AN ANALYSIS OF THE SURFACE FIXED-CHARGE THEORY OF THE SQUID GIANT AXON MEMBRANE

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ABSTRACT The observed shift in threshold potential, after perfusion of the squid giant axon with solutions of low ionic strength, can be predicted by assuming a fixed negative charge on the inside of the membrane. The constant field equation, together with the double-layer potential due to this charge, has been used to determine the change in resting potential during perfusion with solutions of low ionic strength. Neither the modified constant field equation nor Planck's diffusion equation can successfully predict the observed shift in resting potential. It is suggested that a positive charge distribution exists about the sodium channel on the outside of the membrane. The double-layer potential due to this positive charge, together with the independence principle, has been used to predict the relationship between sodium current and membrane potential when the ionic strength and sodium activity of the external solution are decreased. These predictions have been compared with the available experimental observations.

INTRODUCTION

The recently developed internal perfusion techniques for giant axons (Oikawa, Spyropoulos, Tasaki, and Teorell, 1961; Baker, Hodgkin, and Shaw 1962 *a*) have provided the opportunity for further experimental study of the ionic hypothesis (Hodgkin and Huxley, 1952 *b*). A surprising result is the finding that the membrane can fire action potentials from a zero resting potential, when the internal potassium chloride concentration is diluted with sucrose (Baker, Hodgkin, and Shaw, 1962 *b*; Tasaki, Watanabe, and Takenaka, 1962; Tasaki and Shimamura, 1962). Voltage clamp (Moore, Narahashi, and Ulbricht, 1964; Chandler, Hodgkin, and Meves, 1965) and other studies (Narahashi, 1963; Baker, Hodgkin, and Meves, 1964) have shown that there is a shift in the sodium inactivation and conductance curves of the Hodgkin and Huxley (1952 *b*) formulation in the direction of more positive inside potentials on reduction of the internal ionic strength. The shift in these curves explains why the action potential can be initiated from small resting potentials. Baker et al. (1964) have suggested that the shift in the inactivation and conductance curves in the direction of more positive potentials is due to a change in the value of a double-layer potential (Chapman, 1913; Verwey and Overbeek, 1948) at the inside of the membrane. Chandler et al. (1965) evaluated such a double-layer potential, and suggested that this surface fixed-charge theory may account for the deviation between the predictions of the constant field equation (Goldman, 1943; Hodgkin and Katz, 1949; Teorell 1953; Patlak, 1960) and the observed resting potential in perfused axons (Baker et al., 1964). A comparison has been made between the predictions of the surface fixed-charge theory and the experimental results which are at present available.

In general, the constant field equation predicts a rectification of the current carried by an ion species through a membrane. Dodge and Frankenhaeuser (1958, 1959) have shown that the sodium current is instantaneously rectified through the membrane at the node of Ranvier, whereas Hodgkin and Huxley (1952 *a*) have shown that this current is not instantaneously rectified through the membrane of the squid giant axon. The constant field equation predicts a linear relationship between sodium current and voltage when the sodium activity is the same on both sides of the membrane. Frankenhaeuser (1960) has suggested that charge is distributed on the membrane in such a way, that the sodium activity on either side of the membrane is the same. The surface fixed-charge theory allows this distribution of charge to be predicted.

ANALYSIS AND DISCUSSION

The Double-Layer Potential

In the fixed-charge theory (Chandler et al., 1965), the charge on the inside of the membrane (σ_i) is related to the double-layer potential (ψ_0) by an equation derived from the Boltzmann and Poisson relations and the electrostatic conditions at the membrane-solution interface. This equation is

$$\psi_0[(\epsilon_m/4\pi a) + (\epsilon_w/4\pi l)\{\sinh(F\psi_0/2RT)/(F\psi_0/2RT)\}] = \sigma_i \tag{1}$$

where

$$l = [(\epsilon_w RT)/(8\pi CF^2)]^{0.5}$$

and $\epsilon_m/4\pi a$ is the membrane capacitance (1 μf/cm^2); ϵ_w is the dielectric constant of water at temperature T; C is the bulk concentration of electrolyte in moles/cm^3; R and F have their usual meaning. This equation may be written as

$$(\psi_0)^3[(\epsilon_w/96\pi l)(F/RT)^2] + \psi_0[(\epsilon_m l + \epsilon_w a)/(4\pi a l)] - \sigma_i = 0 \tag{2}$$

in which the expansion of (sinh $F\psi_0/2RT$)/($F\psi_0/2RT$) has not been continued beyond the second power. Equation 1 has been solved on the IBM 7044 computer for a range of σ_i from -5 μ coul/cm^2 to -1 μ coul/cm^2, and of C, the concentration of internal KCl, from 4 to 1000 mM. The double-layer potential increases with a decrease in the internal potassium activity, for any particular value of the fixed charge (Fig. 1). As the value of the charge increases, the curvature in the relation between double-layer potential and potassium activity decreases for $(a_K)_i$ less than 100 mM.

It has been shown that the threshold for firing the action potential becomes more positive as the ionic strength is decreased (Baker et al., 1964). The double-layer potential shift with change of ionic strength predicts the shift in threshold potential when the fixed charge σ_i, equals $-2.2\ \mu$ coul/cm^2 (Chandler et al., 1965). Fig. 2 compares the shift in double-layer potential and threshold potential at different ionic strengths for different values of the fixed charge. The double-layer potential was adjusted to the threshold potential at $(a_K)_i$ equals 224 mM for each value of σ_i. The curve for σ_i equals $-2.2\ \mu$ coul/cm^2 gives the best fit to the experimental points out of the various σ_i curves calculated.

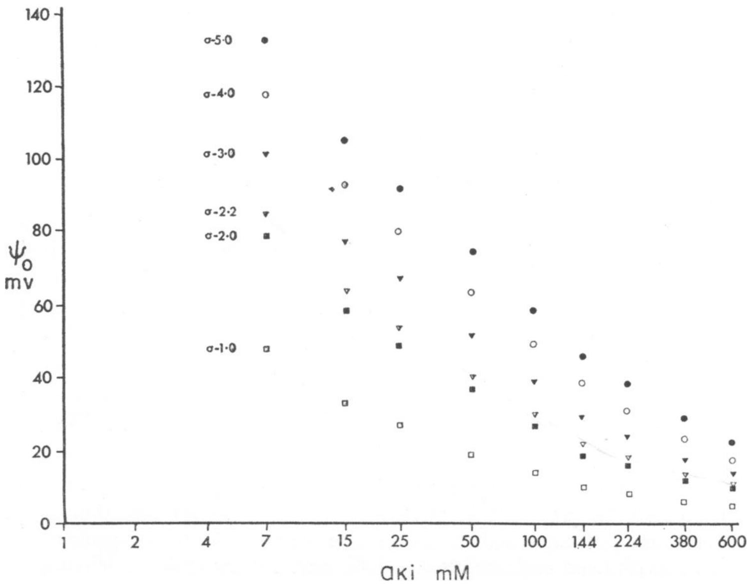

Figure 1 Dependence of double-layer potential ψ_0 on the ionic strength of a potassium chloride solution. Abscissa, potassium activity $(a_K)_i$, inside the axon. Ordinate, double-layer potential ψ_0. The double-layer potential for different values of fixed negative charge in microcoulombs/cm^2 is shown. The activity was estimated from the values tabulated by Robinson and Stokes (1959) for potassium chloride and from the Debye-Hückel theory.

The Constant Field Equation and the Double-Layer Potential

If it is assumed that there is a double-layer potential at the inside of the axon membrane, then the ionic concentrations in the perfusion solution near the inner edge of the membrane will be different from those in the bulk of the perfusion solution.

According to the Boltzmann relations, the cations will be concentrated by an amount, ρ, and the anions diluted by a similar amount, where

$$\rho = \exp\left(-F\psi_0/RT\right) = \frac{(a_{\mathrm{K}})_i''}{(a_{\mathrm{K}})_i} = \frac{(a_{\mathrm{Cl}})_i}{(a_{\mathrm{Cl}})_i''} \tag{3}$$

and the double primed values refer to activities at the inner edge of the membrane.

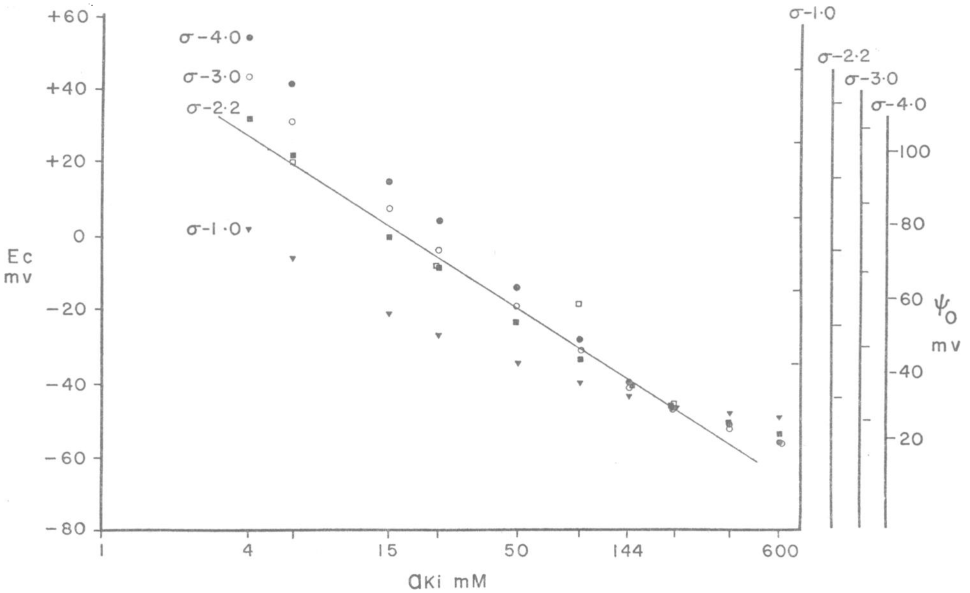

FIGURE 2 Comparison between the shift in double-layer potential ψ_0 with dilution of potassium chloride and the shift in threshold potential (E_C) for initiation of the action potential. Abscissa, potassium activity $(a_{\mathrm{K}})_i$ inside the axon. Left-hand ordinate, threshold potential E_C. Right-hand ordinate, double-layer potential for different values of the fixed negative charge. This ordinate has been fitted to the experimental value of the threshold potential at 224 mM for each value of the fixed charge. Open squares, threshold potential given by Baker et al. (1964). The different values of the fixed negative charge σ_i are given in microcoulombs/cm².

The total potential between internal and external bulk solutions is given in this model by the constant field equation with corrections for the altered activities at the membrane, together with the double-layer potential.

$$V = RT/F)\ln[\{P_{\mathrm{K}}(a_{\mathrm{K}})_E + P_{\mathrm{Na}}(a_{\mathrm{Na}})_E + P_{\mathrm{Cl}}(a_{\mathrm{Cl}})_i/\rho\}/ \\ \{P_{\mathrm{K}}(a_{\mathrm{K}})_i + P_{\mathrm{Cl}}(a_{\mathrm{Cl}})_E/\rho\}] \tag{4}$$

where $(a)_E$ refers to the activity of an ion species on the outside of the axon, $(a)_i$ to the activity on the inside. P_{K}, P_{Cl}, P_{Na} are the permeability coefficients (Hodgkin and Katz, 1949). It has been suggested (Chandler et al., 1965) that this equation may account for the shift in resting potential with dilution of the internal solution observed by Baker et al. (1964). Equation 4 has been computed for different values of membrane charge, and of the ionic activities on either side of the membrane.

The terms in equation 4 which include the chloride activity may be neglected when ρ equals 7 (Fig. 3) for they are no longer numerically significant. When σ_i equals -3.0 μ coul/cm^2, the chloride activity may be ignored when the internal potassium activity is less than 90 mM; when σ_i equals -1.0, the chloride activity may not be ignored until the potassium activity is less than 7 mM. The effect of this change in ρ on the predictions of the constant field equation, with and without double-layer correction, is shown in Fig. 4. The shift in membrane potential predicted by the constant field equation, increases with a decrease in $(a_K)_i$ towards a constant value given by

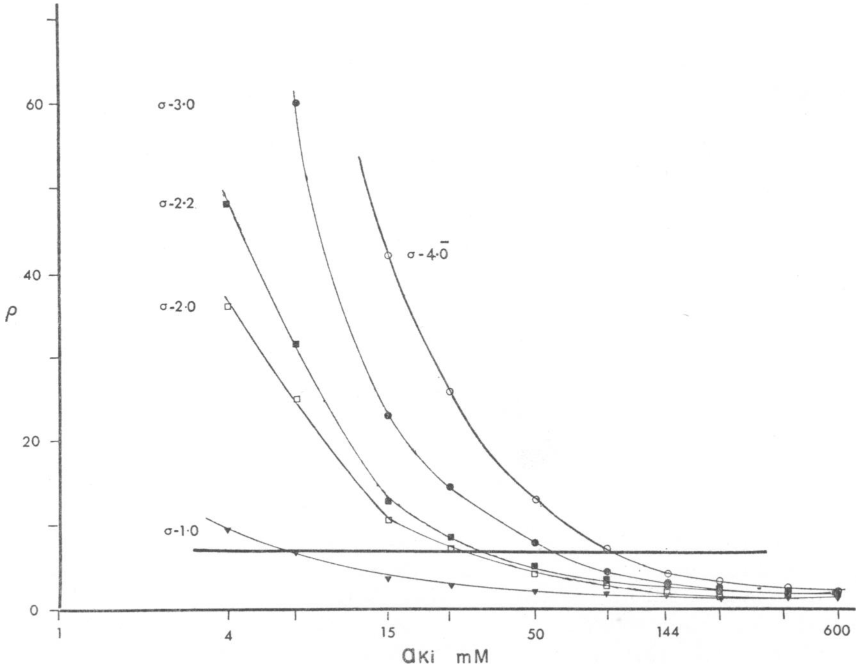

Figure 3 Change in the value of ρ given by equation 3, with a change of ionic strength. Abscissa, potassium activity $(a_K)_i$ inside the axon. Ordinate, value of ρ. The value of $(a_K)_i$ at $\rho = 7$ is shown for different values of the fixed charge. The charge σ_i is given in microcoulombs/cm^2.

$$V = (RT/F)\ln[\{P_K(a_K)_E + P_{Na}(a_{Na})_E\}/\{P_{Cl}(a_{Cl})_E\}] \qquad (5)$$

The change in membrane potential predicted by the constant field equation with double-layer correction is more positive than that given by the field equation alone, for all values of the charge considered. At the various values of $(a_K)_i$, at which ρ is greater than 7, the shift in membrane potential is about 58 mv/tenfold change in $(a_K)_i$ (Fig. 4), that is

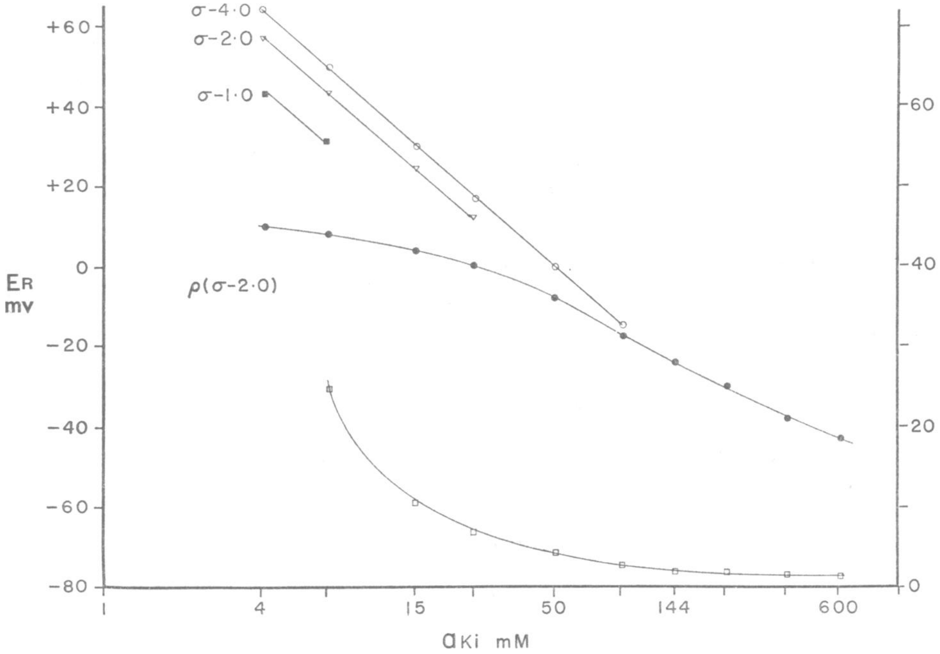

FIGURE 4 Theoretical predictions of the variation of resting potential E_R, with a decrease of the ionic strength of the internal potassium chloride solution. $(a_K)_E$ = 40 mM, $(a_{Na})_E$ = 282 mM, $(a_{Cl})_E$ = 322 mM. Abscissa, potassium activity $(a_K)_i$. Left-hand ordinate, resting potential E_R. Right-hand ordinate, value of ρ given by the open squares for a fixed negative charge of -2.0 μ coul/cm^2. Filled circles, resting potential change predicted by the constant field equation with $P_{Cl}/P_K = 0.10$, $P_{Na}/P_K = 0.05$. The resting potential approaches +13 mv, given by equation 5. Straight lines, predictions of the constant field equation with $P_{Cl}/P_K = 0.18$, $P_{Na}/P_K = 0.05$ corrected for double-layer potential shift with the value of the fixed charge indicated in microcoulombs/cm^2. The resting potential predicted by this equation varies as an ionic electrode when ρ is greater than about 7.

$$V = (RT/F) \ln [\{P_K(a_K)_E + P_{Na}(a_{Na})_E\}/\{P_K(a_K)_i\}] \quad (6)$$

An attempt has been made to see whether equation 4 can predict the observed shift in membrane potential with internal dilution (Baker et al., 1964). Figs. 5 and 6 show the experimental results together with the theoretical predictions. The values given by the constant field equation for low internal ionic strength are less than the observed shift in potential, whereas the predictions of the field equation with double-layer correction are too positive. The best fit was obtained with the smallest value of charge considered, σ_i equals 1.0 μ coul/cm^2. The fixed charge which gave the best fit to the shift in threshold potential with internal dilution, -2.2 μ coul/cm^2, was unable to predict the shift in the resting potential.

The threshold potential depends mainly on parameters controlling sodium permeability in the Hodgkin and Huxley (1952 *b*) analysis, whereas the resting potential is dependent on the relative permeabilities of sodium, potassium, and chloride. The -2.2 μ coul/cm^2 charge may be confined to the vicinity of the sodium channel, a different charge distribution occurring at the potassium and chloride channels (Chandler et al., 1965). In these circumstances the modified constant field equation 4, would be unable to predict successfully the shift in resting potential with dilution of the internal solution.

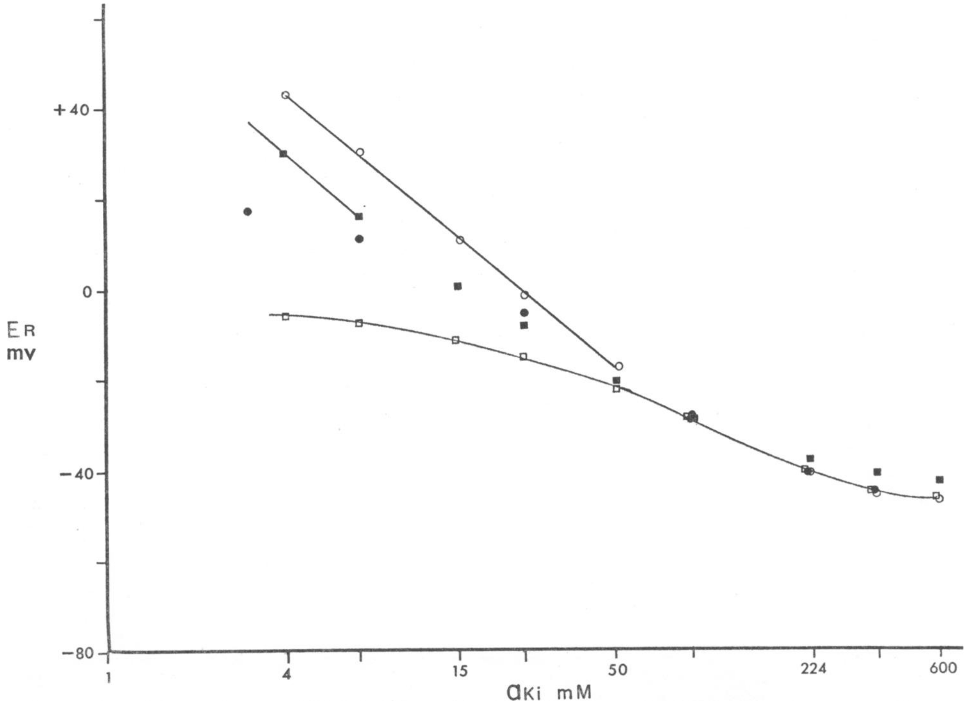

FIGURE 5 Theoretical and experimental predictions of the variation of resting potential E_R, with a decrease of the ionic strength of the internal potassium chloride solution. $(a_K)_E$ = 13.6 mM, $(a_{Na})_E$ = 308 mM, $(a_{Cl})_E$ = 322 mM. Abscissa, potassium activity $(a_K)_i$, ordinate, resting potential E_R. Open squares, resting potential change predicted by the constant field equation with $P_{Cl}/P_K = 0.10$, $P_{Na}/P_K = 0.05$. The resting potential approaches -2.7 mv, given by equation 5. Filled squares and open circles, predictions of the constant field equation with $P_{Cl}/P_K = 0.18$, $P_{Na}/P_K = 0.05$ corrected for double-layer potential shift with fixed charge values of -1.0 and -2.2 μ coul/cm^2 respectively. Filled circles, experimental resting potential change determined by Baker et al. (1964).

Planck's Diffusion Equation

It has been shown that there is a constant electrical field through a membrane separating two solutions of equal total concentration of electrolytes (Teorell, 1953; Finkelstein and Mauro, 1963). Under these circumstances, the general equation given by Planck (1890) for the potential across the membrane, reduces to the constant field equation of Goldman (1943). It might be expected that the constant field equation would no longer apply if during perfusion of the squid giant axon the electrolyte concentration on one side of the membrane was greatly different from that on the other. To test this, Planck's diffusion equation has been solved on the IBM 7044 computer using an approximation technique. Planck's equation gave results which were identical with those given by the constant field equation without

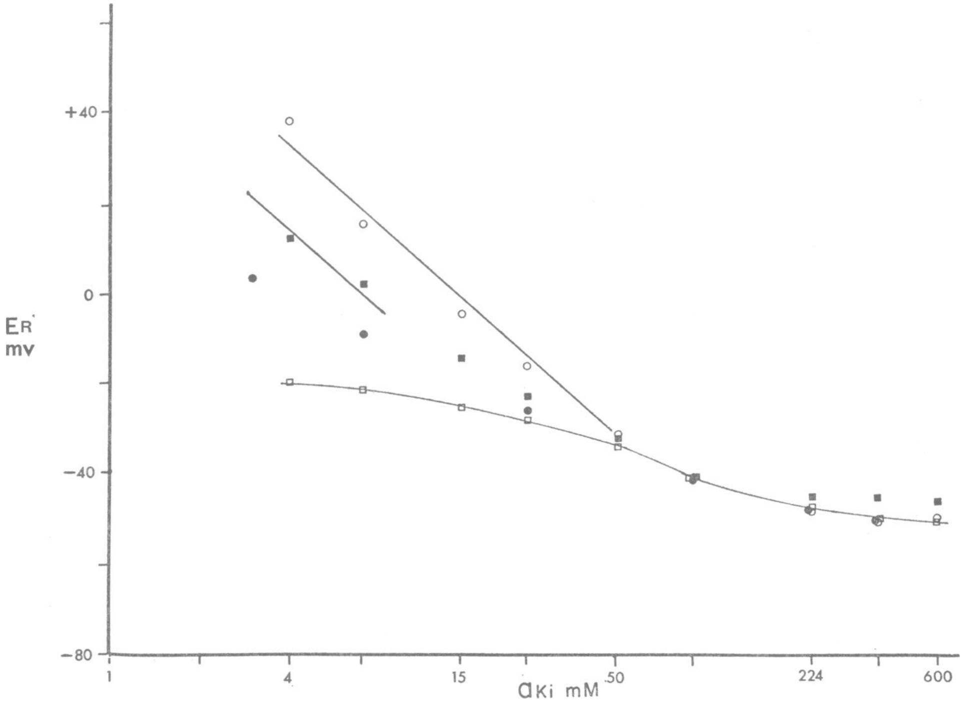

FIGURE 6 Theoretical and experimental predictions of the variation of resting potential E_R, with a decrease of the ionic strength of the internal potassium chloride solution. $(a_K)_E = 0$, $(a_{Na})_E = 322$ mM, $(a_{Cl})_E = 322$ mM. Abscissa, potassium activity $(a_K)_i$. Ordinate, resting potential change predicted by the constant field equation with $P_{Cl}/P_K = 0.10$, $P_{Na}/P_K = 0.05$, and given by the open squares. The resting potential approaches −17 mv, given by equation 5. Filled squares and open circles, predictions of the constant field equation with $P_{Cl}/P_K = 0.18$, $P_{Na}/P_K = 0.05$ corrected for double-layer potential shift with fixed charge values of −1.0 and −2.2 μ coul/cm², respectively. Filled circles, experimental resting potential change determined by Baker et al. (1964).

double-layer potential correction, for internal potassium activities down to about 25 mM. At lower activities, the Planck equation gave a more negative potential that that given by the constant field equation, as shown in Fig. 7. This deviation is to be expected at the lower activities because of the great difference in ionic strength between the solutions on either side of the membrane. As Planck's equation gives a more negative resting potential than that predicted by the constant field equation, at low internal activity, it is unable to predict the experimental results obtained by Baker et al. (1964).

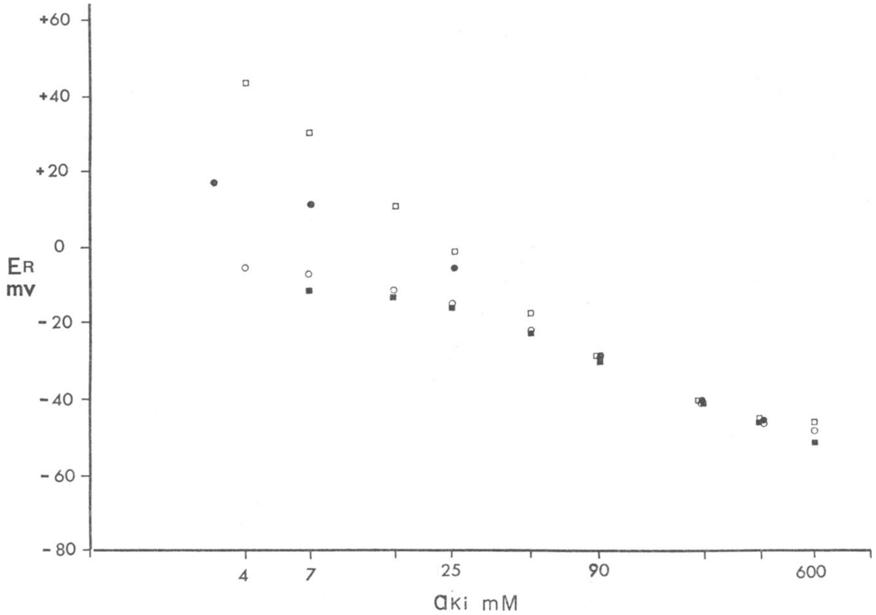

FIGURE 7 Comparison of the predictions of the constant field equation, with and without double-layer potential correction, and the predictions of Planck's diffusion equation, when the internal potassium chloride is diluted. $(a_K)_E = 13.6$ mM, $(a_{Na})_E = 308$ mM, $(a_{Cl})_E = 308$ mM. Abscissa, potassium activity, $(a_K)_i$. Ordinate, resting potential, E_R. Open circles, resting potential change predicted by the constant field equation with $P_{Cl}/P_K = 0.10$, $P_{Na}/P_K = 0.05$. Open squares, predictions of the constant field equation with $P_{Cl}/P_K = 0.18$, $P_{Na}/P_K = 0.05$ corrected for double-layer potential shift with fixed charge of $-2.2\ \mu$ coul/cm^2. Filled circles, experimental results of Baker et al. (1964). Filled squares, predictions of Planck's diffusion equation, with $\mu_{Cl}/\mu_K = 0.10$, $\mu_{Na}/\mu_K = 0.05$.

The Independence Principle and the Double-Layer Potential

Hodgkin and Huxley (1952 *a*) derived a relation between the current carried by an ion species through the membrane at a particular membrane potential, and the current carried by the same ion species when the activity of the ion was altered on one side of the membrane. This relation was derived on the assumption that the currents carried by the ion in both directions across the membrane were independent. This relation predicts, that for a change in sodium activity outside the membrane,

$$I'_{\mathrm{Na}}/I_{\mathrm{Na}} = [\{(a_{\mathrm{Na}})_E'/(a_{\mathrm{Na}})_E\} \cdot \{\exp{(V - E'_{\mathrm{Na}})F/RT} - 1\}/\{\exp{(V - E_{\mathrm{Na}})F/RT} - 1\}] \quad (7)$$

where I_{Na} is the sodium current, E_{Na} the sodium electrode potential, and the values after the change in sodium activity are labeled with a prime. Frankenhaeuser (1960) has pointed out that the independence principle also applies to the constant field equation, and that relation 7 was used by Hodgkin and Huxley (1952 *a*) because it incorporates instantaneous rectification of the sodium current, which is revealed in the squid axon membrane on changing the sodium activity.

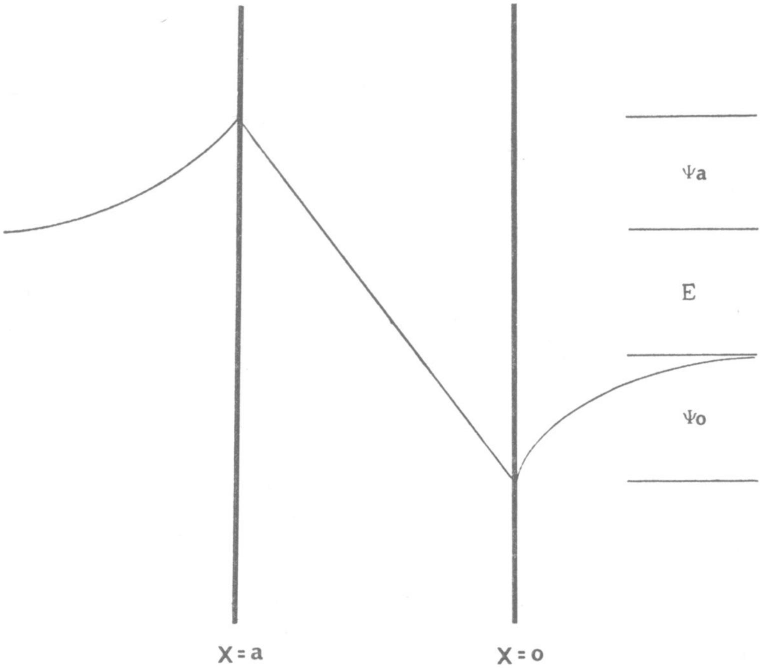

Figure 8 Double-layer potential at the outside of the membrane, $x = a$, and at the inside $x = 0$, for a distribution of positive charge on the outside and negative charge on the inside of the membrane.

If there are fixed charges distributed somewhere on the membrane, relation 7 need only be modified when the change in sodium activity involves a change in ionic strength of the solution near the fixed charges (Frankenhaeuser, 1960). For the most general case of a fixed-charge distribution on both sides of the membrane, it is convenient to derive a relation similar to 7 from the constant field assumption.

This assumption gives the sodium current (Hodgkin and Katz, 1949) as

$$I_{Na} = [(P_{Na}F^2V/RT)\{(a_{Na})_i - (a_{Na})_E \cdot \exp(-VF/RT)\}/\{1 - \exp(-VF/RT)\}] \quad (8)$$

where the sodium activities (a_{Na}) are those in the bulk of the solution. If it is assumed that there is a positive charge distribution on the outside of the membrane,

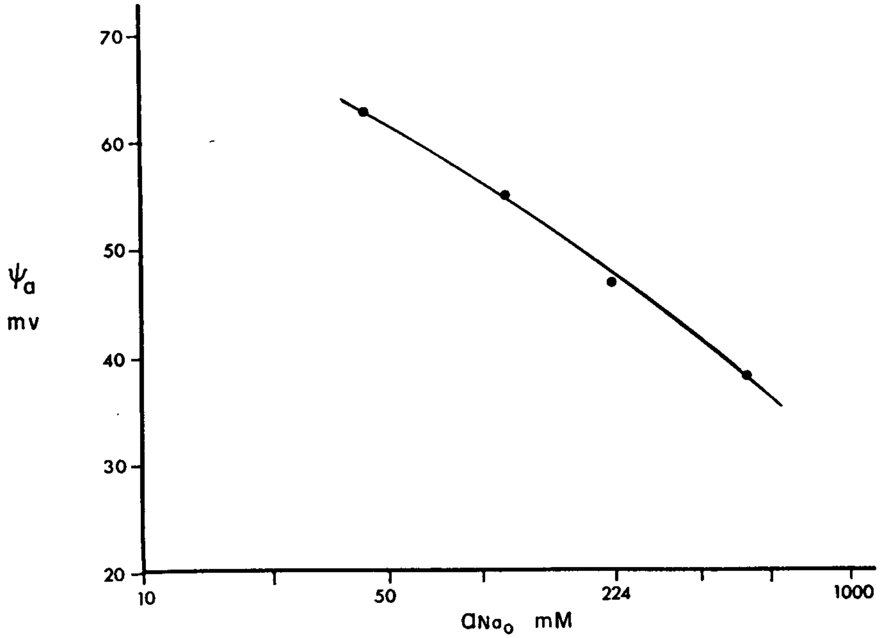

FIGURE 9 Theoretical shift of the double-layer potential at the outside of the membrane due to a decrease in the ionic strength of the external solution. Abscissa, external sodium activity $(a_{Na})_E$. Ordinate, double-layer potential calculated on the basis of a positive charge on the outside of the membrane of +6.7 μ coul/cm². The activity was estimated from the values tabulated by Robinson and Stokes (1959) for sodium chloride and from the Debye-Hückel theory.

and a negative charge distribution on the inside, then the double-layer potentials outside ψ_a and inside ψ_0 will be as shown in Fig. 8. When equation 8 is modified to incorporate the double-layer potentials due to the membrane charge, then

$$I_{Na} = [\{P_{Na}F^2(E + \psi_0 - \psi_a')/RT\}\{(a_{Na})_E \exp(-\psi_0F/RT) - (a_{Na})_E \exp(-\psi_aF/RT) \exp(-E - \psi_0 + \psi_a)/RT\}/\{1 - \exp(-E - \psi_0 + \psi_a)F/RT\}] \quad (9)$$

$$P_{Na} = [I_{Na}RT \exp(\psi_aF/RT)\{\exp(E + \psi_0 - \psi_a)F/RT - 1\}/\{F^2(E + \psi_0 - \psi_a)(a_{Na})_E\}\{\exp(E - E_{Na})F/RT - 1\}] \quad (10)$$

The total double-layer potential, $\psi = \psi_0 - \psi_a$, changes to $\psi' = \psi_0 - \psi_a'$, if there is a change in the external sodium activity together with a change in ionic strength, and equation 10 gives

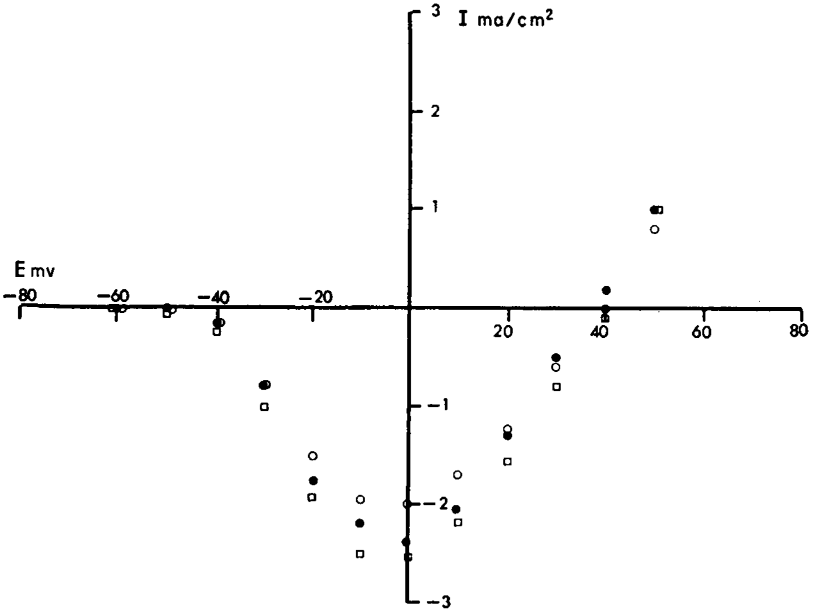

FIGURE 10 Comparison of the predictions of the independence principle with and without double-layer potential correction, when the external sodium chloride is diluted to one-half. Abscissa, membrane potential during a voltage-clamp. Ordinate, sodium current during a voltage-clamp. Open squares are the predictions of sodium current from application of the independence principle to the sodium current in normal solution. Open circles are the predictions of the sodium current from the independence principle corrected for the shift in double-layer potential, with $\sigma_0 = +6.7\ \mu$ coul/cm². Filled circles give the sodium current as determined in the experiments of Adelman and Taylor (1964).

$$I'_{Na}/I_{Na} = [\{\exp(\psi - \psi')F/RT\}\{(E - \psi')/(E - \psi)\}\{(a_{Na})_E'/(a_{Na})_E\} \cdot \{\exp(E - E'_{Na})F/RT - 1\}\{\exp(E - \psi)F/RT - 1\}/ \{\exp(E - E_{Na})F/RT - 1\}\{\exp(E - \psi')F/RT - 1\} \quad (11)$$

where the values labeled with a prime occur after the change in sodium activity.

If there were no change of ionic strength with the change in sodium activity, $\psi = \psi'$, and equation 11 would revert to equation 7. The predictions of the independence principle with or without the presence of a double-layer are the same, if the ionic strength of the solution remains the same.

The lack of instantaneous rectification of sodium current through the squid axon membrane in normal ionic solutions may be explained by assuming that there is a distribution of negative charges about the sodium channel on the inside of the membrane and of positive charges about the channel on the outside (Frankenhaeuser, 1960). There is no instantaneous rectification when the sodium activities are the same on each side of the membrane (equation 9).

If it is assumed that the charge about the sodium channel on the inside of the membrane is -2.2 μ coul/cm^2, then the double-layer potential at the inside of the membrane (ψ_0) in normal solution is about -19 mv. As the sodium electrode potential is about 56 mv (Brinley, 1965), the double-layer potential at the outside of the membrane (ψ_a) is $+38$ mv. If this potential is inserted into equation 1, with a total electrolyte concentration outside the membrane of 500 mM, the charge distribution about the sodium channel on the outside of the membrane, σ_0, is $+6.7$ μ coul/cm^2.

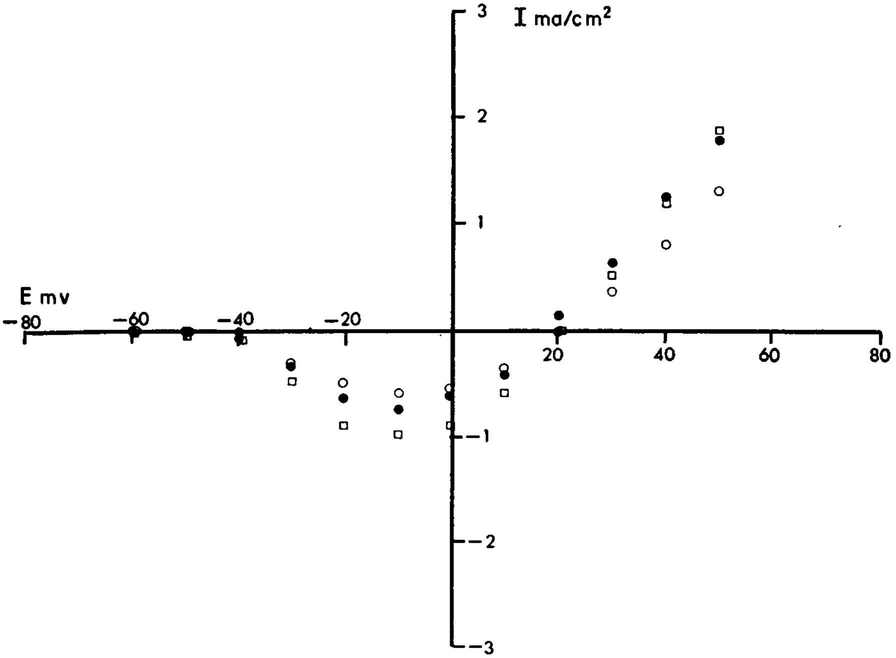

FIGURE 11 Comparison of the predictions of the independence principle with and without double-layer potential correction, when the external sodium chloride is diluted to one-quarter. Abscissa, membrane potential during a voltage-clamp. Ordinate, sodium current during a voltage-clamp. Open squares are the predictions of sodium current from application of the independence principle to the sodium current in normal solutions. Open circles are the predictions of the sodium current from the independence principle corrected for the shift in double-layer potential, with $\sigma_0 = 6.7$ μ coul/cm^2. Filled circles give the sodium current as determined in the experiments of Adelman and Taylor (1964).

If the external sodium activity is decreased by replacing sodium chloride with sucrose, the double-layer potential will increase by 25 mv/tenfold decrease in sodium activity (Fig. 9), modifying the predictions of the independence principle. The predictions of this principle, with and without correction for the double-layer potential, have been applied to the experimental results of Adelman and Taylor (1964). These authors replaced the external sodium chloride with sucrose, and measured the sodium current across the membrane at different membrane potentials. These results are compared with the predictions of the independence principle, with and without correction for the double-layer potential, in Figs. 10 and 11.

The predictions deviate at the most by 0.5 ma/cm^2, and there is good agreement between the predictions of the corrected independence principle and the experimental results. However, it is not possible to accept this correlation without reservation, as there was a considerable shift with time in the I_{Na}/V relation observed by Adelman and Taylor (1964). Further experiments are required to prove the existence of a positive charge on the membrane surface.

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