introduction

In understanding or predicting the behavior of cells, it is often important to be able to relate the flow of the various ion species across the membrane to the potential difference across the membrane and the ionic concentrations in cytoplasm and the external medium, not only because the magnitude of the flow of the individual species may be *per se* important to understanding cellular function, but also because the resting potential of a cell is that at which the total ion flow across the cell's membrane equals zero.

In 1943, David Goldman (1943) attempted to find such a theoretical relationship which might explain the nonlinear current-voltage relationship observed in squid axon. To make the problem analytically solvable required that he make a number of simplifications, including the "constant field" assumption, which he clearly pointed out, warning of the limited validity of his results. Of course, at that time the important effect of gating was completely unrecognized. His equation was

$$I = \frac{zPVF^2}{RT} \cdot \frac{C_{ext} - C_{int} \cdot \exp(zVF/RT)}{1 - \exp(zVF/RT)} \tag{1}$$

I being the current through the membrane of an ion species of permeability P, valence z and concentrations C_{ext} and C_{int} in the external and internal baths.

Hodgkin and Katz (1949) later used the Goldman equation to calculate the resting potential of cells. Their equation, known as the "Goldman-Hodgkin-Katz Equation," as well as the Goldman equation itself and other equations derived from it continue to be widely used in interpreting electrophysiological data, despite the limitations of their validity.

In this paper, an alternative treatment, based on fundamental physiochemical principles, is developed, and its predictions are compared with available experimental data.

Theory

The Conductance Approach of Hodgkin and Huxley

Hodgkin and Huxley (1952) described the Na^+ and K^+ currents in squid axon by phenomenological equations of the form

$$I_{Na} = G_{Na}(V - E_{Na}) \tag{2a}$$
$$I_K = G_K(V - E_K) \tag{2b}$$

where E_j is the Nernst (equilibrium) potential of the

species:
$E_j = (RT/zF)\log(C_{j\text{ext}}/C_{j\text{int}})$, with $z = +1$ for univalent cations. Since the sense of I_j will reverse as V passes through E_j, E_j is also termed the *reversal potential*. They expressed the chord conductances, G_j, by empirical equations to describe their voltage- and time-dependence. From single channel recordings, it has since been found that the apparent change in channel conductance is largely the result of the opening and closing of voltage-sensitive gates, and that when the gate is open, the *I-V* relationship frequently tends to be linear over a considerable range, but may depart from linearity as the result of adsorbed divalent cations, e.g. Ca^2 and Mg^2 (Offner, 1970, 1972; Offner & Kim, 1976), as well as concentration effects to be discussed later.

Effect of Concentration on Conductance

These equations do not, however, give any information as to the effect of ionic concentration, except to the extent that a change in concentration *ratio* will affect the Nernst potential of a species. The Goldman equation, on the other hand, predicts that, if the concentration ratios are kept constant, ion flow will be directly proportional to the concentration. This, however, is only an approximation, valid only at low concentrations because of saturation effects (*Vide infra*).

The observed current outflow I_j of a cation, species j (assumed in the following to be univalent) through a membrane channel, is the net result of two currents, $\vec{I}_j$ from the internal to the external bath, and $\overleftarrow{I}_j$, from the external to the internal; then

$$I_j = \vec{I}_j - \overleftarrow{I}_j. \tag{3}$$

At the reversal potential, $\vec{I}_j$ and $\overleftarrow{I}_j$ are equal (Hodgkin & Keynes, 1955). The two component currents are, for ion concentrations C_j sufficiently low to avoid the effect of saturation

$$\vec{I}_j = P_j\left(\frac{RT}{F}\log C_{j\text{int}} - \frac{RT}{F}\log C_{j\text{ext}} + V\right)\cdot C_{j\text{int}} \tag{4a}$$

$$\overleftarrow{I}_j = P_j\left(\frac{RT}{F}\log C_{j\text{ext}} - \frac{RT}{F}\log C_{j\text{int}} - V\right)\cdot C_{j\text{ext}}. \tag{4b}$$

$\frac{RT}{F}\log C_{j\text{int}}$ and $\frac{RT}{F}\log C_{j\text{ext}}$ are the electrochemical potentials of the ion species in the two baths,[1] the expressions within the parentheses thus being the total driving force across the channel in each sense; P_j is the *permeability*, which may be expressed in siemens per mole of concentration of the diffusible ion species.

Putting Eqs. (4) in Eq. (3) gives

$$I_j = P_j\left(\frac{RT}{F}\log C_{j\text{int}} - \frac{RT}{F}\log C_{j\text{ext}} + V\right)\cdot(C_{j\text{int}} + C_{j\text{ext}}) \tag{5a}$$

which may be written

$$I_j = P_J(V - E_J)\cdot(C_{j\text{int}} + C_{j\text{ext}}) \tag{5b}$$

with E_J = the Nernst potential [Eq. (2)]. Thus Eqs. (5) show that, neglecting saturation, the conductance of the channel is proportional to the sum of the internal and external concentrations of the diffusible ion species.

Effect of Channel Saturation

For Eqs. (5) to be useful at other than low concentrations, the conductance must be corrected for saturation resulting from the limited rate at which ions can enter and pass through the channel. If ions could enter as rapidly as they reach the channel's mouth in their random motion in the bathing medium, there would be no saturation effect. But this is not the case: cations will dwell at negatively charged sites at the channel's mouth, and other ions will be unable to enter the channel until sites are vacated as thermal agitation and electrical forces move ions within and across the channel. The result is a limiting conductance that can only be approached asymptotically as the concentration of the diffusible species increases.

This process is similar to the partial blocking effect of Ca^{2+} and other ions by adsorption at the channel's mouth [Eqs. (6 and 7)] which follow an adsorption isotherm, an ion being able to enter the channel only during T_0, the fraction of the time the channel's mouth is free to accept the ion: $T_0 = C_{j/h}/(C_j + C_{j/h})$; $C_{j/h}$ is the concentration at which G_j has half its maximum (extrapolated) value [for $C_j = C_{j/h}$, $C_{j/h}/(C_j + C_{j/h}) = \frac{1}{2}$]. The values of the internal and external concentrations are therefore respectively multiplied by the factors $C_{j/h}/(C_{j\text{int}} + C_{j/h})$ and $C_{j/h}/(C_{j\text{ext}} + (C_{j/h})$

$$G_j = P_j\cdot C_{j/h}[C_{j\text{int}}/(C_{j\text{int}} + C_{j/h}) + C_{j\text{ext}}/(C_{j\text{ext}} + C_{j/h})] \tag{6}$$

for the j^{th} ion species, then

$$I_j = G_j(V - E_j). \tag{7}$$

[1] For greater accuracy, ionic concentrations should be expressed in terms of *activity*.

Nonlinearities Resulting from Saturation

$C_{j/h}$ will have its minimum value $C_{j/h0}$ when V is in the vicinity of the reversal potential, at which point ions become bound to, or leave the charged sites at the channel's mouth only as a result of thermal agitation (Brownian movement); in this region the *I-V* relation will be substantially linear. The situation changes, however, when there is a net current flow through the channel: at the interface at which the current flow is into the channel, additional ions will leave the binding site at the mouth to enter the channel, so that the rate at which ions can be accepted from the bath is increased, thus increasing its $C_{j/h}$; conversely, at the opposite interface current flows from the channel to the mouth, *increasing* the rate at which ions are bound to the site at the mouth, and thus *decreasing* the rate at which ions can be accepted from that bath, thus reducing its value of $C_{j/h}$. This effect should be linear with current at both interfaces, so that

$$C_{j/h\text{int}} = C_{j/h0} + k_j I_j \tag{8a}$$

$$C_{j/h\text{ext}} = C_{j/h0} - k_j I_j. \tag{8b}$$

Since with current flow through the channel the value of $C_{j/h}$ will now be different at the two interfaces, we re-write Eq. (6) as

$$G_j = P_j[C_{j/h\text{int}} C_{j\text{int}}/(C_{j\text{int}} + C_{j/h\text{int}}) + C_{j/h\text{ext}} C_{j\text{ext}}/(C_{j\text{ext}} + C_{j/h\text{ext}})]. \tag{9}$$

The value of I_j as a function of V may then be obtained, either by substituting the expression for G_j from Eq. (9) in Eq. (7), and solving the resulting equation simultaneously with Eqs. (8), or by an iterative solution, as shown in Appendix I.

Nonlinearities from Change of Interface Concentration

As the current through a channel increases, the concentration C_j of the ion species passing through the channel will be decreased in the immediate vicinity of the channel's mouth at the interface *from which* the ions are flowing into the channel; and increased at the interface *into which* they are flowing. For example, if there is current outflow from the internal to the external solution, the concentration $C_{0j\text{int}}$ at the mouth of the channel at the internal interface will be reduced from its value $C_{j\text{int}}$ remote from the internal interface, and $C_{0j\text{ext}}$, that at the external increased from its value $C_{j\text{ext}}$, by an amount proportional to the current:

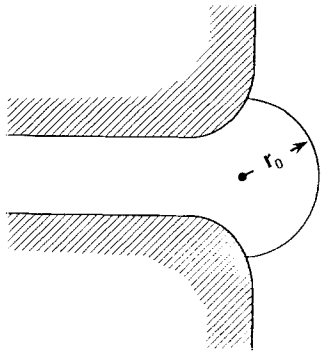

Fig. 1. The mouth of a channel, as assumed for simplified analysis. The ions are assumed to have left the channel when they cross a hemispheric surface into the bathing solution; the radius of this surface is r_0.

$$C_{0j\text{int}} = C_{j\text{int}} - \Phi_{ji} I_j \tag{10a}$$

$$C_{0j\text{ext}} = C_{j\text{ext}} + \Phi_{je} I_j. \tag{10b}$$

Since the reversal potential $E_j = (RT/F)\log(C_{j\text{ext}}/C_{j\text{int}})$, its value will be affected by the concentration change at the interfaces.

The interface concentration parameter Φ_j, which gives the change in concentration for unit change in current, should be independent of voltage. However, since it will depend upon the structure of the channel at the interface, Φ_{je} and Φ_{ji} may have different values.

Estimation of the Value of the Interface Concentration Parameter

When cations of a given species flow from a channel into the bathing solution, they will displace other cations, of both the same and other species, from the immediate vicinity of the channel's mouth. The *total* cation concentration will, however, change only slightly, remaining substantially equal to the anion concentration, with only a minute departure from electroneutrality, producing an electric field resulting from the diffusion of the entering cations into the bulk solution; the ion concentration at the opposite interface changes in a symmetrical manner.

In effect, the bathing solutions themselves cannot distinguish electrically between univalent cation species, so that their flow from the channel into the solution, or the converse, may be treated, so far as the solution phase is concerned, as the diffusion of a neutral species, which, with the aid of some structural approximations, is subject to a simple analysis.

Figure 1 is a schematic of the mouth of a chan-

nel, assumed for mathematical simplicity to interface with the bathing solution across a hemispherical surface of radius r_0. The density of ion flow, J, through the surface, is

$$J_0 = I_j/2\pi r_0^2 F. \tag{11}$$

The flow density at any larger radius r is then

$$J_r = J_0 \cdot (r_0/r)^2. \tag{12}$$

By Fick's law, the flow density is related to C_{jr}, the concentration of the ion species at radius r, by

$$J_r = -D_j \mathrm{d}C_{jr}/\mathrm{d}r. \tag{13}$$

D_j is the Einstein diffusion coefficient, $\sim 10^{-5}$ cm^2 sec^{-1}. Then

$$J_r \int \mathrm{d}r = -D_j \int \mathrm{d}C_{jr}. \tag{14}$$

Integrating from $r = r_0$: $C_{jr} = C_{jr\,0}$, to $r = \infty$: $C_{jr} = C_j$, gives the value of δC_j, the difference between the concentration of the diffusing ion species in the bath, *treated as a neutral species*, at a point remote from the channel's mouth, and the concentration at $r = r_0$, at the mouth

$$\delta C_j = C_j - C_{j\,0} = -J_{j\,0} r_0/D_j. \tag{15}$$

As the ions of species j flow into the bath, if there are n other cation species in the bath, those of species j will displace other cations, species i, in the vicinity of the mouth in a ratio proportional to their relative concentrations times their mobilities, $C_j + \sum_n C_i D_i/D_j = C_T$. Thus of the total cation concentration in the bath, the fraction of the j^{th} species remaining in the region will be $1 - C_j/C_T$. Therefore the value of δC_j, as calculated by Eq. (15), must be multiplied by this factor. δC_j, expressed as a function of I_j, the current through the channel is then

$$\delta C_j = I_j \cdot (1 - C_j/C_T)/2\pi D_j F r_0 \tag{16}$$

F being the value of the Faraday. Expressing I_j in picoamperes, C in molarity, and r_0 in Å units

$$\delta C_j \approx 0.016\, I_j \cdot (1 - C_j/C_T)/r_0. \tag{17}$$

Thus the interface parameter, Φ_j in Eqs. (10), is

$$\Phi_j = (1 - C_j/C_T)/2\pi D_j F r_0 \approx 0.016 \cdot (1 - C_j/C_T)/r_0 \tag{18a}$$

or, with concentrations expressed in millimolarity

$$\Phi_j \approx 16(1 - C_j/C_T)/r_0. \tag{18b}$$

Assuming the area of the mouth of a Na^+ channel is 30 Å^2, consistent with the estimate of Hille (1971), gives $r_0 \approx 2.2$ Å; the actual value of r_0 may be considerably larger.

Substitution of the values of C_j from Eqs. (10) into Eqs. (5) will then give the value of I_j corrected for the interface concentration effect. Since the value of E_j is now a logarithmic function of Ij, the resulting equation cannot be solved explicitly; an iterative solution is included in *Appendix I*.

Comparison of Flow Equations with Experiment: the Interface Parameter

The most relevant experimental data I have found for verifying Eq. (7), with the effective concentrations and reversal potential corrected by Eqs. (10), is that of Fig. 1*B*, from Smart (1987). The concentration ratios of the upper and lower curves were highly asymmetrical: the outside-out patch (upper curve): $[K^+]_{int}$, 150 mM; $[K^+]_{ext}$, 4 mM; $[Na^+]_{int}$, 3 mM; $[Na^+]_{ext}$, 150 mM. The concentrations for the lower curve, inside-out patch, were the converse. The middle curve was for (presumably) symmetrical $[K^+] =$ 150 mM. These values were used for C_{ext} and C_{int} in Eqs. (10). P_K and C_{Kh} were calculated using Smart's value, 225 pS, for the conductance under symmetrical conditions, and the estimated maximum conductance for asymmetrical conditions from the outside-out curve. The values of these calculated parameters are quite sensitive to the estimate of these latter parameters, but the calculated currents are relatively insensitive, provided that the values of P_K and $C_{K/h}$ are obtained from the same calculations.

Smart's Fig. 1*B* is reproduced in Fig. 2, with calculated values for the data points of the upper curve shown by x symbols. The values used for the parameters were $P_K = 1.9$, $C_{K/h} = 90$ mM, $\Phi_{ext} = \Phi_{int} = 2$, and $P_{Na} = 0.07\, P_K$, a ratio consistent with experimental data, e.g., Reuter and Stevens (1980). Only the value of Φ was adjusted to fit the data; the value used, $\Phi = 2$, implies an effective channel radius $r_0 = 8$ Å. Since experimental conditions for the lower curve (inside-out patch) were symmetrical with those of the upper curve, the calculated values would also be the symmetrical, and are therefore not repeated. The fact that the two experimental curves are essentially mirror images suggests that the assumption that $\Phi_{ext} = \Phi_{int}$ is justified, and that the effective areas of the two mouths of the channel are about the same.

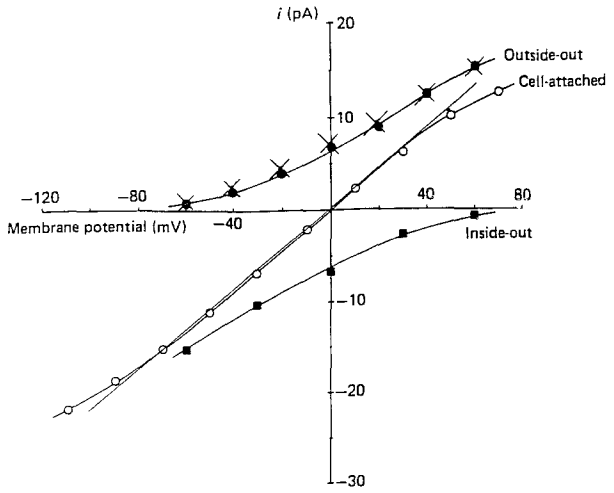

Fig. 2. Currents through single Ca^{2+}-activated K^+ channels in rat sympathetic ganglia neurones, as recorded by Smart (1987). Upper curve: $[K^+]_{int}$ = 150 mM, $[Na^+]_{int}$ = 3 mM; $[K^+]_{ext}$ = 4 mM, $[Na^+]_{ext}$ = 150 mM. Lower curve: $[K^+]_{int}$ = 4 mM, $[Na^+]_{int}$ = 150 mM; $[K^+]_{int}$ = 150 mM, $[Na^+]_{int}$ = 3 mM. Middle curve: $[K^+]_{ext}$ = 150 mM; internal concentrations were not determined, $[K^+]_{int}$ was assumed to be 150 mM. ×'s on upper curve are calculated values. *See* Smart (1987) for further details.

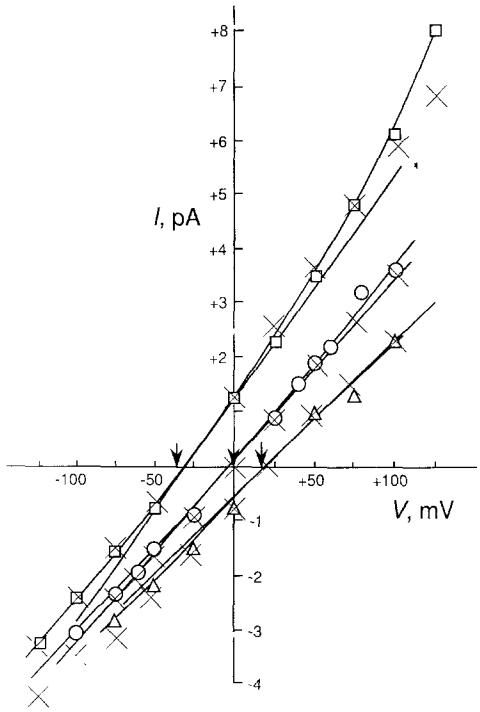

Fig. 3. Na^+ currents through single channels in muscle membrane, as recorded by Horn and Patlak (1980). □: $[Na^+]_{int}$ = 500 mM (340 mM activity); ○: 100 mM (75 mM activity); △: 40 mM (32.4 mM activity. ×'s: values calculated using Eqs. (7)–(9), with k_{Na} = 4. For all curves, $[Na^+]_{ext}$ = 100 mM (75 mM activity). Straight lines were calculated using Eq. (4). Heavy lines: Horn and Patlak's "best fit" to their experimental data. The middle curve data (○, symmetrical $[Na^+]$) shows some asymmetry across the *V* axis. This could result from differences in the composition of the external and internal solutions (external, 103.6 mM Cl^-, 1.8 mM Ca^{2+}; internal, 95 mM F^-, 0 Ca^{2+}); or possibly from a difference in the values of k_{Na} at the two interfaces, resulting from structural differences.

Comparison with Experiment: The Concentration-Conductance Relationship

Horn and Patlak (1980) recorded single channel Na^+ currents in muscle membrane as a function of voltage. Their results for three different concentration ratios are shown in Fig. 3 (Fig. 4 of their paper). Their data is useful in comparing the effect of concentration on conductance predicted by Eq. (7), with experiment. For all their curves, the external Na^+ concentration was 100 mM, with internal concentrations of 500, 100, and 40. In using these data, the concentrations were corrected by using activity coefficients corresponding to the respective concentrations: 0.68, 0.75, and 0.81, giving ion *activities* of 340, 75, and 32.4, respectively; in what follows, in all equations the "concentrations" are the corrected values, i.e., the activities. Using these values in Eq. (6) with $P_{Na} = 9 \times 10^{-10}$ S-M^{-1} and $C_{Na/h}$ = 28 M*, the value of the conductance, G_{Na}, is calculated at the reversal potential for each curve, there being no nonlinearity effect due to current flow at this point. The values obtained are, G_{Na} = 41.6 pS for the top curve, 36.7 pS for the middle curve, and 27.6 pS for the bottom curve. The linear *I-V* relationship of Eq. (7) using these values is plotted in Fig. 3.

The data of Horn and Patlak (1980) exhibit considerably nonlinearity, especially the upper curve, in which $[Na]_{int}$ was large compared with the calculated value of $C_{Na/h}$. The × symbols in Fig. 3 give the currents calculated at their data points using Eqs. (7)–(9), with k_{Na} = 4 mM-pA^{-1}. There should be no interface effect, as discussed above, because Na^+ was essentially the only cation present in the baths: $C_j/C_T \approx 1$, and thus $\Phi_{Na} = 0$ [Eqs. (18)].

The Horn and Patlak data have also been used to calculate the *I-V* relationship as predicted by the Goldman equation, Eq. (1). The results of such a calculation are plotted in Fig. 4 (dashed line). In making these calculations, the permeability *P* was calculated by differentiating Eq. (1), setting the conductance, as determined from the middle curve (symmetrical concentrations), equal to *dI/dV*, and

* The saturation concentration, and thus the value of $C_{Na/h}$, could be more accurately determined by using the experimental protocol described in Fig. 3 of Behrens *et al.*, 1989; also, *see* Appendix I.

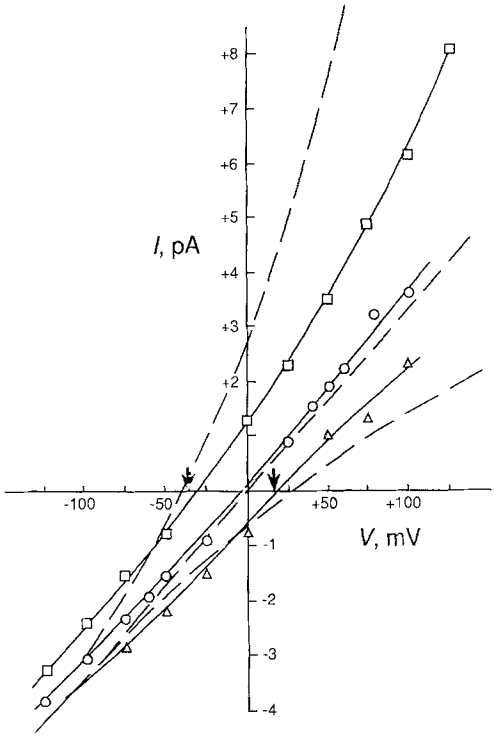

Fig. 4. Same as Fig. 3, but with flow calculated by the Goldman equation (dashed lines). Permeability was calculated from data of middle curve (symmetrical concentration).

solving for P. It is clear that the Goldman equation provides a much poorer approximation of the ion currents than does the conductance approach, Eq. (7).

Resting Potentials

In the absence of any external perturbation, the cell's voltage will in its steady state be that at which the sum of the current flows of all diffusible ion species equals zero: its "resting potential." Using the Goldman equation, and considering only passive ion flows, Hodgkin and Katz (1949) wrote for the resting potential, where the diffusible species are K^+, Na^+, and Cl^-

$$V_0 = \frac{RT}{F} \log \frac{P_K[K]_{ext} + P_{Na}[Na]_{ext} + p_{Cl}[Cl]_{ext}}{P_K[K]_{int} + P_{Na}[Na]_{int} + p_{Cl}[Cl]_{int}}. \tag{20}$$

This relation, usually termed the Goldman-Hodgkin-Katz (GHK) equation, has since been widely used to relate resting potentials to ion concentrations and permeabilities; since the effect of Cl^- on V_0 is usually much smaller than that of K^+ and Na^+, Cl^- is often not included. As seen above, the actual ionic currents may depart very far from those predicted by the Goldman Equation, Eq. (1), and therefore calculations based on the GHK equation must be equally suspect.

A more accurate formulation may be based on Eq. (2). For membranes in which the channels are not voltage sensitive, the resting potential (the potential at which $\Sigma I_j = 0$) may then be written for the diffusible ion species K^+ and Na^+, as given by Kuffler and Nicholls (1976)

$$V_0 = (G_{Na}E_{Na} + G_K E_K)/(G_{Na} + G_K). \tag{21}$$

As an example of a typical cell, $[Na]_{ext}$ = 150 mM, $[Na]_{int}$ = 10 mM, giving E_{Na} = 68 mV; and $[K]_{ext}$ = 145 m, $[K]_{int}$ = 5 mM, giving E_K = −86 mV. At rest, G_K in most cells is much larger than G_{Na}. Assuming $G_K = 10 \times G_{Na}$, then V_0 = −72 mV.

Equation (21) considers only the *passive* ion flow across the membrane, as does Eq. (17). For the true steady state to exist in a cell, this ion flow must be accompanied by equal and opposite *active* transport. Since Na,K-ATPase actively transports Na^+ and K^+ in a ratio Na : K = $r \approx 3:2$, their *passive* flow, as given by Eqs. (2) must, in the steady state, be in this same ratio. Therefore, including this active transport, Eq. (21) becomes[2],

$$V_0 = (G_{Na}E_{Na} + rG_K E_K)/(G_{Na} + rG_K). \tag{22}$$

Assuming $r = 3:2$, and the same values are used as above for the other parameters, then V_0 = −76 mV. Thus the activity of the Na,K-ATPase makes the cell potential more negative; or, as we will see later, in some cases *less positive*. The action of Na,K-ATPase is therefore said to be "negative electrogenic," a term which has often been misinterpreted to mean that the cell potential will *necessarily* be negative.

A model of a cell to which Eq. (22) would apply is shown in Fig. 5A, and a schematic electrical diagram illustrating the current pathways, in Fig. 5B.

Equations (21) and (22) are formulated in terms of the *conductances* of the permeable ion species, and thus are valid only at the concentrations at which the conductances have been determined. The effect of concentration on the resting potential is obtained by expressing G_j in terms of the permeability: $G_j = P_j(C_{jint} + C_{jext})$ [cf. Eq. (5b)] at concentrations where saturation is not a significant factor, or, for higher concentrations, by Eq. (6); at the resting potential of a cell, the effect of nonlinearity will be negligible

[2] Mullins and Noda (1963) made a similar correction to the GHK equation.

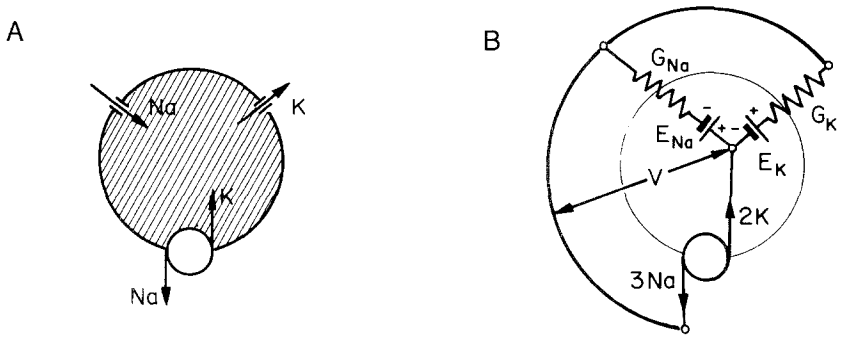

Fig. 5. (*A*) Cell, not of epithelial type, surrounded by a common body fluid, through which the ion channels and the Na,K-ATPase are joined electrically, with negligible resistance. The Na,K-ATPase transports Na^+ ions out of the cell, and K^+ ions into the cell, with the Na : K ratio. In the steady state, the active transport of each ion species equals its passive flow. The conductance of the ion channels is usually voltage dependent. (*B*) Equivalent circuit of ion flow in cell shown in *A*. Symbols are as defined in the text. *V*, the cell potential, is given by Eq. (4).

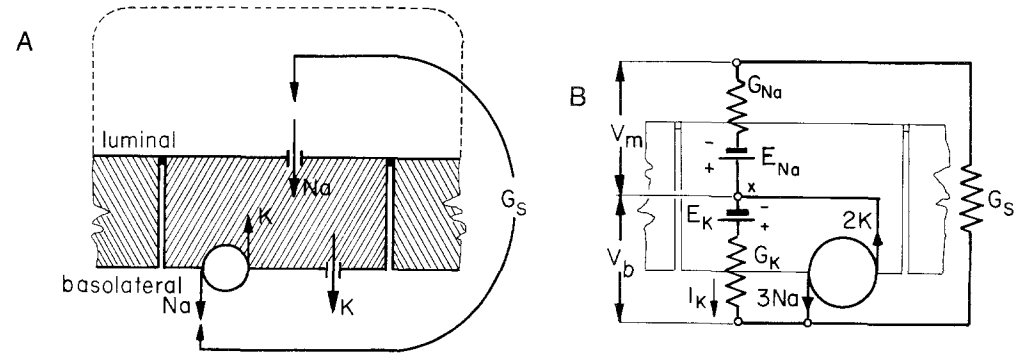

Fig. 6. (*A*) Sheet of epithelial cells of the type commonly found in the body. Na^+ channels are found in the mucosal membrane, with K^+ channels and Na,K-ATPase in the basolateral membrane. The mucosal and basolateral membranes are in contact with different media, which communicate through different pathways which include intercellular leakage. (*B*) Equivalent circuit of epithelium shown in *A*. Na^+ ions flow from the medium bathing the mucosal membrane through channels having a total conductance G_{Na}, into the cell; they are then transported out of the cell into the basolateral medium by Na,K-ATPase in the basolateral membrane. The basolateral and mucosal media communicate electrically through nonspecific external "leakage" pathways having a total conductance G_s. The Nernst potential E_{Na} resulting from the Na^+ concentration ratio across the mucosal membrane, provides the driving force for the Na^+ ion current through the two series conductances G_{Na} and G_s, having an effective total conductance $G^*_{Na} = 1/(1/G_{Na} + 1/G_s)$. Then $I_{Na} = G^*_{Na}(V_b - E_{Na})$. K^+ flows out of the cell under the driving force of its Nernst potential E_K, through the basolateral membrane (conductance G_K), to the basolateral medium: $I_K = G_K(V_b - E_K)$. This K^+ is then transported back into the cell by the ATPase.

because of the low current flow through the channels.

Epithelial Cells

Epithelial cells form a sheet with tight junctions, separating two media, one of which, as in frog skin, may be external. Figure 6A illustrates the functioning of the usual epithelial cell, and Fig. 6B, its equivalent circuit; Na^+ flows into the cell through channels in the mucosal membrane, conductance G_{mNa}, and are transported out across the basolateral membrane by Na,K-ATPase, with K^+ transported in, in the usual 3 : 2 ratio; these K^+ ions then return to the basolateral medium through K^+ channels in that membrane.

The Na^+ flow across the mucosal membrane is

$$I_{mNa} = G_{mNa}(V_m - E_{Na}). \tag{23}$$

The return flow of Na^+ ions to the basolateral medium through the external path, conductance G_s, produces a potential difference $\Delta V = I_{Na}/G_s$ between the mucosal and basolateral solutions; the potential V_b across the basolateral membrane is the sum of these two potentials. This is conveniently expressed by defining the *effective* Na^+ conductance, G^*_{Na}, to be that of the combination of channels themselves, plus the return path

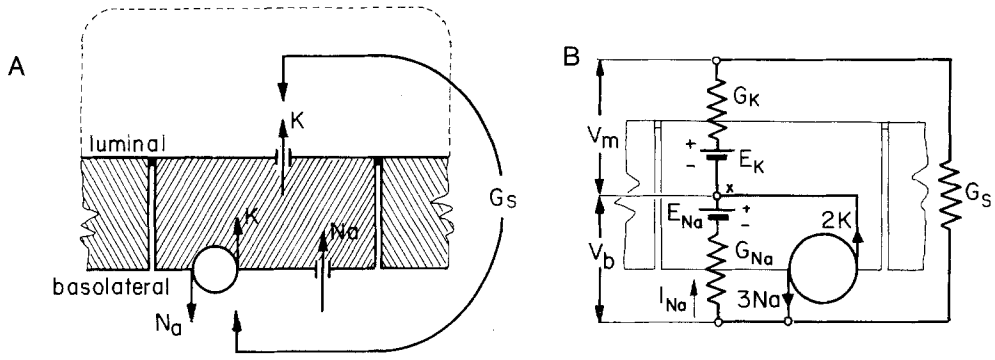

Fig. 7. (*A*) Sheet of epithelial cells differing from those shown in Fig. 6, except that K^+ and Na^+ channels are transposed. (*B*) Equivalent circuit of epithelium shown in *A*. Na^+ ions flow into the cell from the medium bathing the basolateral membrane through channels having a total conductance G_{Na}: $I_{Na} = G_{Na}(V_b - E_{Na})$; they are then transported out of the cell, back into the basolateral medium by Na,K-ATPase in the basolateral membrane, with K^+ ions transported into the cell from the basolateral medium. The basolateral and mucosal medial communicate electrically, as in Fig. 6, through nonspecific external "leakage" pathways having a total conductance G_s. The Nernst potential E_K across the basolateral membrane provides the driving force for the K^+ ion current through the two series conductances G_{Na} and G_s, having an effective total conductance $G_K^* = 1/(1/G_K + 1/G_s)$: $I_K = G_K^*(V_b - E_K)$. As applied to the media, G_s is the conductance of the SM-perilymph barrier.

$$G_{Na}^* = 1/(1/G_s + 1/G_{Na}). \tag{24}$$

Substituting G_{Na}^* for G_{Na} in Eq. (22) gives the value of V_b

$$V_b = (G_{Na}^* E_{mNa} + rG_K E_K)/(G_{Na}^* + rG_K) \tag{25}$$

and for V_m, the voltage across the mucosal membrane,

$$V_m = V_b G_{Na}^*/G_{Na}. \tag{26}$$

This reduction of the effective value of G_{Na} thus increases the effective value of G_K/G_{Na}, and thus further increases the negativity of V_b. A cell of this type, with parameters as in the first of the above examples: $E_K = -86$ mV, $E_{Na} = 68$ mV, $G_K/G_{Na} = 10$, but with $G_s = G_{Na}/9$, increases the negativity of V_b to -85 mV.

The short-circuit current I_{sc} across epithelia, as sometimes studied experimentally, may also be obtained by this analysis. In this case, $G_s = 0$, so that $V_m = V_b = V$. Since in these epithelia $I_{sc} = I_{Na}$, I_{sc} may be calculated by substituting V_0 from Eq. (22) into Eq. (2a), giving

$$I_{scNa} = rG_K G_{mNa}(E_{Na} - E_K)/(G_{Na} + rG_K). \tag{27}$$

Figure 7A illustrates an epithelial cell in which the K^+ and Na^+ channels are transposed between the basolateral and mucosal membranes, so that I_K, rather than I_{Na}, now flows through the return path (Fig. 7B). Equation (24) is therefore replaced by

$$G_K^* = 1/(1/G_s + 1/G_K) \tag{28}$$

giving

$$V_b = (G_{Na}E_{Na} + rG_K^* E_{mK})/(G_{Na} + rG_K^*) \tag{29}$$

and

$$V_m = V_b G_K^*/G_K. \tag{30}$$

The effect of G_s is now to decrease the value of G_K^*, the effective value of G_K, thus *decreasing* the negativity. Assuming the same value of G_s ($G_{Na}/9$), and $G_K/G_{Na} = 10$, we now have $G_s = G_K/90$, $G_K^* = G_K/91$; V_b then becomes 12 mV *positive*.

It is thus seen that, even in cells having Na^+ and K^+ channels having the usual conductances ($G_K \gg G_{Na}$), epithelial cells may be positive with respect to one of the bathing solutions.

Epithelial Cells of the Cochlea

The scala media (SM), the central compartment of the mammalian cochlea, is unique in having a rather high positive potential (endocochlear potential, EP+ $\approx$ 80 mV) (Von Bekésy, 1952). The source of the EP+ was shown by Smith (1957) to be the stria vascularis (StV) in the wall of the SM. It was concluded by Kuijpers and Bonting (1970*a*,*b*), and Johnstone and Sellick (1972) that the EP+ must due to the action of a "positively electrogenic ion pump"; i.e., one having a K : Na transport ratio *greater* than

unity. Sellick and Johnstone (1974) modified this model, proposing that the EP+ is produced by a K-ATPase, an enzyme otherwise unknown in vertebrates, in the mucosal wall of the boundary cells of the StV.

This model was generally accepted for over 20 years, until, based on the analysis of epithelial potentials presented above, it became evident that the EP+ could be produced by the action of Na^+ channels along with the normal Na,K-ATPase in the basolateral membrane of the marginal cells (MC), and with channels above to pass K^+ ions in the mucosal membrane. Experiments, which have already been published elsewhere (Offner, Dallos & Cheatham, 1987), have verified the model. Using the analysis presented above, and using data from the literature relative to the EP+ of the SM, as well as that of Offner et al. (1987) (from which the data used below is taken, unless otherwise cited), the ionic conductances of the marginal cells may be calculated.

The K^+ and Na^+ Conductances

In the steady state, the K^+ outflow *from* the SM through the marginal cells of the SM must equal the flow of K^+ *into* the SM across the mucosal membrane of the MCs of the StV. This is in turn equal to the active transport of K^+ into the MCs from the perilymph, across their basolateral membranes, there being no evidence of the presence of Na,K-ATPase elsewhere in the endolymph-perilymph barrier of the SM (Schulte & Adams, 1989). The active transport of K^+ into the MC by Na,K-ATPase is accompanied by the transport of Na^+ out of the MC, into the perilymph, in the ratio ~3 Na^+ : 2 K^+ (Fig. 7).

Konishi, Hamrick and Walsh (1978), using radioactive tracers, measured the flow of K^+ across the SM-perilymph barrier; their results may be expressed as producing a rate of change of $[K^+]_{end} = 1.2 \times 10^{-5}$ M · min^{-1} · mV^{-1}, or 1.9×10^{-3} M · min^{-1} † for an EP+ = 70 mV and $E_K = -78$ mV. On this basis, there should be a corresponding inflow of Na^+ equivalent to a rate of change of $[Na^+]_{end} = 2.8 \times 10^{-3}$ M · min^{-1}. This will not actually appear in the endolymph, however, since substantially all the Na^+ entering through the basolateral membrane of the MCs will be pumped out by the Na,K-ATPase in the membrane. The Na^+ inflow must therefore be measured with the Na,K-ATPase inactivated. Bosher (1979) accordingly determined the rate of change of $[Na^+]_{end}$ under anoxia to be 6×10^{-6} M · min^{-1} · mV^{-1}; Konishi et al. (1978) obtained a similar value with anoxia. Assuming $[Na^+]_{end} = 1$ mM, the rate of change of $[Na^+]_{end} = 7.8 \times 10^{-4}$ M · min^{-1}, 28% of the required Na^+ flow. The Na^+ flow was, however, determined in the SM, but that calculated is the flow into the MCs; the discrepancy is due principally to the limited Na^+ permeability of the mucosal membrane of the MCs.

The potential within the MC is ~9 mV more positive than the EP+ within the SM. Assuming that $[Na^+]_{end} = 1$ mM (Konishi et al., 1978; Boser, 1979), then $[Na^+]_{MC} = 0.7$ mM, and $E_{Na} = 140$ mV; the Na^+ electrochemical potential difference is then 140–79 = 61 mV. The calculated rate of change of concentration within the MC of 2.8×10^{-3} M · min^{-1} therefore requires the Na^+ permeability of the basolateral membrane of the MC to be 4.6×10^{-5} M · min^{-1} mV^{-1} and the mucosal membrane, 6.9×10^{-6} M · min^{-1} mV^{-1}.

The observed 9 mV across the mucosal membrane of the MC is the result of the normal K^+ flow across the membrane. Using the K^+ flow as determined by Konishi et al., this implies a K^+ permeability of $\sim 2.1 \times 10^{-4}$ M · min^{-1} · mV^{-1}, which is 30 times the Na^+ permeability calculated above for the mucosal membrane of the MC. It thus appears that only nominally K^+ channels may be present in that membrane and that the Na^+ flow may be through the same channels.

In addition to K^+ and Na^+ flow across the endolymph-perilymph barrier of the SM, there are also H^+ and HCO_3^- as products of the metabolic activity of Na,K-ATPase; and Cl^-, including cotransport with K^+ and/or Na^+. The available data, however, do not permit calculations to be made on these processes.

Discussion

There are many different types of ion channels, differing both from species to species, as well as from cell type to cell type in a given species; there also may be a number of different types of channels passing a given ion species in the same cell (notably, K^+ channels). Thus, we cannot expect to find any generally applicable mathematical model which will *precisely* predict ions currents, but may hope to find one which will be sufficiently accurate to be useful.

The simple phenomenological Eqs. (2) have been useful in predicting the *I-V* relationship in membranes in which the channel conductance is not voltage sensitive, but do not predict the effect of concentration on the membrane conductance. This is included in Eq. (7), which, with Eqs. (8) and (9),

† Concentrations and flow rates are given in moles per liter. Assuming the volume of the SM is 50 μL, the actual flow rates would be 5×10^{-5} times the values shown.

also includes the effect of saturation at higher ionic concentrations. Equations (2), along with derivative Eqs. (21) and (22), are useful when the values of the parameters G_j are known; Eq. (7) allows their use to be extended to changes in concentration, within the range of a substantially linear *I-V* relationship.

When the concentration ratio of a permeable cation species across a channel is large, the *I-V* relationship can become significantly nonlinear; if a second, impermeable species is also present, the departure from linearity may be explained, in part at least, by a change in the concentration of the permeable species at the channel mouth-bath interfaces [Eqs. (10)–(19)] which results in a lowering of dI/dV as the magnitude of I increases. Equation (7) also becomes nonlinear at higher current flows, especially at higher concentrations, even if there is only one cation species present, as the result of the effect of current flow on channel saturation [Eq. (9)]; the nonlinearity due to this effect is, however, in the opposite sense, resulting in an *increase* in dI/dV with I.

Only two reports of experimental results have been found in the literature which provide sufficient data to permit comparison with the theoretical equations; these tend to support the validity of the model. Since only one cation species was present in the experiments of Horn and Patlak (1980), only the second source of nonlinearity (saturation) should be effective in their data.

The data of Smart has been analyzed assuming only the first source of nonlinearity (interface concentration) to be effective; calculations including saturation nonlinearity with parameter k_j of the same value as used for the Horn and Patlak data made only a small change in the calculated values. It would, however, be essential for obtaining a more conclusive comparison, to perform a similar experiment, but with the low permeability cation species (in this case Na^+) replaced by a non-ionic solute; e.g., sucrose, as used by Horn and Patlak.

Smart's data also did not provide information to determine the value of $C_{j/h}$. While the data of Horn and Patlak permits its estimation, a more accurate value would be desirable; as, for example, by using the protocol employed by Behrens et al. (1989).

Equation (7) would clearly be more accurate than the Goldman equation in predicting *I-V* relationships, even without the correction for saturation ($C_{j/h}$), and thus Eqs. (21) and (22) are preferable to the GHK equation, Eq. (17), for expressing resting potentials. It would, however, be desirable to be able to include this term, if the effect of the change in ionic concentrations is to be included; it would therefore be useful to have data on the half-saturation concentration for channels in a number of different preparations.

Further data exhibiting nonlinearities due to changes in local concentrations at the channels' mouths, similar to those found by Smart (1987) and by Behrens et al. (1989) with high concentration ratios, but with adequate data to permit determination of $C_{j/h}$ an k_j, would also be useful, the effective value of r_0 possibly providing information as to the nature of the channel-solution interface. Univalent cation species for which a channel has low permeability can, in theory at least, affect the flow of cations for which a channel is selectively permeable (Offner & Kim, 1976); this has not been included in this paper; its possible effect should be experimentally investigated. A suggested protocol for more accurately determining the various parameters used in the equations derived in this paper is given in Appendix II.

The experimental work on the cochlea on which the analysis was based was performed by Dr. Peter Dallos and his associates; he, as well as Dr. Robert Linsenmeier, contributed substantially to the author's understanding of epithelia in our many discussions. I wish to thank Drs. John Troy and Jay Yeh for their helpful criticism of the manuscript.

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Appendix I

Iterative Solution of Nonlinearities of the *I-V* Relationships

(* = value of parameter)

1	$C_{int} = *$	Internal concentration of permeable cation
2	$C_{Ti} = *$	Total internal cation concentration
3	$C_i = C_{int}$	Initialize C_i
4	$C_{ext} = *$	External concentration of permeable cation
5	$C_{Te} = *$	Total external cation concentration
6	$C_e = C_{ext}$	Initialize C_e
7	$C_h = *$	Half-saturation concentration at zero current
8	$C_{hi} = C_h$	Initialize C_{hi} (corrected value of C_h, internal)
9	$C_{he} = C_h$	Initialize C_{he} (corrected value of C_h, external)
10	$k = *$	Proportionality factor of current on saturation
11	$\Phi_{int} = *$	Interface parameter—internal
12	$\Phi_{ext} = *$	Interface parameter—external
13	$V = *$	Voltage difference across channel ($V_{int} - V_{ext}$)
14	$P = *$	Permeability of permeable ion species
15	CONV = *	Convergence parameter—approximately 10^{-5}
16	$I = 0$	Initial trial value of current
17	$I_0 = I$	
18	$C_i = C_{int} - \Phi_{int} I(1 - C_{int}/C_{Ti})$	
19	$C_e = C_{ext} + \Phi_{ext} I(1 - C_{ext}/C_{Te})$	
20	$V_T = P(V + 26 \cdot P \cdot \log(C_{int}/C_{ext})$	
21	$I = V_T[C_i C_{hi}/(C_i + C_{hi}) + C_e C_{he}/(C_e + C_{he})]$	
23	$C_{hi} = C_h + k \cdot I$	
25	$C_{he} = C_h - k \cdot I$	
26	If abs$(I - I_0) >$ CONV, go to statement 17	
27	Print I	

This routine may be used to calculate either the saturation or interface effects separately by setting either the Φ's or k equal to zero. The value of CONV should be chosen so that a value one tenth as large makes a negligible difference in the results.

Appendix II

Protocol for Determining Parameters of Conductance and *I-V* Equations

Purpose

1. To determine the values of the parameters in the conductance-concentration and the voltage-current equations.

2. To compare the values of conductance and currents over a range of concentrations and currents with predictions of equations, with these values of parameters.

3. To compare values of parameters for channels from various tissues, and for different ion species (probably only Na^+ and K^+).

4. To determine if there is inter-ion species effect on conductance.

Definitions

Conductance, $G_j = dI_j/dV$.

Permeability, P_j: parameter which, when multiplied by concentration, gives the conductance, G_j of the channel, as the concentration and current approach zero: P_j = limit of $dI/C_j dV$ as $C_j \to 0$ and $V \to E_j$, the reversal potential.

$C_{j/h}$: The total concentration ($C_T = C_{jint} + C_{jext}$) at which G_j has half its maximum value as $C_T \to \infty$; $C_{j/h0}$ the value of $C_{j/h}$ at $I_j \approx 0$.

k_j: Proportionality factor giving change in C_j/h with I_j.

Φ_j: Proportionality factor for change in C_j with I_j (affecting Nernst potential).

Experimental Protocols

All experiments were performed using patch-clamp. For gated Na channels, inactivation should probably be eliminated by Pronase, or other agent which does not affect the open-channel conductance.

Inter-ion effect

With equal ionic concentration (e.g., 100 mM Na^+), as well as equal concentrations of a non-ionic solute (e.g., sucrose) on both sides, measure currents through channel at small ± voltages: e.g., +10 and −10 mV.

Repeat, but with sucrose replaced by an essentially impermeable univalent cation (e.g., Rb^+ or Cs^+).

The current may be reduced; if significantly, this must be taken into account in other experiments, e.g., on Φ_j.

Determination of $C_{j/h}$ (Concentration for Half-Maximum Conductance)

With equal concentrations of permeable ion species (K^+ for K channels, Na^+ for Na channels) on both sides, and with non-ionic solute to make up to normal osmotic strength, measure current for small voltages + and −, as above; repeat at several different ionic concentrations, e.g., 20, 50, 150, 250 mM.

A similar experiment should be performed, with different concentrations on the two sides, but the same *total* concentrations: e.g., 75 and 225 mM, comparing the results with the above for 150 mM (in each case, the total concentrations would be the same: 300 mM, so that the parameter values should be the same). In these experiments, the voltage should be displaced by the same amount as before, as measured from the zero current (reversal potential) value.

The two paramters P_j and $C_{j/h0}$ can be determined from the data from any two of the experiments (e.g. 20 and 150 mM); the values of the parameters so obtained can then be used to *predict* the currents to be expected at other concentrations, to check the accuracy of Eqs. (6) and (7) of this paper.

Similar experiments may be performed on channels of various kinds. It should be of interest to find how $C_{j/h0}$ varies, if at all, with the nature of the cell from which the channels are derived.

Determination of k_j, the Nonlinearity Saturation Parameter

Perform experiments similar to the last (unequal concentrations on the two sides), but for a series of voltage steps, up to values sufficient to produce relatively high channel currents. The value of $k_{j/h}$ is found by substitution of values into the equations, using the previously determined values of $C_{j/h0}$ and P_j. These data can be obtained during the previous runs for determining those parameters with unequal concentrations.

Determination of Φ_j, the Interface Concentration Parameter

These experiments would be essentially the same as the preceding, and should probably be performed on the same preparation, but with an impermeable univalent cation species replacing the sucrose in the bath having the lower permeable ion concentration. In this way, any nonlinearity resulting from k_j can be included in the analysis of the data, permitting a more accurate calculation of Φ_j.

In all experiments, calculations should be based on ion *activities*, rather than actual concentrations.