EFFECTS OF REPLACEMENT OF EXTERNAL SODIUM CHLORIDE WITH SUCROSE ON MEMBRANE CURRENTS OF THE SQUID GIANT AXON

WILLIAM J. ADELMAN, JR., *and* ROBERT E. TAYLOR
From the National Institutes of Health, Bethesda. Dr. Adelman's present address is the Department of Physiology, University of Maryland Medical School, Baltimore

ABSTRACT It was observed that a reduction of the sodium chloride concentration in the external solution bathing a squid giant axon by replacement with sucrose resulted in marked decreases in the peak inward and steady-state outward currents through the axon membrane following a step decrease in membrane potential. These effects are quantitatively acounted for by the increase in series resistance resulting from the decreased conductivity of the sea water and the assumption that the sodium current obeys a relation of the form $I = k_1C_1 - k_2C_2$ where C_1, C_2 are internal and external ion activities and k_1, k_2 are independent of concentration. It is concluded that the potassium ion current is independent of the sodium concentration. That the inward current is carried by sodium ions has been confirmed. The electrical potential (or barrier height) profile in the membrane which drives sodium ions appears to be independent of sodium ion concentration or current. A specific effect of the sucrose on hyperpolarizing currents was observed and noted but not investigated in detail.

INTRODUCTION

Strong evidence for the view that the transient inward current which occurs during a step reduction of membrane potential of the squid giant axon is carried by sodium ions was presented by Hodgkin, Huxley, and Katz (1949), and Hodgkin and Huxley (1952*a*) as a result of experiments in which the external sodium concentration was reduced with substitution of choline ions. In the course of voltage clamp experiments on the nature of the squid axon membrane leakage currents (Adelman and Taylor, 1961) it has been noted that the effects of substituting sucrose for external sodium chloride were different from those using choline chloride substitution. The effects of low sodium on the short time leakage current rectification and the peak transient current were similar, but marked differences in the steady-state outward current curves occurred. In order to examine these effects in more detail we have obtained a number of complete voltage clamp series for a graded series of external sodium concentrations using sucrose replacement. We have tested the hypothesis that all of the effects seen were the result of changes in the series resistance (Hodgkin, Huxley, and Katz, 1952) and changes in the sodium current as predicted by the "independence principle," (Hodgkin and Huxley, 1952*a*).

Giant axons were obtained from the mantle nerves of squid *(Loligo pealii)* and cleaned of surrounding small fibers and loose connective tissue. The experiments were done utilizing the point control system of voltage clamping (for review of method see Moore and Cole, 1963). Corrections for liquid junction potentials in the microelectrode tip were made where applicable (Cole and Moore, 1960*a*). The standard artificial sea water (ASW) used is described elsewhere (Kishimoto and Adelman, 1964). The temperature of the solutions were maintained at 10±1°C. The sucrose used to substitute for sodium chloride in the external medium was not repurified, but recoverability of axons exposed to the solutions employed indicates that no heavy metal poisoning was occurring.

Experiments were performed on a number of axons and the results were similar. The data presented here are all from one axon (61-LR-10) and are taken in the order (*a*) ASW, (*b*) $\frac{1}{2}$ NaCl, (*c*) ASW, (*d*) $\frac{1}{4}$ NaCl, (*e*) ASW, (*f*) $\frac{1}{10}$ Nacl, (*g*) ASW.

The membrane currents in the voltage clamp were obtained during a test pulse which followed a hyperpolarizing prepulse of 8 msec. duration. The effect of this prepulse was to set the initial conditions to a standard value by removing resting sodium inactivation and reducing the initial potassium conductance (Hodgkin and Huxley, 1952*b;* Taylor, 1959) and to delay the onset of the delayed outward current (Cole and Moore, 1960*b*). A prepulse, instead of a steady hyperpolarization, was employed because of the possible cumulative effects of such steady polarization on the membrane (Adelman and Fok, 1964; Ehrenstein and Gilbert, 1964) and to minimize polarization of the internal platinum wire.

EXPERIMENTAL RESULTS

As shown in Figs. 1 and 3, both the delayed outward current (at 5 msec.) and the initial, transient inward current were reduced for depolarizing test pulses when some of the sodium chloride was replaced by sucrose. As expected the resting potential increased somewhat on reduction of external sodium.

We shall not present detailed results of the effect of sucrose substitution on the currents during hyperpolarizing test pulses. Qualitatively there is a kind of reversible breakdown for hyperpolarizing pulses up to about 200 mv above the normal resting potential which is difficult to describe simply. In addition to the very short time rectification (Adelman and Taylor, 1961) there are further changes in the current during a constant step potential change with time constants in the order of milliseconds, seconds, and minutes (Adelman and Taylor, 1962; Ehrenstein and Gilbert, 1964). With substitution of sucrose for 90 per cent of the sodium chloride the process with time constant of less than 100 μsec. appears to be unchanged while there is a marked reduction in the delayed leakage current with time constant in the order of milliseconds.

INTERPRETATION OF RESULTS

Delayed Outward Current. Previous work strongly supports the conclusion that the outward current at 5 msec. following a depolarizing pulse contains only a small component carried by sodium or chloride ions. An irregularity in the outward current in normal ASW is visible on the curves of Fig. 1, between values of E_p of -10 to -40 which we take to be due to the steady-state sodium current. We shall not consider it further. The most obvious possibility for the reduction of the outward current is the presence of a series resistance external to the membrane (Hodgkin, Huxley, and Katz, 1952; Frankenhaeuser and Hodgkin, 1956; Villegas and Villegas, 1960*a, b*) which has a value which is proportional to the resistivity of the solutions employed. Measurements of solutions of the compositions used gave ratios of resistivities for $\frac{1}{4}$ NaCl, $\frac{1}{2}$ Nacl, and $\frac{1}{10}$ NaCl to ASW of 2.06, 3.59, and 5.55. The correction for the series resistance, r_s, is simply to compute the actual membrane potential, E_p, from the relation $E_p' = E_p - I_{ss}r_s$. This is what is ordinarily called a load line correction and will be valid provided that the system is truly in a steady-state, regardless of whether it is a linear system or not (see Taylor, Moore, and Cole, 1960).

It was found that assuming a value for the series resistance in ASW of 3 ohm cm^2 and in $\frac{1}{2}$ NaCl of 6.18 ohm cm^2 could account for the effect of the $\frac{1}{2}$ NaCl solution on the delayed outward current. On the basis of these assumptions, load line corrections for series resistance of 3, 6.18, 10.77, and 16.65 ohm cm^2 for ASW, $\frac{1}{2}$, $\frac{1}{4}$, and $\frac{1}{10}$ NaCl are shown in Fig. 2. A value of r_s for the axon in ASW slightly greater than 3 ohm cm^2 would perhaps increase the over-all fit of the corrected curves for the delayed outward current, but it is considered that this would be an entirely arbitrary procedure. The results as they stand indicate that the predominate effect of the low NaCl solutions is explained by the increase of the series resistance. Whether or not the discrepancy in the $\frac{1}{10}$ NaCl solution is the result of an increase in the series resistance with time is not deducible from these experiments. Alternative possibilities are that the delayed outward current does include a chloride component or that the sucrose has an effect on the membrane to reduce the currents directly. Previous work using choline chloride as a substitute for NaCl (Hodgkin and Huxley, 1952*a*; this laboratory, unpublished) did result in a reduction of outward current, but as these experiments were not conducted with hyperpolarizing prepulses or holding potentials, it is not possible to say whether or not the increase in series resistance with choline could account for the effect.

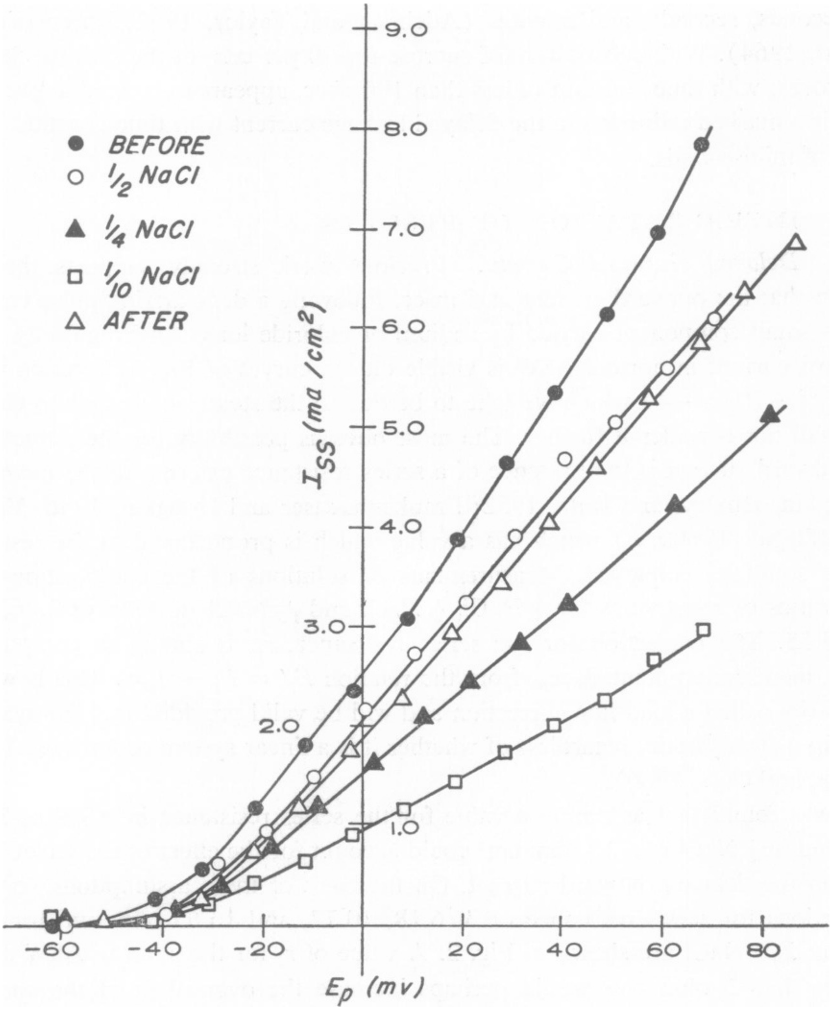

FIGURE 1 Outward curent at 5 msec. following depolarizing step to E_p. This step was preceded by an 8 msec. hyperpolarizing step of 55 mv. Effect of replacement of external sodium chloride with sucrose as indicated. Curves in artificial sea water (ASW) were taken between each run in low sodium chloride but only the first and last are shown. Resting potentials were: ASW, —61 mv.; ½ NaCl, —59 mv.; ASW, —57 mv.; ¼ NaCl, —58 mv.; ASW, —55 mv.; 1/10 NaCl, —61 mv.; ASW, —52 mv.

Peak Transient Current. A number of investigations (see Hodgkin, Huxley, and Katz, 1949, 1952; Adelman and Moore, 1961; and Moore and Adelman, 1961) support the conclusion that the sign of the peak transient current reverses at a value close to that expected from the calculation of the sodium equilibrium potential on the basis of the Nernst relation. We shall assume that the initial transient

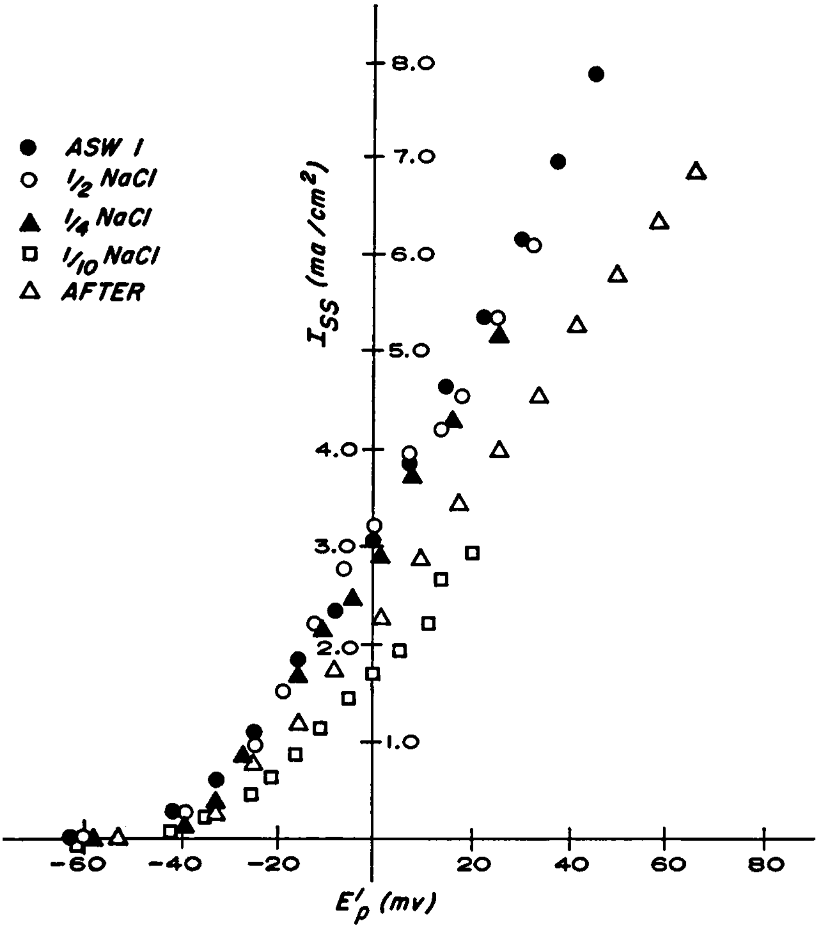

FIGURE 2 Data as for Fig. 1 but corrected for series resistance as described in text. $E'_p = E_p - I_{ss}r_s$.

current is carried by sodium ions. This is not to say that all of the current at the time of the transient peak is sodium. We take current at this time, for a potential step to the empirically determined sodium equilibrium potential, to be the leakage. The points for the leakage shown in Fig. 5, thus determined at various sodium equilibrium potentials do not lie on a straight line (Adelman and Taylor, 1961). It is thus necessary, in order to obtain the best estimate of the sodium current *versus* E_p at the time of the transient peak, to correct for the leakage and for the series resistance.

The total current, I_p, at the time of the transient peak, *versus* the potential during the step, E_p, is shown in Fig. 3. Lowering NaCl by substitution of sucrose produces

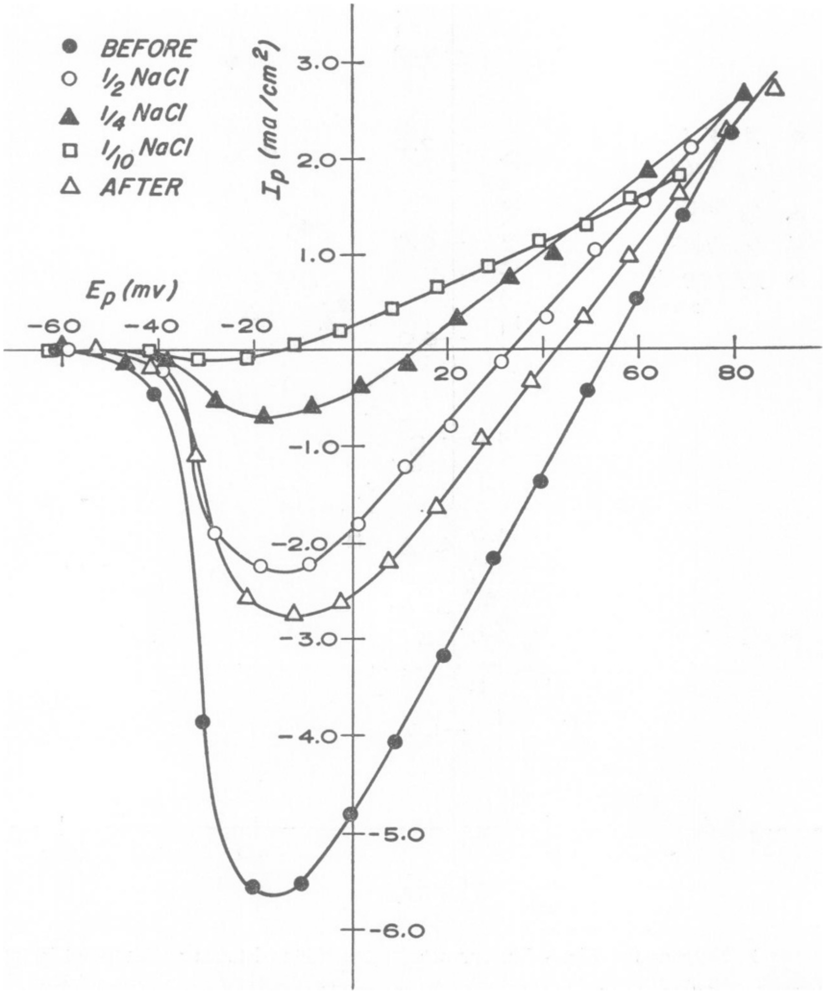

FIGURE 3 Peak transient current during same potential steps as for Fig. 1.

a change in the sodium equilibrium potential and decreases the slope of the linear portion of the current potential curve.

We are assuming that the leakage current does not change in the low sodium solutions. This is likely to be true because most of the leakage current is surely carried by potassium ions. There is no effect on the leakage as measured this way when external sodium is replaced by choline or when the external chloride is replaced by metyl sulfate (Adelman and Taylor, 1961). The small increase in the resting potential of about 5 to 8 mv on replacement of the sodium chloride by sucrose is consistent with a very low permeability of the axon membrane to chloride ions and a leakage conductance to potassium ions about ten times that to sodium ions. We

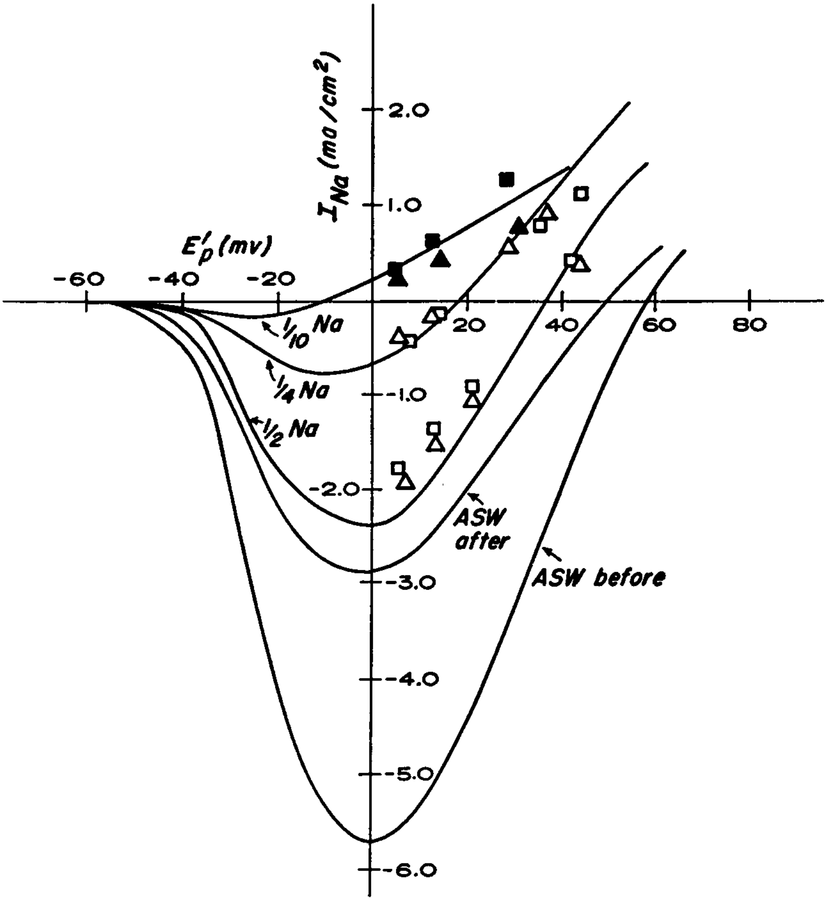

FIGURE 4 Solid line: Data as for Fig. 3 but corrected for series resistance and leakage. Open triangles and squares for ½ Na: Independence principle prediction of current in ½ NaCl from ASW curves before and after, respectively. Open squares and triangles for ¼ Na: Predicted currents for ¼ NaCl. Filled triangles and squares: Predicted currents for 1/10 NaCl. Only first and last ASW curves are shown.

therefore corrected the data shown in Fig. 3 for series resistance and subtracted the leakage as shown in Fig. 5. The results are shown in Fig. 4. We feel that these curves represent a reasonably accurate estimate of the actual transient sodium current as a function of step potential. Caution must be exercised in the application of a series resistance correction for the transient data. As has been emphasized before (Taylor, Moore, and Cole, 1960), the points for the current bear no immediate relation to one another. The form of the empirical equations given by Hodgkin and Huxley (1952*c*) lead to the prediction that if the sodium conductance is zero at the end of the prepulse, then the peak transient sodium current during a potential step will

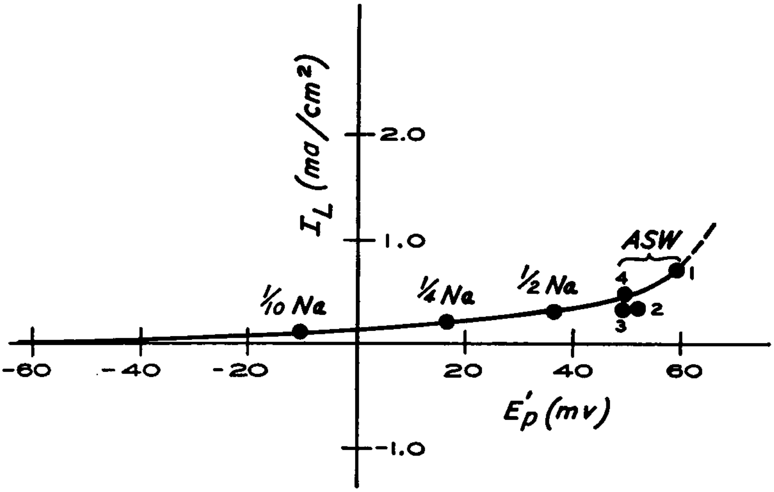

Figure 5 Leakage current, taken to be the value of the current at the time of the peak transient current for a depolarizing step to the sodium equilibrium potential in normal, ½, ¼, and 1/10 sodium concentration.

be proportional to the ratio of the time constants for the off and on processes at that potential during the step. However, with a series resistance present, the potential across the membrane itself is a function of time. Only if the off and on time constants remain almost proportional to each other over a range of potential will the peak current potential curve be nearly a straight line. As can be seen in Fig. 4 after correction for leakage the curves are no longer straight. We see no reason, from our data, why this is not a valid result. Indeed, it is from data such as these that the ratio of the time constants must be derived if we are not to reverse our logic.

The series resistance correction for the remaining linear portion of the current-voltage curve is expected to be reliable, particularly in view of the result of such corrections made for an added series resistance shown in Taylor, Moore, and Cole (1960, Fig. 26).

Independence Principle Predictions. It is difficult to imagine a physical system in which the conductance at a given potential was independent of the concentration of the charge carrier. In order to make any predictions as to the nature of the dependence it is necessary to know or assume something about the nature of the system. In the present case we are assuming at the outset that the sodium concentration does not change the physical structure of the membrane. In the range of concentrations with which we are dealing it is reasonable to expect that the concentration of sodium near the membrane will be the same as in the bulk solution and will not be significantly altered by the current flow. This would probably not be true for concentrations in order of 1mM. Since the diffusion coefficient of NaCl in water is about 1.5×10^{-5} cm^2 sec.,$^{-1}$ and the value of the Farady is 96,500 coul. per mole it requires a concentration gradient of about 0.7mM/cm for diffusion alone to supply enough ions for a current of 1.0 ma/cm^2. If we proceed on the assumption that the concentration changes with current flow *within* the membrane are also unimportant, one might expect the dependence of the membrane on concentration to be given by the independence principle relations as presented by Hodgkin and Huxley (1952). For any system in which a modification of Fick's Law in the form

$$I = k_1(E)C_1 - k_2(E)C_2$$

holds, where C_1 and C_2 are concentrations on either side of the membrane, of the ion carrying the current I, k_1, and k_2 are not functions of concentration, and E is the potential across the membrane, the equations relating current to concentration as derived by Hodgkin and Huxley (1952*a*), will apply. For our purposes we shall rearrange their expression to obtain

$$\frac{I_p}{I_p'} = \frac{\exp\{(E_{Na} - E_p)F/RT\} - 1}{\exp\{(E_{Na} - E_p')F/RT\} - 1}$$

for the ratio of the currents at any given membrane potential E'_p for a concentration of Na ions in ASW giving a sodium equilibrium potential of E_{Na} and in low sodium solution giving E_{Na}'. Using the measured values of E_{Na} rather than the calculated ones should reduce uncertainties from the fact that the activity coefficient of sodium will change due to the change in ionic strength.

In any event, we compared the predictions of the above relation with the values for sodium current obtained after corrections for series resistance and leakage current had been made. The results are shown in Fig. 4. The accuracy of the prediction is not great near either value of E_{Na} and the series resistance correction is certainly not valid for the non-linear portions of the current curves. We have therefore only plotted those points which we would expect to be reasonably accurate. It can be seen that the agreement between predicted and observed values is as good as one has any reasonable right to expect. It would appear that in spite of possible weak points in the development of the arguments for these corrections and the independence principle predictions, the results do indeed give no indications of failure.

DISCUSSION

Steady-State Currents. From the results presented here we conclude that the steady-state current does not depend upon external sodium or chloride ion concentration except for a small deviation observable in the curves of Fig. 1 and the predictable effects of changes in the conductivity of the external solution. This is a satisfactory result and was used as an approximation by Hodgkin and Huxley in their method of separating the total current into sodium and potassium components (Hodgkin and Huxley, 1952*a*, p. 457). It was not possible for them to determine this point accurately because of uncertainties introduced by the change in resting potential with low sodium, unknown series resistance and possible electrode polarization effects. This kind of independence between the movements of sodium and potassium ions gives support to the suggestion that sodium and potassium ions cross the membrane at different places. Whether or not the potassium ions are independent of each other in the sense that the sodium ions are (see next section) remains to be determined.

The current at 4 msec. following a step depolarization is only a quasi steady-state current. Following decrease in current partly attributable to the accumulation of potassium in the Schwann cell region (Frankenhaueser and Hodgkin, 1956), the current continues to drop with maintained depolarization for some time (Ehrenstein and Gilbert, 1964). It has further been observed by these workers and by Armstrong and Binstock (personal communication) that the depolarization resulting from increased external potassium results in a reduction not only of the potassium conductance, but also of the sodium conductance as well. It is therefore of considerable importance that comparisons between experiments with alterations in ionic environment can only be made if the axons have been subjected to the same membrane potentials for times of minutes.

Peak Inward Current. The correspondence of the measured peak inward current with that predicted above strongly supports the view that this current is indeed carried by sodium ions and that a relation of the form $I = k_1C_1 - k_2C_2$ holds with k_1, k_2 independent of concentration.

One way to discuss the implications of this result is to consider that the ions pass through a membrane separating identical solvents by surmounting a series of potential barriers. With the use of absolute reaction rate theory (See Johnson, Eyring, and Polissar, 1954, p. 754) we may derive a current-voltage relation for a single ion species of the form

$$I = (FD/s)\,\frac{C_1 - C_2 \exp\,(V_m F/RT)}{(1/N)\,\sum_{n=1}^{N} \exp\,(V_n F/RT)}$$

where I is the current in amperes, F the Faraday, D a diffusion coefficient, s the distance across which the total potential V_m is measured, C_1 and C_2 the concentrations in the solutions outside the membrane, R the gas constant, T the absolute temperature, and N the number of barriers. We say that the solution on one side of the membrane is at ground potential and at a potential V_m in the solution on the other side. Then V_n is the height of the nth barrier, in volts, measured from ground and under the conditions as set by the total situation, including the applied potential, space charge effects, etc. Under these conditions this relation holds for the steady-state in a quite general way. In this case the steady-state in question is the establishment of concentrations between barriers and the effects of space charge, etc., on barrier heights. These processes are likely to be fast enough that this steady-state is present at the time of the peak inward current during a depolarizing pulse in spite of the fact that the membrane current is changing with time.

The preceding current-voltage relation is not likely to be of much value in considerations of the potential dependence of the current at long times, but we are here considering the concentration dependence. We have seen that the peak inward current depends on the external sodium concentration in a manner which is satisfactorily predicted by the current being proportional to $(C_1 - C_2 \exp (V_m F/RT))$. The remaining factors in the expression given above must then be largely independent of sodium ion concentration. In particular, the magnitudes of the potential barriers, at a given potential across the membrane, must then be independent of concentration and current as well as of the coefficient D. If the potential barriers are close enough together that the V_n may be represented by a continuous function of distance, then this expression reduces to a partial solution of the diffusion equation. If this continuous function is linear, then the constant field equation of Goldman (1943; Hodgkin and Katz, 1949) results. The reason that the constant field equations satisfy the independence principle as we have used it is not because of the constant field but because by definition a constant field cannot be concentration dependent.

We conclude that the potential profile through the membrane, whether expressed in terms of a continuous function of distance or a set of discrete barriers, is not greatly influenced by concentration changes. Any model proposed for the membrane structure must have this property. A single file long pore with all positions filled as discussed by Hodgkin and Keynes (1955) would certainly be expected to have this property.

In order to avoid confusion we mention that the considerations which we have applied here pertain only to a system with sufficient ion content that the local concentration near the membrane is essentially independent of current flow. In the course of experiments using radioactive tracers (Mullins, Adelman, and Sjodin, 1962) we had occasion to observe the currents during depolarizing steps using an axon which had been soaked in sodium-free solution overnight. The usual sort of inward transient currents occurred but no sign of outward transient current for large depolarizations. The sodium equilibrium potential was not measurable. The same effect was reported by Adelman and Gilbert (1963) and Adelman and Fok (1964) on voltage clamping internally perfused axons with artificial axoplasm containing no sodium ions. This result is to be expected. The small number of sodium ions near the inside of the membrane can easily be swept out and the potential at which no sodium current would flow becomes current- and time-dependent.

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