The Effects of External Potassium and Long Duration Voltage Conditioning on the Amplitude of Sodium Currents in the Giant Axon of the Squid, *Loligo pealei*

WILLIAM J. ADELMAN, JR., and YORAM PALTI

From the Department of Physiology, The University of Maryland School of Medicine, Baltimore, Maryland 21201, and the Marine Biological Laboratory, Woods Hole, Massachusetts 02543. Dr. Palti's permanent address is the Department of Physiology, The Hebrew University, Hadassah Medical School, Jerusalem, Israel

ABSTRACT Giant axons were voltage-clamped in solutions of constant sodium concentration (230 mM) and variable potassium concentrations (from 0 to 210 mM). The values of the peak initial transient current, I_p, were measured as a function of conditioning prepulse duration over the range from less than 1 msec to over 3 min. Prepulse amplitudes were varied from $E_m = -20$ mv to $E_m = -160$ mv. The attenuation of the I_p values in high $[K_o]$ was found to vary as a function of time when long duration conditioning potentials were applied. In both high and low $[K_o]$, I_p values which had reached a quasi-steady-state level within a few milliseconds following a few milliseconds of hyperpolarization were found to increase following longer hyperpolarization. A second plateau was reached with a time constant of about 100-500 msec and a third with a time constant in the range of 30 to 200 sec. The intermediate quasi-steady-state level was absent in K-free ASW solutions. Sodium inactivation curves, normalized to $I_{p_{max}}$ values obtained at either the first or second plateaus, were significantly different in different $[K_o]$. The inactivation curves, however, tended to superpose after about 1 min of hyperpolarizing conditioning. The time courses and magnitudes of the intermediate and very slow sodium conductance restorations induced by long hyperpolarizing pulses are in agreement with those predicted from the calculated rates and magnitudes of $[K^+]$ depletion in the space between the axolemma and the Schwann layer.

INTRODUCTION

In 1952, Hodgkin and Huxley (1952 *a*) demonstrated that the inward transient current in the voltage-clamped squid giant axon is normally carried by sodium ions. Recently, it has been shown that the peak amplitude of this inward transient current, I_p, is inversely related to the external potassium con-

centration, [K_o] (Adelman and Senft, 1968; Adelman and Palti, 1969). Furthermore, external potassium was shown by Adelman and Palti (1969) to affect the time and voltage dependency of the transient sodium current. In the Hodgkin and Huxley formalism (1952 *c*), a parameter, *h*, was included to account for the phenomenon of the voltage- and time-dependent inactivation of sodium currents with membrane depolarization. Adelman and Palti (1969) found that the value of *h* reaches different steady-state levels within 30 msec in external solutions having constant sodium concentrations and variable potassium concentrations. The inhibitory effect of external potassium on the sodium current was attributed by Adelman and Palti to an effect of [K_o] on sodium conductance represented by the *h* and perhaps $\bar{g}_{Na}$ parameters.

However, Narahashi (1964) described an effect of [K_o] on the transient sodium current which has a time constant approaching the 1 sec range. Slow sodium inactivation also has been suggested by Cole (1958). The object of this paper is to describe the effects of external potassium ions on long time constant sodium conductance mechanisms. Such mechanisms have been suggested (Baker, Hodgkin, and Meves, 1964; Adelman, Dyro, and Senft, 1965 *a*, *b*) as being responsible for the long duration action potentials seen in axons perfused internally with low ionic strength media and externally with potassium-free seawater solutions (Narahashi, 1963).

METHODS

Experiments were performed upon single giant axons obtained from *Loligo pealei* at the Marine Biological Laboratory, Woods Hole, Mass. All methods have been described previously (Adelman and Palti, 1969).

Membrane currents were measured in the voltage clamp as a function of external potassium ion concentration. External sodium concentration was kept constant at 230 mM. Tris chloride was added to the solutions to maintain the osmolarity and specific conductivity approximately equal to those of the 430 mM sodium seawater (Adelman and Palti, 1969). Potassium was substituted for given fractions of the $Tris^+$ concentration to make up the experimental solutions. These solutions have been described by Adelman and Palti (1969, Table I).

All axons were voltage-clamped at holding potentials, E_{HP}, equal to the resting potential, E_{RP}, obtained for each of the test solutions. Prepulses and test pulses were made from E_{HP} and are given in the Results in absolute potential units, E_{pp} and E_p, respectively.

RESULTS

Fig. 1 illustrates the relationship between [K_o] and the efficacy of a given prepulse in overcoming resting sodium inactivation. The data shown in Fig. 1 A were obtained from a typical axon when it was externally perfused with K-free artificial seawater having a sodium concentration of 230 mM. In Fig. 1 B the same axon was externally perfused with 50 mM K, 230 mM Na ASW. In Fig.

l the peak value of the initial transient current, I_p, was obtained at the end of a prepulse of variable amplitude and duration by test pulsing to a membrane potential of zero (Hodgkin and Huxley, 1952 *b*, cf. Fig. 3). However, the thin

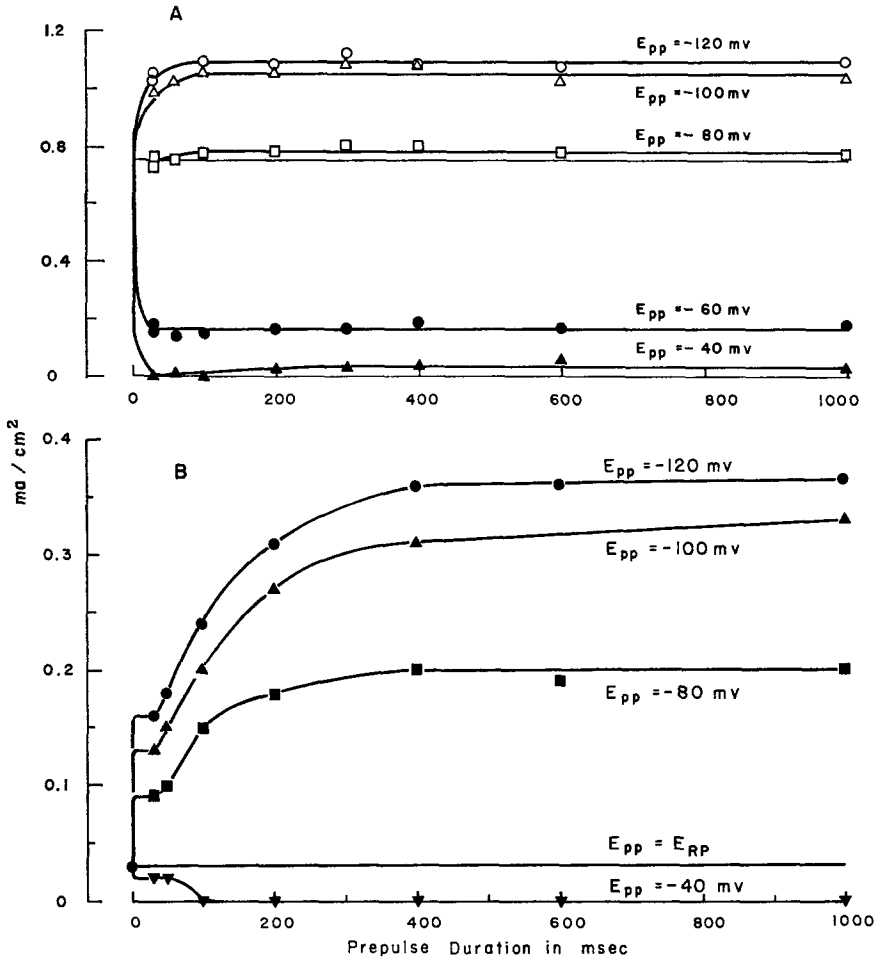

FIGURE 1. Time course of typical variations in the peak value of the initial transient membrane current, I_p, in ma/cm² as a function of the duration of a conditioning prepulse potential step. The values of the prepulse step potential, E_{pp}, are given at the right of each curve. I_p was always measured upon stepping from the prepulse potential to the test potential of zero millivolts. Axon 68–20. A, axon in K-free, 230 mM Na ASW; between pulses the membrane was held at the resting potential, $E_{HP} = E_{RP} = -78$ mv. B, axon in 50 mM K, 230 mM Na ASW, $E_{HP} = E_{RP} = -44$ mv.

line in Fig. 1 A and the line for $E_{pp} = E_{RP}$ in 1 B refer to the values of I_p obtained without prepulse conditioning. Recently, it has been shown (Adelman and Palti, 1969) that I_p values reach a steady state in a variety of K_o solutions as prepulse durations approach 30 msec. The values of I_p at the left of each

curve were obtained immediately following prepulse durations of 30 msec. The initial values given to the far left of the curves in Fig. 1 thus represent this quasi-steady state achieved for each E_{pp}. Notice that these values are much smaller for given E_{pp} values in 50 mM K (Fig. 1 B) than for comparable values of E_{pp} in zero K (Fig. 1 A).

Fig. 1 A demonstrates that for zero $[K_o]$ and any prepulse potential the values of I_p remain constant as the duration of the prepulse becomes greater than 30 msec. Such behavior is predicted by the Hodgkin and Huxley theory (1952 *c*). When external potassium is added, there is a great decrease in I_p values even following 30 msec hyperpolarizing prepulses. However, as illustrated in Fig. 1 B, there is a gradual restoration of the peak values of the sodium current as the duration of the hyperpolarizing prepulses is increased beyond 30 msec. Notice that another set of I_p plateau values is reached within 600 msec. However, the restoration of I_p values in Fig. 1 B does not result in values comparable to those obtained in K-free 230 mM Na ASW for the same E_{pp} values. It is apparent from Fig. 1 B that the time constant of this restoration process varies between 50 and 200 msec. These values are roughly 10–100 times those obtained by Hodgkin and Huxley (1952 *b*) for τ_h, and should be compared with Narahashi's values (1964).

An increase in the prepulse duration, t_{pp}, from 500 msec to 5 sec results in no significant change in the amplitude of I_p. This indicates that the restoration process has achieved a second plateau or steady state. Fig. 2 illustrates typical curves showing the relation between the peak amplitude of the initial transient current, I_p, and the conditioning voltage, E_{pp}. In Fig. 2 prepulse durations were 3 sec, and the curves are plotted for a variety of $[K_o]$.

Notice in Fig. 2 that the E_{pp} value at which $I_p/I_{p_{max}} = \frac{1}{2}$ shifts to the left as $[K_o]$ increases. This shift is roughly proportional to log $[K_o]$. Also notice that the slope of this relation is less steep for high $[K_o]$ than it is for low $[K_o]$.

Fig. 3 illustrates the effect of prepulse durations from 3 sec to 3 min on subsequent I_p values in 230 mM Na ASW solutions containing zero (A), 50 (B), and 100 mM K(C). It is apparent that the I_p values which had reached a plateau for prepulses of 1 sec duration change again as the prepulse duration is increased from 3 sec to 3 min. Fig. 3 suggests that a new plateau is established as prepulse durations approach 2 min. The thin line in Fig. 3 A is for E_{pp} = resting potential (−60 mv); i.e., I_p is obtained upon stepping the membran potential from $E_{HP} = -60$ to zero with no prepulse. Figs. 3 B and 3 C illustrate the effect of increasing $[K_o]$. The primary result is that the over-all level of sodium current is decreased when prepulses of only a few seconds are used. However, as prepulse durations are increased, restoration of I_p values is achieved.

Fig. 4 shows the absolute value of I_p following a prepulse of −120 mv plotted as a function of prepulse duration, t_{pp}. In this figure $[K_o]$ = 50 mM.

The figure demonstrates that three plateaus are obtained. Each succeeding plateau is achieved at t_{pp} values which are roughly 100-fold longer than those of the preceding plateau.

By assigning the value of unity to the I_p maxima obtained in K-free ASW for each of the three plateaus, it is possible to compare the effects of E_{pp} on the shape of these three plateaus. Fig. 5 illustrates typical plots of these relations obtained for a variety of $[K_o]$. It is apparent that the attenuated values of I_p

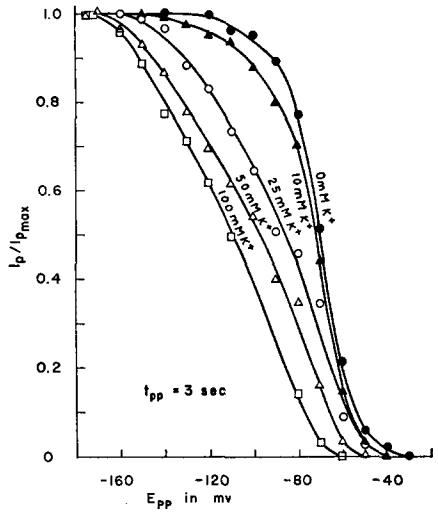

FIGURE 2. Typical relations between normalized values of the peak amplitudes of initial transient currents (initiated with a standard test pulse) plotted as a function of $[K_o]$ and the voltage of an immediately preceding 3 sec prepulse. The ordinate plots the ratio, $I_p/I_{p\max}$, where $I_{p\max}$ is set to unity for each curve obtained when $E_{pp} = -180$ mv. Values of prepulse potential, E_{pp}, are given on the abscissa, and $[K_o]$ values are indicated adjacent to the curves. The test pulse stepped from the prepulse potential to zero millivolts in each case. Axon 68–11.

obtained following 30 msec prepulses in high $[K_o]$ increase as the prepulse duration is increased. For $t_{pp} = 2$ min, the three curves, obtained in ASW solutions having zero, 50, and 100 mM K, almost superpose. However, the curves converge only for E_{pp} values of -120 mv or above. At E_{pp} values of about -80 mv they reflect quite different current values at different $[K_o]$. It is thus apparent that long duration anodic hyperpolarization tends to restore sodium conductance in high $[K_o]$ solutions. However, only the combination of very long duration and very large hyperpolarizing potentials is sufficient to completely overcome the depressant effect of high $[K_o]$.

Fig. 6 (upper trace) shows that, when an axon is externally perfused with K-free, 230 mM Na ASW, a resting potential of -76 mv is achieved (cf.

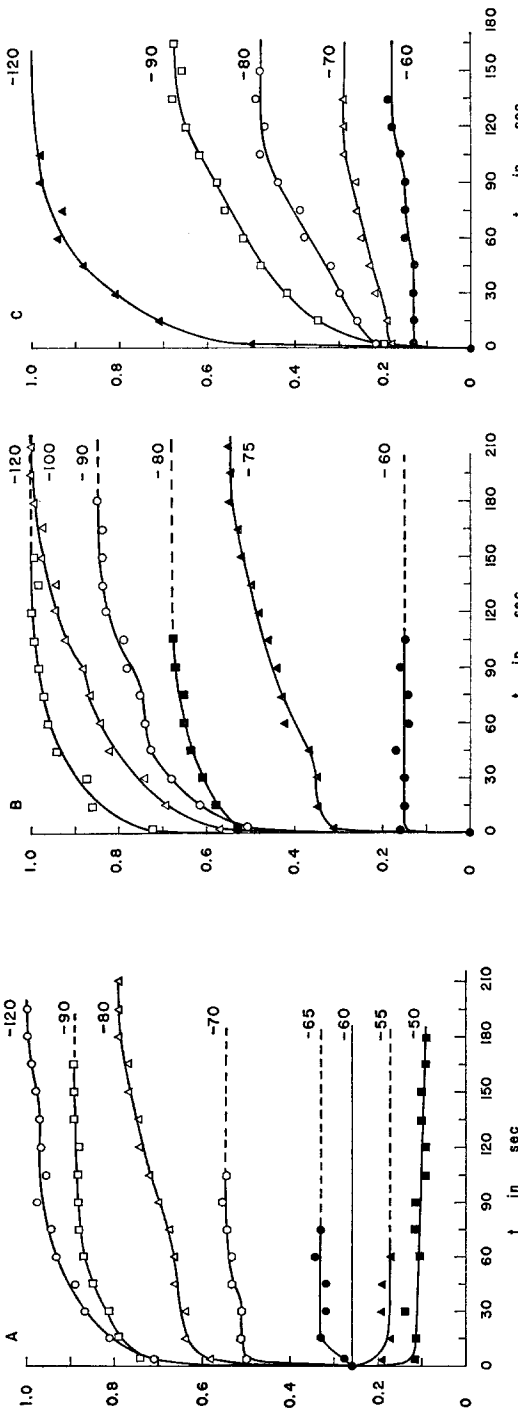

FIGURE 3. Time course of typical variations in normalized I_p (A, B, or C) was normalized so as to have the maximum I_p value obtained in each solution equal to unity. C, axon in 100 mM K, 230 mM Na ASW. Each family of curves values plotted as a function of long duration prepulse potential conditioning. Compare with Fig. 1 and see text. Axon 68–38. A, axon in K-free, 230 mM Na ASW. B, axon in 50 mM K, 230 mM Na ASW.

Goldman, 1943; Hodgkin and Katz, 1949). When the external solution is changed from K-free to 25 mM K, 230 mM Na ASW, the resting potential declines. The lower curve reflects I_p values obtained in a manner revealing some of the combined effects of membrane potential, time, and $[K_o]$. The protocol required that the axon be clamped every 30 sec to a holding potential of −76 mv for 3 sec after which a brief test pulse of zero millivolts was applied

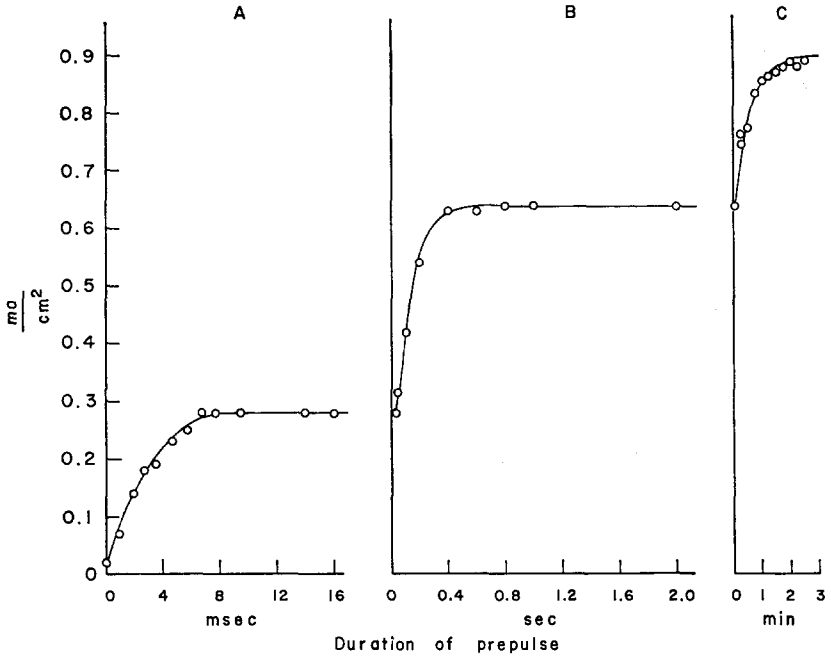

FIGURE 4. Typical I_p values in ma/cm² plotted as a function of the duration of a prepulse having a constant E_{pp} of −120 mv. Axon 68-17 exposed to 50 mM K, 230 mM Na ASW. A, prepulse durations varied between 0 and 16 msec. B, prepulse durations varied between 30 msec and 2 sec. C, prepulse durations varied between 1 sec and 3 min.

so as to determine I_p. Initially, the value of I_p obtained in K-free 230 mM Na ASW was set to one (I_{p_o}). All other subsequent I_p values were plotted as a ratio of I_p/I_{p_o}. The values of I_p/I_{p_o} obtained from a depolarized axon membrane in 25 mM K decrease despite the 3 sec repolarizing prepulses to − 76 mv. This inability of a 3 sec prepulse to completely restore I_p is predicted from the results illustrated in Fig. 5. Notice that the decline in I_p values is not a mirror image of the change in E_m (upper curve) and thus must reflect both voltage- and time-dependent effects. It is apparent that the decline in I_p values continues for a number of minutes after E_m has reached its new plateau value. This time is related to the third time constant shown in Figs. 3 and 4 C.

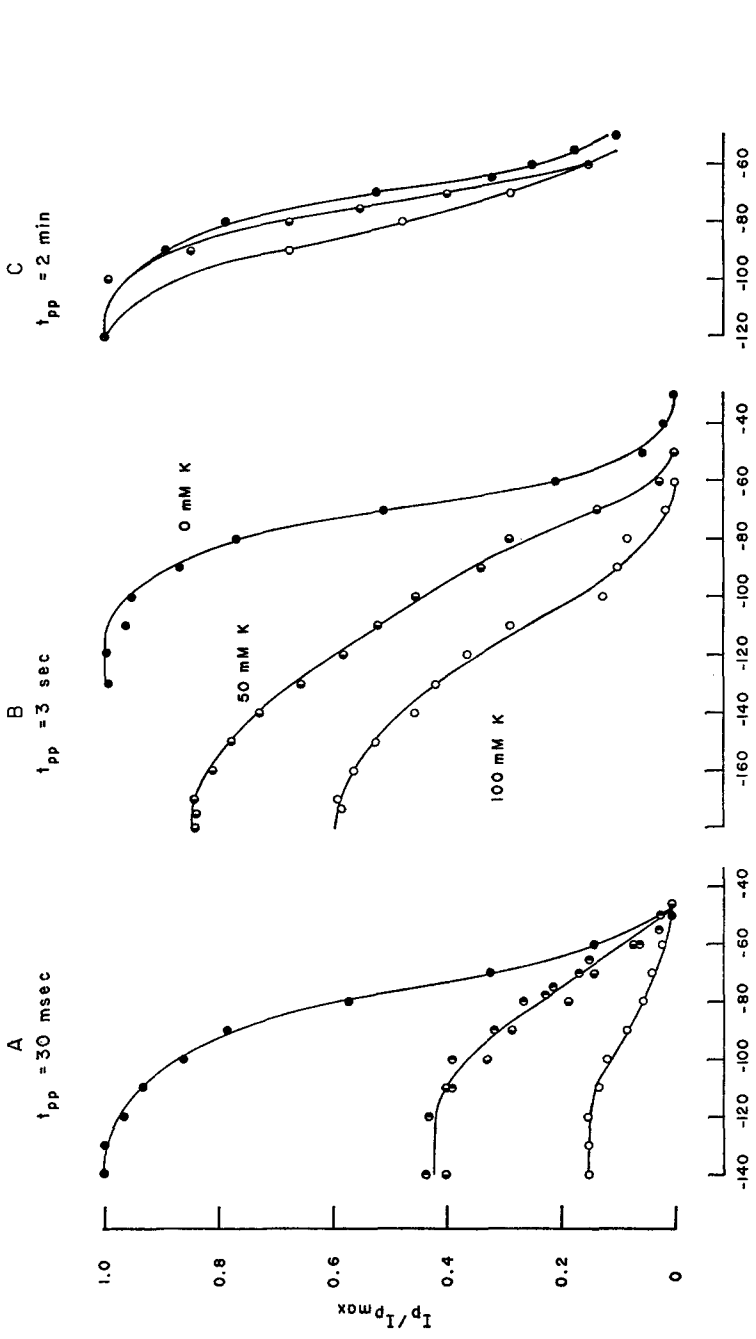

FIGURE 5. Comparison of relative effects of conditioning prepulses of three different durations, t_{pp}, on $I_p/I_{p_{max}}$ ratios plotted as a function of prepulse potential and $[K_o]$. $I_{p_{max}}$ was taken as unity for $[K_o] = 0$ and $E_{pp} \geq |-120$ mv$|$ for each prepulse duration. Filled circles, K-free, 230 mM Na ASW, half-filled circles, 50 mM K, 230 mM Na ASW, open circles, 100 mM K, 230 mM Na ASW. A, t_{pp} = 30 msec, axons 68-17 and 68-34. B, t_{pp} = 3 sec, axon 68-11. C, t_{pp} = 2 min, axon 68-38.

Even continuously voltage-clamping the membrane to −76 mv (E_m in K-free ASW) results in only partial restoration of I_p values when compared to those values obtained in K-free ASW. This partial restoration is again predicted by

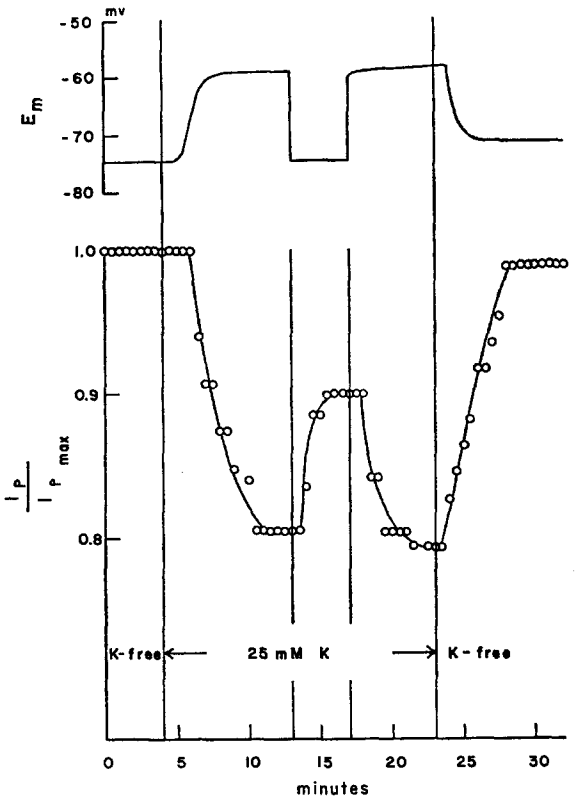

FIGURE 6. Demonstration that the effect of external potassium ion on sodium conductance is not solely a potential phenomenon. For a detailed description of the figure see text. Upper figure, membrane potential, E_m, plotted as a function of time. Lower figure, ratio, $I_p/I_{p_{max}}$, of the peak value of the initial transient current to the maximum value, $I_{p_{max}}$, obtained initially in K-free, 230 mM K, ASW, plotted against time. Axon 68-25. The two long unbroken vertical lines indicate solution changes, and the two shorter vertical lines indicate the period during which the membrane was clamped continuously at a holding potential of −76 mv (except for the brief test pulses used to determine the I_p values).

Fig. 5. The time course of the partial restoration achieved by steady potential conditioning is again similar to the time course illustrated in Figs. 3 and 4 C. The right-hand part of Fig. 6 illustrates the reversibility of the process.

DISCUSSION

It has been demonstrated that the amplitude of the initial transient sodium current initiated upon membrane depolarization is decreased when $[K_o]$ is

increased. This attenuation can be at least partly reversed by voltage conditioning of long duration. The degree of restoration is a function of the duration and the potential value of the conditioning prepulses used. Within the Hodgkin and Huxley (1952 *c*) framework, the observed changes in sodium current induced by high $[K_o]$ must be attributed either to a change in the effective membrane conductance to sodium ions, or to a change in the actual sodium driving force across the axon membrane. Let us assume, for the moment, that the changes in $[K_o]$, which do not involve significant changes in the ionic strength, E_{Na} and conductivity of the medium (Adelman and Palti, 1969),

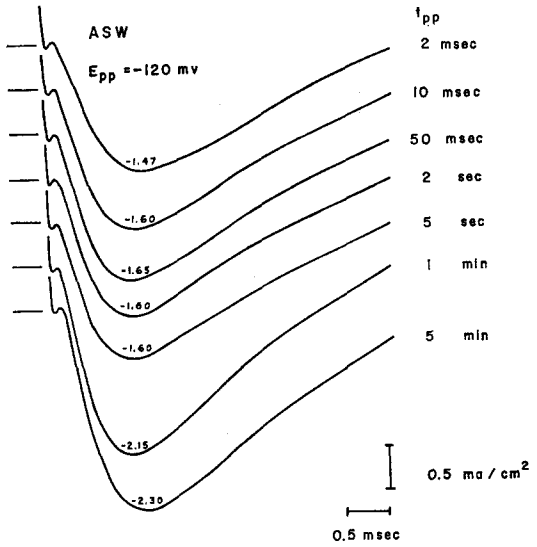

FIGURE 7. Oscilloscope traces of axon membrane currents generated upon stepping E_m to zero at the end of −120 mv conditioning prepulses of various durations, t_{pp}. Values of I_p in ma/cm² are given above the troughs of the curves. Values of t_{pp} are given to the right of each curve. Axon 68-28 in ASW.

do not introduce a considerable error in the measurement of E_m or the ability of the voltage clamp system to clamp the membrane to this E_m. The changes in membrane sodium current obtained upon voltage-clamping the axon membrane to the same E_m in different $[K_o]$ solutions must therefore be due to changes in the membrane sodium conductance, namely the $\bar{g}_{Na}$, m, or h parameters.

Obviously, the slow changes in I_p as a function of prepulses of long duration, which range from hundreds of milliseconds up to minutes, do not fall within the τ_m and τ_h ranges (<1–10 msec) considered by Hodgkin and Huxley (1952 *b*) and by Adelman and Palti (1969). Therefore, these slow changes must be regarded as resulting from some mechanisms which affect the apparent sodium conductance with completely different kinetics. Fig. 7 illustrates initial transient currents recorded from a typical axon in ASW upon stepping E_m to zero potential (E_p) at the end of a prepulse of variable duration (t_{pp} = from 2 msec to 5 min) and having an E_{pp} value of −120 mv. Fig. 7 demonstrates that while the amplitude of I_p increases with t_{pp}, as predicted

by Figs. 1 A and 3 A, the time course is hardly affected. In other words, these long duration processes do not seem to affect the voltage- and time-dependent parameters m and h as described by the Hodgkin and Huxley (1952) framework. In principle, the changes in sodium conductance described in this work should be ascribed to changes in a parameter with the same properties as $\bar{g}_{Na}$ since such changes should not result in alterations in the voltage dependency and time course of I_{Na}. However, Figs. 2 and 5 illustrate that the voltage dependency of the peak amplitude of the sodium conductance varies with long duration conditioning. Figs. 3 and 4 illustrate that conductance changes are also a function of time.

Since $\bar{g}_{Na}$ is defined within the Hodgkin-Huxley framework as being independent of voltage and time, one seems to be left with only one alternative, namely, that a new function or process should be incorporated within the equational framework to account for the observed changes in sodium conductance. In the Hodgkin-Huxley framework, sodium conductance is represented by:

$$g_{Na} = \bar{g}_{Na} \cdot m^3 \cdot h. \quad (1)$$

The new process may be described by multiplying the conductance by a time- and voltage-dependent factor, f, so that:

$$g_{Na} = f \cdot \bar{g}_{Na} \cdot m^3 \cdot h \quad (2)$$

The f factor appears to vary slowly with time in a double sigmoidal manner as reflected by the changes of I_p as a function of t_{pp} and E_{pp} shown in Figs. 3 and 4. Inasmuch as I_p as a function of t_{pp} develops three plateaus, we may readily assume that we are dealing with two processes in addition to the h process. Thus the f factor appears to represent two processes, which will be referred to as p and q. Both the new processes, p and q, rise in a sigmoidal fashion with increasing prepulse duration in a manner similar to the h process. As the p process does not affect I_p values in K-free ASW, it seems that this process specifically counteracts the inactivation effect of $[K_o]$ on sodium conductance.

Further restoration of sodium conductance begins to appear when the duration of conditioning prepulses exceeds the 1 sec range. This effect is described by the q process. This process does not seem to be closely related to $[K_o]$ as a similar activation of sodium conductance occurs in K-free ASW. The very slow q process becomes complete only when sufficiently intense hyperpolarizing prepulses in the minute range are used. One might, therefore, assume that the magnitude of the q process depends, at least in part, on the quantity of charge transported through the membrane. External potassium, therefore, seems to have a significant effect on the h and p processes, but no significant effect on the very slow q process. However, the last conclusion may

be wrong if [K] at the external surface of the axon membrane is not zero when $[K_o] = 0$. The $[K_o]$ effect on the h process has been discussed in detail by Adelman and Palti (1969). Therefore, we shall limit this discussion mainly to the p and q processes. The $[K_o]$ effect on the apparent sodium conductance may be the result of one or more different types of mechanisms of which we shall consider the following: (*a*) an inhibitory or competitive effect of potassium ions on a properly sensitive element in the sodium conductance system, (*b*) a modification of the sodium-carrying system by the persistent membrane depolarization in high $[K_o]$, (*c*) a change in E_{Na}, (*d*) a change in the actual potential difference across the membrane with no change in measured E_m, and (*e*) changes in electrode polarization, and/or electrode-solution interface impedance.

Let us now consider the first possible mechanism. The attenuation of I_{Na} by high $[K_o]$ may be the result of association of K^+ with a site which as a result of this process cannot accept Na^+ for transfer across the cell membrane (direct competitive inhibition) or the result of a reaction of K^+ with a membrane site which by an allosteric mechanism interferes with Na^+ transfer (see Adelman and Palti, 1969). It has been demonstrated in this paper that conditioning by hyperpolarizing prepulses of 100 msec or longer removes a substantial fraction of the attenuation of sodium conductance brought about by high $[K_o]$. Within this framework such a restoration process may result from depletion of the $[K_o]$ in the Frankenhaeuser-Hodgkin space.

Such a mechanism would be the inverse of the K^+ accumulation process associated with excitation and described by Frankenhaeuser and Hodgkin (1956). Indeed, by using the same model and the assumptions of Frankenhaeuser and Hodgkin (1956) the change in potassium concentration in the Schwann space, $[K_s]$, during hyperpolarization can be calculated (see Appendix). Fig. 8 plots $[K_s]$ as a function of time during a hyperpolarizing pulse of -120 mv. The figure illustrates that within 50–500 msec $[K_s]$ is reduced from 100 mM to about 30–60 mM, depending on the permeability values of the membrane and Schwann cell parameters chosen for the calculation. The calculated values of the time constants for $[K_s]$ changes are very similar to the observed time dependency of the p process. The magnitudes of the calculated (K_s) changes are also in agreement with those predicted from the experimental results shown in Fig. 5. In Fig. 5 the I_p values obtained after 3 sec of hyperpolarization in 100 mM $[K_o]$ ASW are seen to correspond roughly to I_p values that would be obtained in an ASW solution containing about 40 mM K^+ after 30 msec hyperpolarization. This finding indicates that the restorative effect of long lasting (hundreds of milliseconds) hyperpolarizing currents roughly corresponds to the effect of lowering $[K_o]$ by 60 mM. Therefore, assuming that the values of the parameters chosen for the calculation

(see Appendix) are correct, one can ascribe the sodium current restorative effect to (K_s) depletion by means of hyperpolarization.

The slow, K^+-independent, restoration of sodium conductance brought about by long duration hyperpolarizations (the *q* process) may be related to alterations in the sodium inactivation curve caused by changes in other ions such as Ca^{++} in the Schwann space.

It was suggested earlier in this paper that a second possible mechanism for [K_o] effects on sodium conductance might involve slow changes in membrane dipole orientations associated with the long term membrane depolarization resulting directly from membrane exposure to increased [K_o]. Ordinarily, on a time average basis, a substantial fraction of the membrane dipoles (natural or induced) presumably are oriented to some degree by the very high electric field (10^5 v/cm) within the membrane (Goldman, 1964; Berg, 1968). Membrane depolarization or hyperpolarization obviously alters this field and thus varies the degree of dipole orientation. Such a transition of states is very rapid (within microseconds). However, a fraction of the dipoles may reorient very slowly as is the case with wax electrets (Eguchi, 1925; Berg, 1968), for example. Such slow changes may give rise to the slow membrane conductance changes associated with high [K_o] and long duration conditioning potentials. However, the fraction of slowly reorienting dipoles must be very small as no significant changes in membrane capacity were observed upon increasing [K_o] from 10 to 100 mM (Palti and Adelman. *J. Membrane Biol.* Accepted for publication.)

The third mechanism suggested for the [K_o] effect on sodium currents considered the possibility that the effect was the direct result of changes in the sodium reversal potential, E_{Na}. However, it has already been shown by Adelman and Palti (1969) that the changes in E_{Na} are small in high external potassium (4 mv in 50 mM K ASW). Therefore, the changes in E_{Na} in high [K_o] solutions can be responsible for only a small fraction of the observed changes in I_{Na} amplitudes reported in this work.

The fourth possible mechanism mentioned above considered that in high [K_o] or during long duration potential conditioning the actual potential difference across the axon membrane might not be equal to the potential difference measured between microtip and reference electrodes. Changes in the Gouy-Chapman double layer (Abramson, Moyer, and Gorin, 1964) or in the resistance in series with the membrane (Hodgkin, Huxley, and Katz, 1952) could bring about such errors. If high [K_o] produced lower actual potential differences across the membrane than the values measured, obviously one would need higher apparent membrane polarizations to obtain currents of the same amplitude as in low [K_o]. However, significant changes in the double layer in high [K_o] ASW solutions are highly unlikely, primarily because the ionic strength of all solutions used was kept reasonably constant.

Changes to be expected in the series resistance in high [K_o] or during long duration conditioning pulses are also small. The resistance changes due to ionic alteration in the space between axolemma and Schwann cells were calculated to be considerably less than 1% of the total resistance in series with the membrane.

The fifth and final mechanism considered to be responsible for the effects of high [K_o] or of long duration potential conditioning suggested that such effects might be due to changes in electrode polarization and/or in electrode-solution impedances. In the voltage-clamping system used in the experiments reported in this paper, the current electrodes were completely separated, electronically and geometrically, from the potential-measuring electrodes. Therefore, it is unlikely that any polarization or impedance changes of the current electrodes (resulting from the long duration pulses) could affect the E_m electrodes. Thus, errors resulting from current-sensitive E_m feedback control seem unlikely. In addition, there was never any indication of instability of the clamp system during a test pulse following long duration conditioning. Fig. 7 illustrates typical initial transient currents initiated by depolarizing test pulses following a variety of short and long duration conditioning pulses (up to 5 min). These currents show no signs of notching or oscillations and are taken as evidence for voltage clamp stability (cf. Taylor, Moore, and Cole, 1960).

The currents associated with the slow buildup of electrode polarization at the current electrodes can be considered as a DC bias. Since such processes decay at the end of the prepulses with the same slow rates as those with which they buildup, they cannot be expected to affect the values of the initial transient currents associated with the depolarizing test pulses used to measure I_p.

The slow changes in sodium conductance associated with high [K_o] and long duration conditioning potentials, as described above, may provide an insight into the mechanisms underlying some slow or long duration bioelectric phenomena such as long duration action potentials. In this example, the rate of decline of the plateau of long duration action potentials obtained in perfused axons (Narahashi, 1963; Baker et al., 1964; Adelman et al., 1965) corresponds roughly to the time course of the p process. Therefore, these phenomena might be considered as resulting from slow changes in sodium conductance corresponding to those described in this work.

APPENDIX

The effects of long duration hyperpolarizing, conditioning potentials on [K] in the space between the axon membrane and the Schwann cells can be calculated with the use of the Frankenhaeuser and Hodgkin model (1956). We will use their basic assumptions (hypothesis 1): (*a*) Diffusion of K^+ away from the axon is restricted by a thin outer layer separated from the excitable membrane by an aqueous space most

probably a few hundred A thick. (*b*) The potassium ion concentration (mole/cm³) in this space, $[K_s]$, at any time depends on the initial steady-state concentration, $[K_{s_o}]$, and the integral, over time, of the K^+ fluxes into and out of the space.

The increase or decrease, $\delta[K_s]$, of K^+ concentration in the space from $[K_{s_o}]$, resulting from a difference between inflow and outflow of K^+ from the space, can be calculated from:

$$d\delta[K_s]/dt = (M_{K_{si}} - M_{K_{so}})/\theta \tag{3}$$

where θ is the space thickness (in cm), $M_{K_{si}}$ and $M_{K_{so}}$ are the net fluxes (mole $cm^{-2} \cdot sec^{-1}$) of K ions from the space into the axon and from the space through the outer layer into the Schwann cells and external solution, respectively (flow into the space and K^+ accumulation in the space are taken as positive).

The net flux of ions driven by diffusion and electrical forces operating around the axon membrane is given by (Hodgkin and Huxley, 1952 *a*):

$$M_{K_{si}} = G_K(E - E_K)/F \tag{4}$$

where G_K is the axon membrane K^+ conductivity in ohm^{-1}, E is the potential difference across the axon membrane (v), E_K is the reversal potential of the potassium current, and F = 96,500 coul/mole. Note that the apparent G_K is a function of $[K_o]$ (see equation 8). However, for a given $[K_o]$ and hyperpolarizing potentials, n tends to zero so that potassium current is carried mainly through the leakage conductance. Therefore, under these conditions, G_K is practically independent of time.

The diffusion of K ions from the space to the outer layer is given by (Frankenhaeuser and Hodgkin, 1956):

$$M_{K_d} = -\delta[K_s] \cdot P_{K_s} \tag{5}$$

where P_{K_s} is the outer layer K^+ permeability in $cm \cdot sec^{-1}$. If it is assumed that the values of the resistances in series with the axon membrane are small as compared with the membrane resistance then the potential difference between internal and external current electrodes approaches E. Thus, the K^+ flux carried to the space by an electric current flowing from an external electrode is given by:

$$M_{K_e} = -G_t \cdot E \cdot t_K/F \tag{6}$$

where G_t is the axon membrane conductance, and t_K is the transport number of K^+ in the solution used. Note again that G_t is a function of $[K_o]$ and also that for hyperpolarizing currents M_{K_e} signifies an influx of K ions into the space.
As $M_{K_{so}} = M_{K_d} + M_{K_e}$, from equations 3 through 6 we get:

$$\delta[K_s] = \int_o^t [G_K \cdot (E - E_K) - G_t \cdot E \cdot t_K - \delta[K_s] \cdot P_{K_s} \cdot F)/F \cdot \theta] \cdot dt \tag{7}$$

The values of G_K, G_t, and E_K were experimentally determined as functions of $[K_o]$. The conductances were evaluated by measuring the voltage clamp currents generated, upon step membrane hyperpolarizations, by axons placed in 230 mM Na ASW

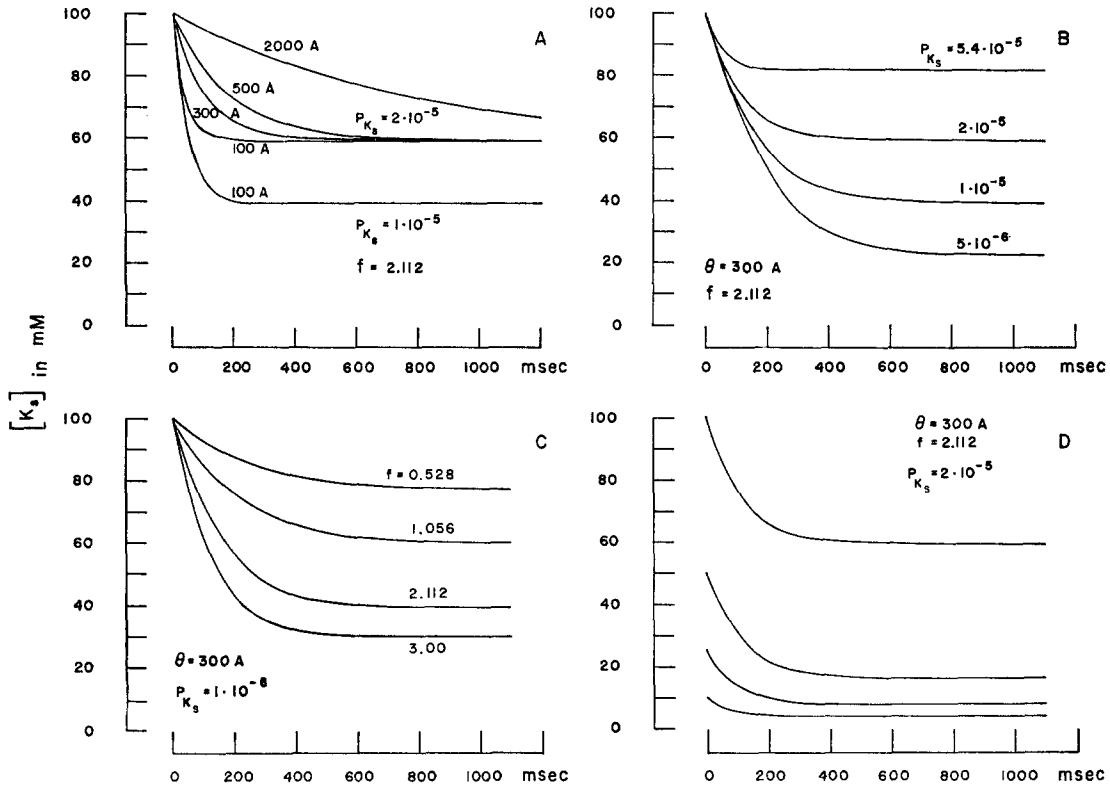

FIGURE 8. Potassium ion concentration, mM/liter, in the space between the axolemma and Schwann cell layer, $[K_s]$, calculated by means of a digital computer from equation 7 as a function of time after onset of a −120 mv hyperpolarizing pulse. Note $[K_s]$ depletion which reaches a plateau level with time constants in the order of 50–250 msec. A, $[K_s]$ values plotted as a function of space thickness, θ. The value of nerve conductance factor, f, used in the computation was 2.112. The outer layer K permeability, P_{K_s}, was 2.10^{-5} cm sec^{-1} for the upper plateau and 1.10^{-5} cm sec^{-1} for the lower plateau. The lower plateau level illustrates the steady state $[K_s]$ obtained for any θ with a smaller P_{K_s} value. B, $[K_s]$ values as a function of outer layer K permeability, P_{K_s}. θ = 300 A and f = 2.112. C, $[K_s]$ values plotted as a function of nerve conductance factor f (see equation 8). θ = 300 A, P_{K_s} = 1.10^{-5} cm sec^{-1}. D, $[K_s]$ values plotted as a function of time for the four different initial $[K_o]$ used experimentally: 100 mM, 50 mM, 25 mM, and 10 mM.

solutions containing from zero to 200 mM [K]. The osmolarity of solutions containing less than 200 mM [K] was maintained by adding proper amounts of $Tris^+$. The experimental relationship found between G_t (and thus also G_K)[1] and $[K_o]$ was:

$$G_t = 10^{-3}(0.094 + 0.336 \log [K_o])f \qquad (8)$$

[1] For E = −120 mv, within a few milliseconds, G_t can be considered equal to the membrane leakage conductance, G_l. G_K is then the fraction of G_l carried by K ions as given by the [K]-dependent term in equation 8.

where $200.0 \geq [K_o] \geq 5.0$; f is a conductance factor (ohm^{-1}) specific to any axon (the experimental values of f varied between 0.5 and 2). In solving equation 7, the conductance values were varied in accord with equation 8 as $[K_s]$ varied with time. The values of P_{K_s} taken were in the range 1 to $5.4 \cdot 10^{-5}$ cm sec^{-1} (see Frankenhaeuser and Hodgkin, 1956) and the space thickness 100 to 2000 A. When these values were used, the integral of equation 7 was numerically solved by the finite increment method using an IBM 360/44 digital computer. As $[K_s] = [K_{s_o}] - \delta[K_s]$, from solution of equation 7 one can easily determine the [K] in the Schwann space.

Fig. 8 A illustrates the change in $[K_s]$ as a function of time after the onset of a hyperpolarizing conditioning potential ($E = -120$ mv) for different values of Schwann space thickness, θ. It may be seen that the calculated $[K_s]$ values decrease exponentially to about 50% of the original values with time constants usually ranging between 40 and 250 msec, depending on the values of θ and P_{K_s} used. The wider the space, the longer the time constant, τ. The steady-state value of $[K_s]$ is little affected, however. The magnitude and the rate of decline of $[K_s]$ in a 300 A space are in good agreement with the observed sodium conductance restoration (see Fig. 1).

Fig. 8 B plots $[K_s]$ as a function of conditioning pulse duration for different P_{K_s} values[2] ($0.5 \cdot 10^{-5}$ to $5.4 \cdot 10^{-5}$ cm·sec^{-1}) when $\theta = 300$ A. It is seen in Fig. 8 B that when P_{K_s} values are $1\text{–}2 \cdot 10^{-5}$ cm·sec^{-1} both the magnitude and rate of decline of $[K_s]$ are again in agreement with the experimental values of sodium conductance restoration.

Fig. 8 C plots $[K_s]$ during hyperpolarization as a function of the conductance factor, f, which expresses the relative over-all level of axon conductance. When $f \geq 1$ the computed changes in $[K_s]$ correspond well with the sodium restoration process during hyperpolarization.

Fig. 8 D, which plots the change of $[K_s]$ as a function of time for different initial values of $[K_o]$, illustrates that the predicted change in $[K_s]$ is a direct function of $[K_o]$ as is the restoration of experimental sodium conductance during hyperpolarization.

This work was supported by United States Public Health Service, National Institutes of Health Research Grant NB 04601, and by International Postdoctoral Research Fellowship 1F05TW1220.
Received for publication 26 May 1969.

REFERENCES

ABRAMSON, H. A., L. S. MOYER, and M. H. GORIN. 1964. Electrophoresis of Proteins. Hafner Publishing Co., Inc., New York.

ADELMAN, W. J., JR., F. M. DYRO, and J. SENFT. 1965 *a*. Long duration responses obtained from internally perfused axons. *J. Gen. Physiol.* **48**(5, Pt. 2): 1.

ADELMAN, W. J., JR., F. M. DYRO, and J. SENFT. 1965 *b*. Prolonged sodium currents from voltage clamped internally perfused squid axons. *J. Cell. Comp. Physiol.* **66**(Suppl. 2): 55.

ADELMAN, W. J., JR., and Y. PALTI. 1969. The influence of external potassium on the inactivation of sodium currents in the giant axon of the squid, *Loligo pealei*. *J. Gen. Physiol.* **53**:685.

ADELMAN, W. J., JR., and J. SENFT. 1968. Dynamic asymmetries in the squid axon membrane. *J. Gen. Physiol.* **51**(5, Pt. 2):102 s.

[2] The P_{K_s} value selected by Frankenhaeuser and Hodgkin (1956) is $5.4 \cdot 10^{-5}$ cm·sec^{-1}. However, their value was based on data obtained at about 18°C while our working temperature was 3–4°C.

BAKER, P. F., A. L. HODGKIN, and H. MEVES. 1964. The effect of diluting the internal solution on the electrical properties of a perfused giant axon. *J. Physiol. (London).* **170**:541.

BERG, H. C. 1968. Membrane dipole potentials. *Biophys. J.* **8**:1051.

COLE, K. S. 1958. Effects of polarization on membrane ion currents. Abstracts of the Biophysical Society 2nd Annual Meeting. Cambridge, Mass. 22.

COLE, K. S. 1968. Membranes, Ions and Impulses. University of California Press, Berkeley.

EGUCHI, M. 1925. On the permanent electret. *Phil. Mag. Sixth Ser.* **49**:178.

FRANKENHAEUSER, B., and A. L. HODGKIN. 1956. The after-effects of impulses in the giant nerve fibers of *Loligo. J. Physiol. (London).* **131**:341.

GOLDMAN, D. E. 1943. Potential, impedance, and rectification in membranes. *J. Gen. Physiol.* **27**:37.

GOLDMAN, D. E. 1964. A molecular structural basis for the excitation properties of axons. *Biophys. J.* **4**:167.

HODGKIN, A. L., and A. F. HUXLEY. 1952 *a*. Currents carried by sodium and potassium ions through the membrane of the giant axon of *Loligo. J. Physiol. (London).* **116**:449.

HODGKIN, A. L., and A. F. HUXLEY. 1952 *b*. The dual effect of membrane potential on sodium conductance in the giant axon of *Loligo. J. Physiol. (London).* **116**:497.

HODGKIN, A. L., and A. F. HUXLEY. 1952 *c*. A quantitative description of membrane current and its application to conduction and excitation in nerve. *J. Physiol. (London).* **117**:500.

HODGKIN, A. L., A. F. HUXLEY, and B. KATZ. 1952. Measurement of current-voltage relations in the membrane of the giant axon of *Loligo. J. Physiol. (London).* **116**:424.

HODGKIN, A. L., and B. KATZ. 1949. The effect of sodium ions on the electrical activity of the giant axon of the squid. *J. Physiol. (London).* **108**:37.

NARAHASHI, T. 1963. Dependence of resting and action potential on internal potassium in perfused squid giant axons. *J. Physiol., (London).* **169**:91.

NARAHASHI, I. 1964. Restoration of action potential by anodal polarization in lobster giant axons. *J. Cell. Comp. Physiol.* **64**:73.

TAYLOR, R. E., J. W. MOORE, and K. S. COLE. 1960. Analysis of certain errors in squid axon voltage clamp measurements. *Biophys. J.* **1**:161.