Kinetics of Excitable Membranes

Voltage amplification in a diffusion regime

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ABSTRACT An understanding of the properties of excitable membranes requires the calculation of ion flow through the membrane, including the effects of nonuniformity in the transverse membrane properties (mobilities, fixed charge, electric field). Permeability is apparently controlled at the external interface. Two factors may be involved here: the statistical blocking of pores by divalent cations, and activation energy. Only the former is included in the present treatment. When the total transmembrane voltage is varied, a redistribution in ionic concentration occurs. This can cause a change in boundary (zeta) potential, large in comparison with the applied voltage change—"voltage amplification." The result is a steep change in membrane conductance. The calculated flow curves are compared with experimental results. The Appendix gives an outline of the numerical method used for solving the boundary value problem with several diffusible ions, across a nonuniform regime.

INTRODUCTION

Excitation in membranes (e.g., axonal) involves two processes, apparently distinct, the establishment of ionic concentration gradients, which requires an active, energy-consuming process; and the excitation process itself, manifested by dramatic changes in ion fluxes and membrane potential. This latter process releases energy, and need not involve any active, metabolic source of energy. Only this portion of the problem is considered in this paper.

Bernstein (1912) first proposed that the excitation process is due to a change in ionic permeability through the membrane. Offner (1939) and Offner, Weinberg, and Young (1940) showed mathematically that such a change, manifested by a change in the transverse electrical resistance of the membrane, could result in conducted activity; while Cole and Curtis (1938) demonstrated such a change in resistance during activity in Nitella, and in the squid axon (1939).

Bernstein's simple hypothesis could not explain the appearance of inductive reactance in membrane (Cole and Baker, 1941), the reversal of the membrane potential found during activity (Curtis and Cole, 1942; Hodgkin and Huxley,
1945), and the accompanying sequence of inward and outward flows. The reversal of potential and the accompanying current flows have been shown by Hodgkin and Huxley (1952) to be the result of the inflow of Na\(^+\) ions; and Offner (1967, 1969 a) has shown that the presence of Na\(^+\) gradient also implies an inductive reactance. A concise quantitative description of the sequence of K\(^+\) and Na\(^+\) currents in the voltage-clamp experiment was provided by Hodgkin and Huxley (1952 a) in their well-known equations. They also showed that the observed action potentials and conduction phenomena could be derived from the voltage-clamp data.

None of this work has, however, given any real clue as to the source of the dramatic changes that occur in the permeability of the K\(^+\) and Na\(^+\) ions. There have been numerous hypotheses, electrical, mechanical, and chemical, based on specific assumed membrane properties.

Several of the phenomena of the voltage-clamp experiments whose explanations have appeared crucial to the understanding of the excitatory phenomena, can be examined by the solution of the steady states of the system. These problems can be solved by the use of total differential equations. This aspect of the problem will be covered in the present paper. Among the phenomena which can be considered by this method of solution are the negative membrane resistance and the rapid change in conductance with transmembrane potential. The solution of the transient problem, giving the time course of the individual ion species currents, requires the solution of the time-dependent partial differential equations of the system. This problem will be treated in a succeeding paper.

When a depolarizing voltage step is applied to a voltage-clamped axon, Hodgkin and Huxley (1952 a) pointed out that the resulting conductivity changes much more rapidly with the amplitude of the step than can be explained by simple theory. Typically the sodium and potassium conductivities may increase as \(e^{av}\), where \(V\) is the amplitude of the voltage step in units of \(RT/F\), and \(a\) is a constant in the range of 5 to 7; while they assumed that with univalent ions, the constant should be unity.

A logical difficulty in interpreting the phenomena is that the voltage step \(V\) is the change in voltage across the entire membrane structure. But the membrane is surely not homogeneous; probably conductance is controlled in a very narrow region. There is considerable evidence that it is the external surface of the plasma membrane that is responsible for permeability control, and that Ca\(^{++}\) (or other divalent cations) play an essential role in the control (cf change in ionic concentrations, effect of tetrodotoxin). One should then logically consider only that portion of the applied voltage step that appears at this boundary, and a priori one would expect the voltage effective in controlling the conductivity to be only a fraction of the applied step, \(V\), thus intensifying the theoretical problem.
But a marked change occurs if one considers the effect of the boundary layer on the ion flow, and the concomitant effect on the distribution of the electric field through the membrane. Then the boundary potential $V_0$ may not vary uniformly with the total membrane potential $V_m$. The disproportionality arises due to a change in the ionic distribution through the membrane. As simplified examples, Fig. 1a shows an uncharged membrane with cations uniformly distributed through its thickness: the electric field then rises linearly through the membrane, while the potential falls quadratically. This may be compared with Fig. 1b in which the cations are all concentrated at the outer surface. The full change in electric field occurs at that point, and the potential then falls linearly towards the inside.

The two figures, (Figs. 1a and 1b), are drawn to have the same potential difference across the membrane. It is evident, however, that the boundary electric fields are very different, and consequently, the boundary potentials (their relationship is given below in equation [6]). While such ionic distributions are idealized, and do not correspond to any actual case, a similar tendency does occur in the actual membrane with boundary layer control of ion flow. Thus the boundary potential, $V_0$, may change much more rapidly than does the total membrane potential, $V_m$, over some ranges of membrane potential, and the rapid change in conductivity with a small voltage step becomes readily explicable in terms of classical theory. In effect, the membrane be-
comes a voltage amplifier, but the amplified voltage does not appear externally: it is the internal voltage difference between the external surface and the external medium. This is, in effect, the zeta potential of the interface.

**DIFFUSION CALCULATION**

Diffusion of ions is classically calculated using the Nernst-Planck equation. This is only an approximation, a more precise treatment being in terms of activation energy (Glasstone, Laidler, and Eyring, 1941). However, for many purposes the two treatments are equivalent. This communication will investigate how well the membrane phenomena can be explained by the classical formulation of the problem.

The Nernst-Planck diffusion equation (Planck, 1890) for diffusion in one dimension is,

\[ j_i = u_i (E c_i - RT \frac{dc_i}{dx}) \]  

\( j_i \) is the flux of the \( i \)th ion species (e.g., moles per second passing through unit area); \( c_i \) the concentration of the species at the space coordinate \( x \); \( E \), the electric field at the point; and \( R \), \( T \), and \( F \), the gas constant, absolute temperature, and the Faraday constant. The proportionality constant \( u_i \) is the mobility of the ion species at the point.

In order to solve the Nernst-Planck equation analytically, Goldman (1943) made the assumption that the electric field is constant across the regime. Planck used the assumption of electroneutrality in order to solve the equation. These two assumptions are equivalent in membranes having a thickness of the order of unit Debye length, and are probably not justified in excitable membranes.

Two additional assumptions have usually been made in diffusion calculations through membranes: that the fixed charge density is uniform across the membrane, and that the ionic mobilities are constant across the membrane.

The calculations to be described have been generalized so that none of these assumptions need be made. The aspects of the problem to be treated here in fact appear to be strongly influenced by the functional form of these parameters. The distributions assumed are not entirely ad hoc, but have at least partial experimental theoretical justification. It is of interest to compare these findings with the analytic treatment (Offner, 1967, 1969), which indicates that nonuniformity in membrane parameters may be implicated in excitability.

The final factor which has been included is the diffusion-blocking effect of divalent cations in the external medium. The effect of \( \text{Ca}^{++} \) (or \( \text{Mg}^{++} \)) in the external axonal medium is well-recognized, and has been studied quantitatively for the squid axon (Frankenhaeuser and Hodgkin, 1957). Removal
of external divalent cations prevents maintenance of membrane polarizability. The effect of Ca\(^{++}\) has been incorporated by assuming that the Ca\(^{++}\) ions are adsorbed on the external surface of the membrane; and that monovalent ions are prevented from traversing the boundary at the site of such an adsorbed ion.

**Assumptions** Several assumptions have been made in carrying out the calculations. In using equation (1), the assumption is made that ionic activities may be replaced by concentrations; that is, that the activity coefficients are unity; or, at least, constant. This is recognized as an approximation, but one which may not result in much error, as concentrations within the membrane are probably quite low.

The boundary conditions at the internal and external interfaces are obtained by assuming the baths to be in instantaneous equilibrium, with an ionic distribution as given by the Gouy-Chapman double layer. This assumption should be justifiable since relaxation times in free solutions are of the order of nanoseconds, as compared to the observed millisecond time scale of the membrane.

The electric field at each point in the membrane is calculated from Poisson's equation, in one dimension,

\[
\frac{-d^2V}{dx^2} = \frac{dE}{dx} = \frac{F}{\epsilon} \sum_i z_i c_i
\]  

where the summation is taken over the \(i\) charged species, each carrying \(z\) units of charge, with \(z\) being positive or negative, according to the sign of the charge. Here \(\epsilon\) is the dielectric constant, which is assumed to be constant through the membrane. In calculating the field in the membrane, in general three charged species are considered: two mobile univalent cation species, which will be taken as \(K^+\) and \(Na^+\), and the negative fixed charge. Then equation (2) is conveniently rewritten for the electric field,

\[
\frac{dE}{dx} = \frac{F}{\epsilon} (c_K + c_{Na} - q)
\]  

where \(c_K\) and \(c_{Na}\) are respectively the cation concentrations, and \(q\), the molar density of fixed negative charge.

**Dimensionless Variables**

In order to simplify calculation, it is convenient to change to a dimensionless system of variables (Verwey and Overbeek, 1948, p. 25). Potential will be measured in units of \(RT/F\); i.e., \(\Psi = V/(RT/F)\). The unit of distance used is the Debye length in the boundary solutions (assuming unity partition coefficient between the solutions and the membrane). Since only univalent
ions are considered (the concentration of Ca++ is sufficiently low not to ap-
preciably affect the Debye length), the Debye length in the boundary solu-
tions is \( \lambda = \sqrt{\frac{eRT}{2F\theta_o}} \), where \( \theta_o \) is the total molar concentration of (uni-
ivalent) electrolytes in the bath. Thus the length dimension \( \xi = x/\lambda \).

The dimensionless electric field is then

\[ \varphi = -d\Psi/d\xi = E\sqrt{\frac{\epsilon/2RT\theta_o}{}} \]

The units of time and mobility are so chosen that unit mobility results in an
ion moving unit distance in unit time, under the influence of unit field.

When these substitutions are made, equation (1) becomes,

\[ J_i = U_i(\varphi C_i - dC_i/d\xi) \quad (4) \]

and for equation (3),

\[ \frac{d\varphi}{d\xi} = \frac{1}{2}(C_h + C_{Na} - Q) \quad (5) \]

the symbols in capitals \((J, U, C, Q)\) now refer to the quantities expressed in
the dimensionless variables, the concentrations being expressed relative to the
total concentration in the external baths, taken as unity.

**Boundary Conditions**

There is considerable evidence that the critical phenomena in controlling
membrane permeability occur at or near the external interface of the mem-
brane. Among such evidence is the effect of external Ca++ (Hodgkin and
Frankenhaeuser, 1957), and the relative independence of the amplitude of a
depolarizing voltage step, with changes in internal K+ (Narahashi, 1963). At
the internal interface, the concentration of permeable cations is considered
to be in equilibrium across the interface.

By the Gouy-Chapman treatment (Verwey and Overbeek, 1948, p. 25),
the electric field is related to the potential at the external interface, by

\[ \varphi_e = -2 \sinh \frac{\Psi_e}{2} \quad (6) \]

while at the internal interface, at \( \xi = d \),

\[ \varphi_i = 2 \sinh \frac{\Psi_i}{2} \quad (7) \]

where \( \Psi \) is the potential with respect to the internal bath, remote from the
interface.

The concentration of each ion species in the solution adjacent to the inter-
face will be increased by the Boltzmann factor \( \exp (-\Psi) \), relative to its
concentration in the free solution. At the internal interface, letting the (frac-
tional) concentration Na⁺ be \( h_{Na} \) in the internal solution, the concentration of each permeable cation species will be

\[
C_{Na}(d) = h_{Na} \exp(-\Psi_d) \quad (8)
\]
\[
C_{K}(d) = (1 - h_{Na}) \exp(-\Psi_d) \quad (9)
\]

The potential at the interface is found from equation (7):

\[
\Psi_d = 2 \sinh^{-1} \varphi_d / 2 \quad (10)
\]

from which the concentrations at the interface are found from the boundary electric field. Since the membrane is considered to be in instantaneous equilibrium with the solution phase at the internal interface, this also gives the concentration of each permeable cation species within the membrane at this point.

A more difficult problem is presented by the external interface. Various mechanisms have been considered for the control of permeability at this point. The presence of external divalent cations, such as Ca++, is well-known to be essential to make the membrane polarizable. Ca++ has a large, tightly bound hydration shell. This apparently makes the membrane impermeable to it. It can, however, serve to block the passage of univalent cations through the membrane, when it is adsorbed at the outer surface of the membrane. It is assumed that when Ca++ is so adsorbed, neither of the permeable cation species can pass through the interface over some specified area. If the pore hypothesis is accepted, then the assumption would be that a Ca++ ion adsorbed at the external entrance to the pore would block the passage.

When this hypothesis of adsorption blocking is accepted, the problem is then to calculate the probability that a Ca++ ion is adsorbed at the pore entrance.

A theory due to Frenkel (1924) is employed. Frenkel states that if an ion is adsorbed on a charged surface, it will remain adsorbed for a dwell time \( t_d \), given by

\[
t_d = \tau \exp(-z\Psi_s) \quad (11)
\]

where \( \tau \) is the resonant period of the adsorbed ion; \( z \), its valence; and \( \Psi_s \) the potential of the surface relative to the free solution, in units of \( RT/F \).

When the pore entrance is free of an adsorbed ion, it will remain free for a time \( t_4 \) inversely proportional to the collision frequency of ions of this species, at the surface. This frequency will be proportional to the concentration of ions in the free solution \( C_e \). That is, the collision frequency \( f_e \) is given by

\[
f_e = k'C_e \quad (12)
\]
where $k'$ is a proportionality constant. Then $t_o$ is proportional to $1/f_o$, and

$$t_o = k''/C_o \quad (13)$$

The fractional time the patch is open to pass permeable ions is then

$$T_o = t_o/(t_o + t_d) \quad (14)$$

Then equations (11) and (13) with equation (14) give,

$$T_o = 1/[1 + k_o \exp (-\varepsilon \Psi)] \quad (15)$$

where $k_o = C_o \tau/k''$. No attempt will be made here to calculate $k_o$ from the physics of the problem, but it will rather be left as a parameter to be chosen in accordance with experiment. Its physical significance should, however, be remembered.

It is now assumed that the Ca++ permeability barrier produces an effective discontinuity in the concentration of the permeable cation species at the external interface ($\xi = 0$); or, more accurately, a concentration which changes from its external to its internal value within a short distance, which may be related to the mean free path of ions in the solution. For the purpose of simplification, this distance is taken as a constant, $\beta$, and the concentration gradient is taken as constant over $\beta$ (Fig. 2). The flow across the boundary during the time the membrane is free of adsorbed Ca++ is then given approximately by the sum of the flow due to the osmotic gradient, and that due to the electrical gradient.

The osmotic gradient is equal to $(C_i(0-) - C_i(0+))/\beta$, where $C_i(0-)$,
Ci(0+) are respectively the ionic concentrations just outside the membrane boundary layer at \( \xi = -\beta \), and just inside the membrane at \( \xi = 0 \). The flow due to the electric gradient is approximated as being equal to the mean electrical field across the boundary, times the mean concentration of the ion species in the boundary layer.

The flow across the boundary, averaged over the adsorption period, is

\[
J_{io} = T_o U_o \left[ \frac{C_i(0-) - C_i(0+)}{\beta} \right] + \frac{1}{2} (\psi(0-) + C_i(0+)) \frac{\varphi(0-)}{\varphi(0+)} \tag{16}
\]

where \( \varphi(0-) \) and \( \varphi(0+) \) are respectively the fields just outside the membrane boundary layer, and just inside the membrane.

It is seen then that \( C_i(0-) \) is the concentration of the ion remote from the interface, multiplied by the Boltzmann factor:

\[
C_i(0-) = C_i(-\infty) \exp(-\psi_o) \tag{17}
\]

If the mole fraction of K+ in the external solution is \( h_k \),

\[
C_k(0-) = h_k \exp(-\psi_o) \tag{18}
\]

\[
C_{Na}(0-) = (1 - h_k) \exp(-\psi_o) \tag{19}
\]

The calculation of \( C_i(0+) \) is obtained from the numerical integration of the equations (Appendix).

The electric field just inside the membrane, \( \varphi(0+) \), is found by integrating Poisson's equation once across the boundary layer. Since the ionic gradients are assumed constant, this gives

\[
\varphi(0+) = \varphi(0-) + \frac{\beta}{4} [C_k(0-) + C_{Na}(0-) + C_k(0+) + C_{Na}(0+)] \tag{20}
\]

The potential change across the boundary layer, \( \Delta\psi_o \), is taken as equal to the average field through the layer, times the thickness of the layer:

\[
\Delta\psi_o = \frac{1}{2} \beta [\varphi(0-) + \varphi(0+)] \tag{21}
\]

Since the ionic gradient cannot actually remain constant across the boundary, but must change with any change in the total membrane potential, equations (16), (20), and (21) must all be regarded as approximations. The approximations will be good, however, if the boundary layer thickness, \( \beta \), is small. The exact form assumed for the boundary layer, as well as its thickness, has only a minor effect on the calculations, as long as \( \beta \) is kept small. Fig. 3 illustrates the over-all picture employed for the membrane with the various parameters.
Another factor which has been considered in the control of permeability at the external interface is the compressing effect of the electric field. A pressure is produced in any substance in the presence of an electric field, equal to $E^2/\epsilon$, where $\epsilon$ is the dielectric constant. With the high electric field existing in the membrane, and particularly at the external interface, this pressure can be quite significant, and may well be one of the factors operating.\(^1\)

The effect of the compressive force can best be treated in terms of its effect on the activation energy of an ion in leaving or entering the membrane (Offner, 1969\(^b\)). This will be considered in a subsequent communication. Since the phenomena to be treated here can be largely explained without including this effect, this factor will not be discussed further in this communication, except to say that its inclusion can further accentuate the voltage amplification.

**Steady State**

The continuity equation states $\partial J_i/\partial \xi = -\partial C_i/\partial t$. Therefore the steady state is defined by the ion flux being equal at all points of the membrane. The value of the flux at $\xi = 0$ is given by equation (16) for each permeable ion species. This is therefore used in equation (4) to eliminate $J_i$, for each species. That is, equation (4) is used to calculate the concentration contours which will result in an ion flux which, at all points, is equal to the flux at the external interface. The value of $\varphi$ at each point is at the same time obtained from equation (5).

\(^1\) Mullins (1959) has proposed a somewhat similar hypothesis, but his effect was calculated on a different basis.
These equations are solved simultaneously, subject to meeting the boundary conditions for equations (8) and (9) at \( \xi = d \). They may, however, be solved numerically using a digital computer. The major problem in their solution is that of the boundary values; that is, to have the conditions at the interfaces conform to those in the two solutions. The methods employed are discussed in the Appendix.

Results of Calculations

Equation (16) showed that the ion flux is directly proportional to \( T_x \), the time a pore is open; which, by equation (15), is exponentially related to \( \Psi_x \), the boundary potential. A primary objective of the calculations is then to determine the relationship between \( \Psi_x \) and \( \Psi_n \), the total membrane potential (in dimensionless form). This relationship is directly influenced by every parameter of the membrane, and not always in any intuitively obvious manner.

The voltage amplification of the membrane, that is the steep change in \( \Psi_x \) with \( \Psi_n \), is due, as has already been mentioned, to a decrease in the cation concentration in the membrane in the vicinity of the external interface, as the (negative) value of \( \Psi_x \) increases. This decrease in ionic concentration results in a decrease in the electric field, which, acting through the remainder of the membrane thickness, results in a lowering in the \( PD \) across the membrane, as compared to the value it would have if the ion concentration were to have remained unchanged. Since the electric field is integrated across the membrane, it is apparent that the thickness of the membrane will be an important parameter: if it is too thin, the integral of the field will result in a negligible decrease in potential. Thus it is found that voltage amplification can only be obtained with membranes of at least several Debye lengths thickness.

Similarly, a substantial decrease in ionic concentration at the external interface implies sufficiently large initial concentrations, and thus a high negative fixed charge concentration near the interface.

The effect of ionic mobility is less obvious. It is evident, however, that for a membrane potential equal to the Nernst potential for any ion species, there will be no flux of that ion, and therefore the distribution of the ion species will be independent of its mobility function. At other membrane potentials the concentration gradient must be related to the local electric field according to equation (4), with \( J_s \) being determined by equation (16). Therefore, in a region of high mobility, the osmotic gradient must adjust itself to more nearly balance the electric field force, as compared to a region of lower mobility for that species.

From the calculations, it appears that voltage amplification is favored by a sharply decreasing mobility, in passing inwards through the interface, for the
ion having the major mole fraction in the external medium; i.e., Na\(^+\) in the usual situation. It is reasonable to expect such a mobility function in the real membrane, because of the much higher ionic mobility in solution, as compared to that in the membrane. The mobility of an ion having a small mole fraction concentration externally appears to have little effect.

Finally, it appears that a positively charged layer just external to the membrane further enhances voltage amplification.

One configuration on which calculations have been made is shown in Fig. 4. The symmetrical negative fixed charge distribution is taken to agree with the usually accepted picture of the membrane structure, with negatively charged polar groups at the two surfaces. Whether the remainder of the membrane should, in fact, be negatively charged is problematical. A thin positive layer is considered to exist on the external surface. This could be due to tightly bound Ca\(^{++}\) ions. For convenience in calculation, the layer is considered to be so thin that there is no potential difference across it; it produces only a step change in the electric field. Since divalent cations do not appear to be essential in the axoplasm, no positive layer is included at the internal interface.

**Single Cation**

The original objective of this investigation was to calculate the normal activity cycle, in which Na\(^+\) and K\(^+\) diffusion exists, and such calculations were first made. However, when the results were discussed with Dr. K. S. Cole, he pointed out that experiments on the squid axon, in which no Na\(^+\) was present, and in which the axoplasm-exoplasm were replaced by equimolar K\(^+\),
showed the same phenomena of "negative resistance" and steep change in conductivity (Ehrenstein and Gilbert, 1966; Gilbert and Ehrenstein, 1969; Lecar et al., 1967). Dr. Cole suggested that such a system be investigated. Since this problem is simpler and illustrates most of the principles, these calculations will be given first.

The configuration for which calculations are presented is that already shown in Fig. 4. The fixed negative charge, $q_b$, in the regions near the interfaces is taken as 120; while that in the central region, $q_e$, is 10. The positive layer at the external interface is such as to produce a 10 unit increase in the electric field, in passing inwards through the layer, implying a total surface charge density of 20. First, calculations are shown for a membrane thickness of six Debye lengths, plus a boundary layer thickness, $\beta$, of 0.1. If the physical thickness of the membrane is 63 Å, the Debye length is 10 Å, and the boundary layer is 1 Å, or about one ionic radius.\(^2\) The selection of a 10 Å Debye length is, in fact, quite arbitrary, since both the effective ionic concentration in the solution adjacent to the external interface and the effective dielectric constant, are both not known with any precision. If the dielectric constant is taken at 78, its value in free solutions, then 10 Å is obtained with a 0.085 molar total concentration of 1-1 electrolytes. This is not unreasonable.

With these assumptions, unit fixed charge density is then one electron charge for $1.93 \times 10^4$ Å\(^3\) and in the interface region (maximum charge density), 160 Å\(^2\) per electron charge. A 10 unit change in field in passing through the surface is obtained with one (positive) electron charge per 96 Å\(^2\) of surface:

\(^2\) Probably $\beta$ should be several times as large, but its value is limited to 0.1 in the calculations to minimize errors already discussed.
FIGURE 6 a

Total potential across membrane

Figure 6 a. Single diffusible cation with equimolar concentration inside and out. Calculated for four values of relative Ca\(^{++}\) concentration. Membrane six Debye lengths thick. a, boundary potential. b, ion flow (current). Calculations for zero Ca\(^{++}\) concentration made for three Debye lengths membrane, because of numerical problems. Failure of flows to pass through origin due to numerical approximations. See text.

or, if it be due to divalent cations, one such ion per 192 A. These concentrations all appear to be within expected physical limits.

The mobility function used is illustrated in Fig. 5. This again was selected rather arbitrarily, on the basis already discussed.
Fig. 6 shows the results of calculations for the above-discussed configuration, with equimolar K+ in the two solutions. In a is plotted the boundary potential as a function of total membrane potential; and in b, ion flow, also vs. total potential. Each includes the results for four values of the Ca++ concentration, in the ratio 3.0:1.0:0.33:0. The practically vertical rise in \( V_0 \) in the region of \( V_m = 3.5 \) is apparent for the relative Ca++ concentration of 1.0. At Ca++ = 0.33, the curve becomes S-shaped and multivalued; such a curve cannot, of
course, be obtained experimentally, nor can the corresponding flow curve, which also shows a similar multivalued nature.

The reduction of the Ca++ to near zero\(^*\) eliminates the region of rapid change in \(V\), and results in a practically ohmic behavior, while the boundary potential remains almost independent of the total potential, at a value of about 2.4.

The multivalued nature of the response is a function of the membrane thickness. The results of similar calculations are repeated in Fig. 7 for a membrane thickness of 2.0 Debye lengths. The elimination of the multivalued response and of voltage amplification, is apparent.

![Figure 7. Ion distribution for membrane of Fig. 6. Ca++ concentration = 1.0. Solid line, \(V_0 = 4.0\). Dashed line, \(V_0 = 3.0\). Curves closely correspond except where shown as separated.](image)

The ionic redistribution within the membrane is illustrated in Fig. 8. This is shown for the membrane configuration of Fig. 4 with a relative Ca++ concentration of 3.0. It is seen that when the boundary potential falls from 4.0 to 3.0, the ionic concentration in the vicinity of the external interface is increased.

The form of the response is also strongly influenced by the ratio of the ionic concentrations in the two baths. This is to be expected, since the voltage amplification effect is due to a redistribution of ions in the membrane, with the concentration at the outer boundary showing a disproportionate increase as the boundary potential is reduced. Such an increase is less readily obtained when the membrane is in contact with the external solution of low ionic strength.

\(^*\) Calculations for relative Ca++ concentration approaching zero were actually made for a membrane of three units thickness, because of nonconvergence of the present program in this particular problem for six units thickness.
strength. However, the various factors already discussed can influence the form of the steady-state ion distribution, and may result in voltage amplification with lowered external cation concentration.

**Two Cations**

Calculations have been made for two diffusible univalent cations (e.g., $K^+$ and $Na^+$) under a wide variety of assumed conditions, including various contours of fixed charge, and of the separate ion mobilities. Calculations have also been made for various membrane thicknesses. The results are illustrated in Figs. 9–11, for a membrane of the same charge distribution as in the single ion case. In Fig. 9 is seen the change in boundary potential, $V_o$, vs. total membrane

![Figure 9. Two diffusible cations, membrane six Debye lengths thick. Boundary potential for value of relative $Ca^{++}$ concentration = 1.0. External $K^+$ mole fraction = 0.025; internal $Na^+ + 0.05. K^+$ mobility assumed 50.0 times $Na^+$.](image)

![Figure 10. Flow of separate ion species, membrane of Fig. 9. Na$^+$ – O. K$^+$ – Δ.](image)
potential, $V_m$, for relative Ca$^{++}$ concentration = 1.0. The appearance of the voltage amplification region at this Ca$^{++}$ concentration is again apparent. The variation in the steady-state ion currents is shown in Fig. 10. The region of

![Figure 11a](image1.png)

**Figure 11a**

![Figure 11b](image2.png)

**Figure 11b**

Figure 11. Shift in ionic distribution with potential. Membrane of Fig. 9, relative Ca$^{++}$ concentration = 1.0. a, $V_o = 4.0$. b, $V_o = 3.0$.

steep change in flow rate is seen in the vicinity of $V_m = 3.5$, corresponding to the region of steep change in $V_o$ with $V_m$. It should be pointed out that these results do not correspond to voltage clamp data, as usually presented, since both the sodium current and the potassium current are the final values they attain; while the peak sodium current is usually given. This cannot be adduced
from the present calculations, but requires rather the solution of the transient problem, by the use of the appropriate partial differential equations. This will be presented in a subsequent communication.

The ionic distribution through the membrane exhibits a dramatic shift, as the membrane potential passes through the critical region. This is shown in Fig. 11, again for the intermediate Ca++ concentration case. In a is shown the distribution of the Na+ and K+ with $V_a = 4.0$; the ionic concentration is predominantly K+. As seen in b, the distribution substantially reverses when the boundary potential falls to 3.0.

The resting potential is obtained from Fig. 10, being the membrane potential at which the K+ outflow is equal to the Na+ inflow; that is, the point where the two flow curves cross. This point can be shifted by changing the relative mobilities of Na+ and K+. Because of the assumed independence of ion fluxes, changing the relative mobilities by a constant factor will result only in the change in the relative fluxes by the same factor, and will have no effect on the ionic distributions, or the electric field distribution.

The effect on the resting potential of various factors, such as the separate ion mobility functions, the ionic concentrations of the two baths, and the fixed charge distribution, may be readily calculated. Although the present calculations do not include the effect of Cl− flow (for which the membrane is assumed to be impermeable), this could readily be included in the calculations by merely extending the treatment to three permeable ion species.

Comparison with Experiment

Since these calculations deal only with the steady-state solution, they are not readily compared with normal voltage clamp results, involving the diffusion of both K+ and Na+; the important changes in Na+ ion flux occur only as transients. The comparison of the single ion results is therefore of more interest.

Fig. 12 has been replotted from Fig. 6 b of Lecar et al. (1967), with the (linear) leakage current subtracted. The experimental conditions were approximately equimolar K+ inside and outside the squid axon. At a, divalent cations (Ca++ and Mg++) are present; while at b, they are absent. Curve a may be compared with Figs. 6 and 7, particularly with the higher Ca++ concentrations of Fig. 6; curve b is to be compared with the zero Ca++ curve of Fig. 6. A close similarity in the form of the response is evident.

Fig. 13 has been replotted from Fig. 1 of Gilbert and Ehrenstein (1969).

Again, the leakage current has been subtracted, although this could only be done very approximately, since the exact value of the leakage current is not readily derivable from the published figure. Curves a and b show the effect of a fivefold decrease in divalent cations in the external solution (taking Mg++ as having half the effect of Ca++, as assumed by the authors). These results may be compared with Figs. 6 and 7; again there is at least a semiquantitative cor-
respondence. Curve c of Fig. 13 is with nominally no external divalent cations present; it continues to show a negative resistance region. The discrepancy with the experimental results shown in Fig. 12, curve b, in which internal Ca$^{++}$ is also removed by perfusion remains to be explained.

**Figure 12.** Experimental results for perfused squid axon, taken from Lecar et al. (1967), Fig. 6 b. Replotted, with linear component of current (approximately) subtracted. External and internal solutions approximately equimolar in K$^+$. a, with Ca$^{++}$ and Mg$^{++}$ present in external solution. b, divalent ions removed.

**Figure 13.** Experimental results for squid axon, taken from Fig. 1 of Gilbert and Ehrenstein (1969). Replotted with linear component of current very approximately subtracted. a and b are for divalent cations in external medium in approximately 5:1 ratio. At c, divalent ions nominally absent.

Inspection of the experimental data obtained with equimolar K$^+$ in the various references cited shows considerable variability. This may be due in part, at least, to the nonphysiological conditions of the experiment. It is thus probably too much to expect anything closer than the semiquantitative correspondence.
DISCUSSION

Perhaps the most important conclusion from the present calculations is the demonstration that the nature of the ionic diffusion process in a thin membrane cannot be understood fully without considering the processes which occur within the membrane; and in particular the ionic redistribution, with the corresponding redistribution in the electric field. The source of the steep change in the conductance of the separate ion species becomes apparent, and the effect of the Ca\(^{++}\) (or other divalent cation) concentration change is at least qualitatively explained.

The ionic redistribution has been shown to give rise to a change in the boundary (zeta) potential, which is assumed to be a principal factor controlling ionic conductance. Since the redistribution process is progressive, the zeta potential, and thus the conductance, changes progressively with time. Thus the time course of the ionic conductance is inherent in the physical chemistry of the system, and requires no separate assumptions or processes.

Some order of magnitude calculations have been given for the surface and volume charge densities represented by the calculated model. Unfortunately, this is not possible for the Ca\(^{++}\) concentration in the external solution. It would be necessary to have at least an approximate value for \(\tau\) in equation (11). While some estimates have been attempted, the physical basis for these is so poor that it is probably better to retain \(k_a\) as a parameter to be determined by experiment.

In this paper, an attempt has been made to treat the problem so far as possible by classical diffusion theory. Some facets of the problem can be treated better by other methods, such as activation theory. These include the differential change in Na\(^{+}\) and K\(^{+}\) conductance, and the Na\(^{+}\) shutoff phenomenon. These will be treated in a subsequent communication, as will a more extensive treatment of the whole transient problem, using the solution of the partial differential equations of the system.

APPENDIX

Numerical Methods

The system of differential equations represented by equations (4) and (5), with arbitrary functional forms for the mobility, \(U_i\), and the fixed charge, \(Q\), can only be solved numerically. However, the more familiar numerical integration methods can be used directly only to solve initial value problems; that is, the value of the function and its derivatives are specified at one boundary. The change in the value of the function is then calculated at succeeding points, until the opposite boundary is reached. In general, then, the value will not match the desired boundary value at this point.

In general, there are two methods by which numerical integration can be made
to yield a solution corresponding to the desired conditions at the two boundaries. These are the so-called “ballistic” method, and the difference equation method. The ballistic method is the simpler for problems of the class herein treated, and was used for the earlier work on the problem. This method will be described here. Convergence of the ballistic method becomes progressively more difficult, however, as the membrane thickness is increased. Therefore a program for the difference equation method, which is more satisfactory for thicker membranes (longer integration paths), has also been developed. This program is still being perfected, however, so that it will be described in a later communication.

With the ballistic method, an initial value solution is used to solve a double boundary value problem by appropriately varying the (starting) initial conditions. With a single dependent variable, it is fairly simple to vary such an initial value solution to converge on the desired boundary value, provided that the value of the function at the far boundary is a monotonic function of its initial value.

For a given value of $\Psi_s$ (this being taken as a parameter for calculating a family of solutions), the value of $C(0^-)$ is given by equation (17)—the subscript is omitted since only a single species is now being considered. The procedure is to assume a value of $C(0^+)$, and from this, using equation (16), to calculate $J_s$. Since a steady state is assumed, $dJ/d\xi = 0$ and the value $J_s$ can be used in equation (4) to integrate equations (4) and (5) across the regime. Thus the concentration, $C(d)$, and electric field, $\varphi_d$, at $\xi = d$ are determined. Call this value of the concentration $C(d)_1$. The value of $\varphi_d$ gives the boundary potential, $\Psi_d$, from equation (7). But this also determined $C(d)$, by equation (8)—call this $C(d)_2$.

In general, these two values of $C(d)$ will not be the same, and the problem is to select $C(0^+)$ to make them equal, within a prescribed criterion of accuracy.

Two methods may be used for varying $C(0^+)$ to make $C(d)_1 - C(d)_2 = \Lambda \rightarrow 0$. $\Lambda$ is then the error in the boundary conditions at $\xi = d$. The first method may be called “bracketing.” Let $C(0^+)$ be the first trial value of $C(0^+)$. Since $\Lambda$ is known to be a monotonically increasing function of $C(0^+)$, if $\Lambda$ is positive, $C(0^+)_2$ must be less than $C(0^+)_1$; while if $\Lambda$ is negative, the converse is true. Then

$$C(0^+)_2 = (1 \pm \frac{1}{2})C(0^+)_1.$$  \hfill (I-1)

The sign is selected as the negative of the sign of $\Lambda$.

The value of $C(0^+)$ used in successive calculations is increased or decreased depending on the sign of $\Lambda$. The size of the increment in $C(0^+)$ is unchanged until the sign of $\Lambda$ changes; the increment is then reduced by half. That is, if the sign of $\Lambda$ did not change during the $n$th calculation, then

$$C(0^+)_n+1 = C(0^+)_n \pm [C(0^+)_n - C(0^+)_{n-1}]$$  \hfill (I-2)

while if the sign of $\Lambda$ had changed, half the bracketed quantity would be used.

The second method is the quasi-derivative method. Starting with $C(0^+)_1$, calculate $\Delta_n$. Add a small increment, $\xi$, to $C(0^+)_1$ and calculate $\Delta_3$. The next value of $C(0^+)$ is then
\[ C(0^+ -) C(0^+) \Delta - \Lambda \cdot \delta \frac{\delta}{\delta \Delta} \tag{1-3} \]

where \( \delta \Delta = \Delta - \Lambda \).

This procedure is repeated until \( \Delta \) is sufficiently small.

The quasi-derivative method converges rapidly for equations having only moderate nonlinearity. However, with the equations involved in the present calculations which are highly nonlinear, convergence may be very slow. With only a single dependent variable the first (bracketing) method converges quite rapidly.

The problem is complicated by the introduction of the second dependent variable (representing two diffusible ions). Now the variation of the initial concentration of either ion will have a strong influence on the error term, \( \Lambda \), for the other ion. The bracketing method converges only very slowly towards a small error, although it has been found to converge fairly rapidly towards a moderate error. On the other hand, the quasi-derivative method converges only very slowly when the error is very large, but rapidly from a moderate error.

A suitable method of calculation has therefore been found to be the use of the bracketing method at the start, until the error terms are of reasonable size. Final convergence is obtained with the quasi-derivative method. The method is to numerically integrate the equations across the regime, with some set upper and lower bounds on the ionic concentrations. These bounds are set well above and below any concentration which would correspond to an actual steady state solution; values of 500 and 0.01 have been used for the two bounds, where the extreme expected values are of the order of 100 and 0.1. Then if either ion species crosses the set bound during the integration, its initial value is changed, according to equation (I-2); the initial value is reduced if the upper bound is crossed, and conversely.

Once the integration proceeds across the regime, so that the values \( C(d)_K \) and \( C(d)_Na \) are calculated, and thus also the error terms \( \Lambda_K \) and \( \Lambda_{Na} \), then the (quasi) partial derivatives of the error terms are calculated as in (1-3), but for the two variables:

\[
\begin{align*}
\frac{\partial \Lambda_K}{\partial C_K(0^+)} &= \frac{\delta \Lambda_K}{\delta_K} \\
\frac{\partial \Lambda_K}{\partial C_{Na}(0^+)} &= \frac{\delta \Lambda_K}{\delta_{Na}}
\end{align*}
\tag{1-4, 1-5}
\]

etc. \( \delta_K \) and \( \delta_{Na} \) are the small increments given to the initial values of concentration.

Then approximately

\[
\begin{align*}
\Delta \Lambda_K &= \frac{\partial \Lambda_K}{\partial C_K(0^+)} \Delta C_K(0^+) + \frac{\partial \Lambda_K}{\partial C_{Na}(0^+)} \Delta C_{Na}(0^+) \\
\Delta \Lambda_{Na} &= \frac{\partial \Lambda_{Na}}{\partial C_{Na}(0^+)} \Delta C_{Na}(0^+) + \frac{\partial \Lambda_{Na}}{\partial C_K(0^+)} \Delta C_K(0^+)
\end{align*}
\tag{1-6}
\]

the approximation being progressively better as the size of the increments is decreased. The procedure is then to set \( \Delta \Lambda_K \) and \( \Delta \Lambda_{Na} \) equal to \( -\Lambda_K \) and \( -\Lambda_{Na} \), and solve the
two equations (I-6) simultaneously to find the appropriate values of the correction
terms, $\Delta C_{K}(0+)$ and $\Delta C_{Na}(0+)$, to be added to the initial concentrations $C_{K}(0+)$
and $C_{Na}(0+)$. This then gives two new initial values. The equations are again inte-
grated across the regime, using these new initial values, and new error terms, $\Delta_{K}$
and $\Delta_{Na}$, computed. The procedure is repeated until the error terms are both within
the prescribed limit; for example, within 1%.

The actual numerical integration may be accomplished by various methods. The
regime is divided into $n$ equal intervals, each of width $h$, so that $n \cdot h = d$. The value
of the concentrations, and the corresponding value of the electric field, are calculated
successively at each of the $n$ stations succeeding the initial position at $\xi = 0$, which
corresponds to the initial (assumed) boundary values, using the system of equations
(4) and (5). In numerically integrating these equations, two methods have been used.
In the first method, the derivatives are computed by a straight-line approximation,
so that at the $j$th station

$$\left(\frac{dC}{d\xi}\right)_j = \frac{(C_{j+1} - C_{j-1})}{2h} \quad (1-7)$$

If we remember that $J_{i}$ is a constant for the steady state, all the terms are then known
from the $0$th through the $j$th station; thus only the $C_{j+1}$ terms are unknown, and
equations (4) can be solved for these concentrations, until the last station at $j = n$
is reached.

A minor complication exists at the start, since the derivative cannot be computed
by equation (1-7) at $j = 0$.

$$\left(\frac{dC}{d\xi}\right)_0 = \frac{(2C_1 - \frac{3}{2}C_2 - \frac{1}{2}C_0)}{h} \quad (1-8)$$

This problem is resolved by a method of successive approximations.

A more accurate, and in fact in some ways simpler method, has been used in later
calculations. This is the method of Runge and Kutta.

The method is described in any text on numerical analysis, and will therefore not
be further described here, except to mention that it is “self-starting,” thus eliminating
the need for the use of successive approximations at the starting boundary. A fourth-
order method is used; in this, the error decreases as $n^4$, whereas in the first-described
method it decreases as $n^2$. This permits fewer divisions to be used in the Runge-Kutta
method, for a given desired accuracy.

After the steady-state ion distribution has been determined, the total membrane
potential, $\Psi_m$, is calculated. The potential just inside the boundary layer is obtained
by adding $\Delta\Psi_{\theta}$, obtained from equation (21), to $\Psi_{\theta}$. To this sum is added the poten-
tial drop through the membrane, which is obtained by taking the negative of half
the double integral of the total charge concentration through the membrane, since
the potential is the negative of the integral of the field, obtained from equation (5). Finally,$\Psi_{\delta}$, the potential drop at the inner interface, obtained from equation (10),
is added:

$$\Psi_m = \Psi_{\theta} + \Delta\Psi_{\theta} - \frac{1}{2} \int_0^d dx \int_0^\theta (C_K + C_{Na} - Q) \, d\xi + \Psi_{\delta} \quad (1-9)$$
A listing of the Fortran program for solution of this boundary-value problem will be made available to anyone desiring to receive it.

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