# Electronic Measurement of the Intracellular Concentration and Net Flux of Sodium in the Squid Axon

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ABSTRACT A unique, rapid, and non-destructive determination of the intracellular sodium concentration of a squid axon may be provided by the "voltage clamp" technique, in which the potential across the axon membrane is under electronic control. The potential at which the early component of ionic current reverses following a membrane potential step was used as an index of the intracellular sodium concentration. Several types of experiments were used to test the applicability of this method for measurement of intracellular sodium and its net flux. The concentration was found to increase from 38 mM for a fresh axon to 50 mM in about an hour. From this change, the net flux for a fresh resting axon was estimated to be 40 pmoles/cm² sec. Rapid stimulation of an unclamped axon produced a marked increase in the rate of sodium accumulation. Rapid pulsing of the membrane in a voltage clamp to potentials more positive than the sodium potential moved sodium out fast enough to produce a definite decrease in internal concentration. The agreement between the results with this method and those with more direct methods is quite satisfactory. An attractive feature of this method of intracellular sodium determination is that the physiological function of the axon is maintained and other measurements may be made concurrently.

## INTRODUCTION

Hodgkin and Huxley (1952 *a*) found that, when depolarizing potential steps were applied to the squid axon membrane in a voltage clamp, the direction of the early component of ionic current reversed when the membrane potential was pulsed beyond the assumed value for the sodium equilibrium potential. The potential at which this reversal occurred followed rather closely the predicted change in the equilibrium potential upon variation of the external sodium concentration. They therefore named the reversal point the

"sodium potential" and designated it by "$E_{Na}$." In the Hodgkin and Huxley method, all potentials were measured with reference to a non-stationary and unknown resting potential. Because the absolute value of the membrane potential was not known, their sodium potential measurements were useful only for changes of sodium concentration.

Transmembrane potential measurements on the squid axon by means of micropipettes filled with various concentrations of potassium chloride have given confidence that the actual membrane potential difference may be measured to within a millivolt or two (Moore and Cole 1960, Cole and Moore 1960 *a*), and allow liquid junction potential correction to be made. On the assumption that the Nernst equation is as valid for changes in the internal concentration as for external changes, we may calculate the internal sodium concentration from sodium potential measurements when the external concentration is known. The net sodium flux may then be inferred from the temporal variation of the intracellular concentration.

The sodium potential has been followed as a function of time in the resting axons in order to obtain a measure of the resting sodium flux which can be then compared with tracer results. Additional studies were made of the internal sodium accumulation upon rapid stimulation of unclamped axons and sodium loss as a result of rapid short pulses (in voltage-clamped axons) to a value in excess of the sodium potential.

Until the early component of the ionic current can be demonstrated to be a flow of sodium ions by less equivocal techniques, probably the best procedure is to compare the various determinations made using this method to other measurements made previously by different techniques.

## METHOD

(*a*) *Preparation and Experimental Apparatus* The squid giant axon was dissected, cleaned, mounted for voltage clamping as has been described in detail (Moore, 1959, Cole and Moore, 1960 *b*, Moore and Cole, 1961), and only a brief summary is required here. A micropipette filled with 3 M potassium chloride was made to penetrate the membrane and a reference "millipipette" (about 0.2 mm in diameter) filled with 0.5 M potassium chloride in agar was placed outside the membrane. These salt bridges were connected to stable electrodes (silver-silver chloride or preferably calomel half-cells). The potential on each of these electrodes was measured with respect to ground by appropriate amplifiers, stabilized against drift by means of electromechanical choppers and auxiliary amplifiers. The potential difference between the amplifier outputs—proportional to the membrane potential—was measured and compared with a command pulse by an electronic feedback circuit. The appropriate current was forced through the membrane *via* an axial wire so as to maintain the membrane potential at the desired level. The current density was obtained from the current flow through known central area. The axon was

routinely left unclamped at its resting potential except for the intervals of a few seconds (every 3 to 5 minutes) during which the axon was clamped and a few pulses applied to determine the potential at which the sodium current reversed.

The accuracy of the method employed here depends on how well the electrode potentials are measured and are made to follow the command signal. Negative feedback was used around high gain amplifiers in order that the precision and stability of the net amplification might be almost entirely a function of the values of precision resistors (Moore and Cole, 1961, Moore, 1961). The cathode ray oscilloscope had less gain stability than the other circuits. However, the complete system was routinely calibrated to within 1 per cent preceding each experiment and rechecked afterwards for assurance of constancy over the experimental period. The measuring system was balanced to zero with the microtip in the sea water near the reference electrode before penetration. Chopper stabilization reduced the drift of the system to about −1 mv (equivalent membrane potential) for an indefinitely long period. Ripple (from the line or power supply) was of the same order of magnitude; synchronization of pulses with the AC line made this factor constant throughout an experiment.

The actual membrane potential was taken as the measured value plus the liquid junction potentials of 1.0 mv (for sea water to 3 M KCl) and 3.0 mv (for axoplasm to 3 M KCl) (Cole and Moore, 1960 *a*). The algebraic signs of these are such that the absolute value of the resting membrane potential is larger than the measured value; *i.e.*, it is 4 mv more negative than the measured potential.

(*b*) *Measurement of* $E_{Na}$ A typical family of current patterns as a function of time after application of various potential steps is seen in Fig. 1. The early component of ionic current reverses direction as increasing step amplitudes carry the membrane potential through about +50 mv. Hodgkin and Huxley (1952 *a*) interpreted their similar voltage clamp currents in terms of concentration cells and conductance paths for sodium, potassium, and other unidentified ions. At rest, the conductance of the potassium path is much greater than that of the sodium path and the measured internal potential is near the −70 mv of the potassium battery. When a step of potential takes the potential of the axoplasm in a positive direction by an amount of 25 or more mv, there is a rapid increase of the conductance of the sodium channel to the extent that it becomes large compared to the potassium channel resistance and sodium becomes the dominant current. This is a transient phase and the sodium conductance and current wane (sodium "inactivation") and the potassium conductance and current become large and a steady state is reached in which the ratio of the conductance of these ions reverts to a value approximating that at rest. However, during the brief interval of high conductance to sodium, it is possible to measure the potential at which the early transient current reverses; *i.e.*, the sodium potential.

Fig. 2 shows typical current patterns in the neighborhood of the reversal potential at higher sensitivity and sweep speed than in Fig. 1. There is definitely a minimum in the lower trace whereas the upper trace continually rises following the initial transient. The value for the sodium potential was estimated from these records as 52.5 ± 1 mv. It appeared that readings to the nearest 0.5 mv were reproducible and reliable on axons with high sodium conductivities but the accuracy deteriorated in poorer axons with higher leakage currents. The sodium conductivity and the sensi-

tivity of the method also decrease in sodium concentrations of one-half normal or less. Changes in $E_{Na}$ over a period of a few minutes can be measured with considerably better accuracy than the absolute value of $E_{Na}$ because the system non-linearity and drift errors are less than the over-all calibration error.

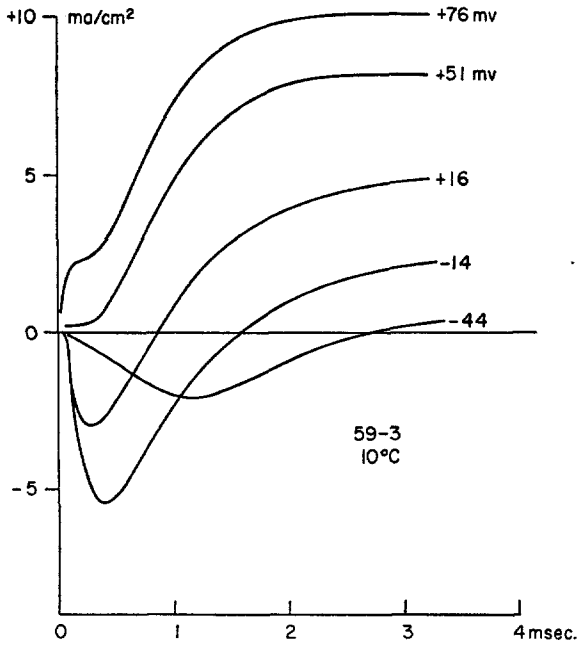


FIGURE 1. A family of current patterns through the membrane as a function of time for various membrane potentials after a step change. The potential of the axoplasm (with respect to an external ground, liquid junction corrections applied) during the pulse is given to the right of each curve. The interior was held at −74 mv between pulses. Figure reproduced courtesy *Proc. IRE* (Moore, 1959).

(*c*) *Calculation of* $[Na]_i$ The equilibrium potential for sodium is given by the Nernst equation:

$$E_{Na} = \frac{RT}{F} \ln \frac{[Na]_o \gamma_o}{[Na]_i \gamma_i} \tag{1}$$

where $[Na]_o$ and $[Na]_i$ represent the external and internal axoplasm sodium concentrations. The simplifying assumption that the activity coefficients $\gamma_o$ and $\gamma_i$ are identical in the two phases may be made until a direct measurement of the axoplasm coefficient provides a better ratio. The validity of this equation appears to be established by experiments in which the external sodium concentration was varied (see Hodgkin-Huxley, 1952 *b*, and Results in this paper). The Nernst equation was rearranged into the Boltzmann form for calculation of the intracellular sodium

concentration:

$$[\mathrm{Na}]_i = [\mathrm{Na}]_o e^{-(E_{\mathrm{Na}}F/RT)} \tag{2}$$

A number of factors must be considered in assessment of the accuracy of the internal sodium concentration determination. Instrumentation calibrations indicated that these errors rarely exceeded 1 or 2 mv. Probably more important were uncertainties in the determination of $E_{\mathrm{Na}}$ and questions of the liquid junction corrections and applicability of the Nernst equation. An error as large as 5 mv in a 50 mv value for $E_{\mathrm{Na}}$ would correspond to about a 10 per cent error in the estimation of the sodium concentration.

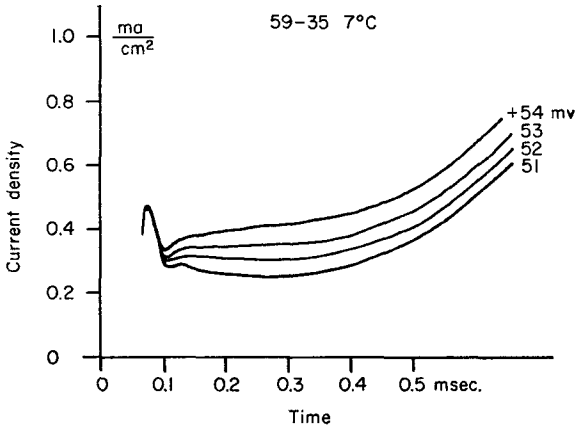


FIGURE 2. Membrane current records similar to those in Fig. 1, but at high sensitivity and sweep to show the reversal in the early current. Only the ends of the preceding capacitive currents are shown.

The variations in concentration from axon to axon may have been considerably greater than the instrumental errors. Although great care was exercised in the dissection process, the amount of insult and injury to the axon during the cleaning process could not be controlled. The time to clean the axon, place it in the chamber, and place all the electrodes, internal and external, varied from about 1 to 3 hours.

(*d*) *Estimation of Net Sodium Flux* The net sodium flux, $M_{\mathrm{Na}}$, was estimated from the time rate of change of $[\mathrm{Na}]_i$ and the volume of axoplasm, $V_{ax}$, surrounded by 1 cm² of membrane by means of the equation:

$$M_{\mathrm{Na}} = \frac{\Delta[\mathrm{Na}]_i}{\Delta t} \times V_{ax} \tag{3}$$

The accuracy of the flux determination depends mainly upon how well small changes in $E_{\mathrm{Na}}$ can be measured. That is, for the flux measurement, errors in the absolute value of $E_{\mathrm{Na}}$ (caused by inaccurate calibrations, liquid junctions, etc.) are secondary

to considerations of sensitivity and reliability in the determination of $E_{Na}$. We found that the $E_{Na}$ declined about 2.5 mv in the usual 20 min. measuring period. The uncertainty of this change was probably ±30 per cent for a single experiment and somewhat less for the averaging procedure used in the hypothetical curve (Fig. 5).

There was always some taper in the axon and often a constriction (at the point of crossover of the fin nerve) in the measured region. Therefore the measured values of membrane area and corresponding axoplasm volume had an accuracy of about ±10 per cent, at best. The per cent of the axoplasm volume displaced (and/or occupied) by the axial wire was usually less than 4 per cent and is not considered significant in view of the other uncertainties.

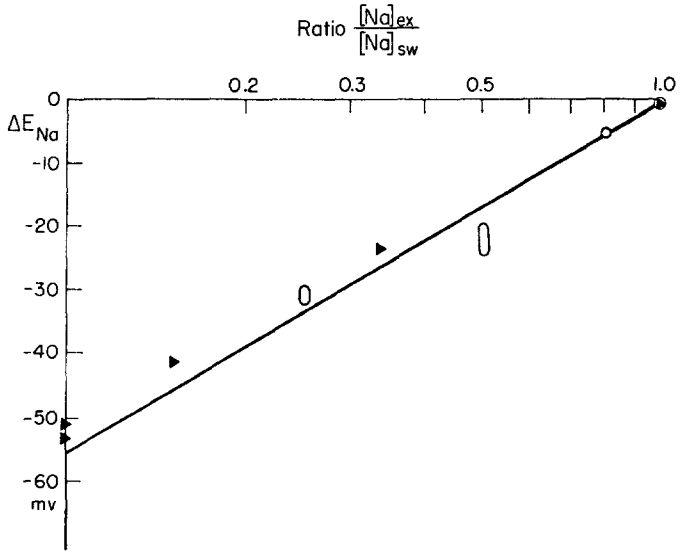


FIGURE 3. Change in the potential at which the early current component reverses as the external sodium is reduced and replaced by choline.

(*e*) *Solutions* The artificial sea water composition used in these experiments is essentially identical to Woods Hole sea water. A table of normal constituents is given in the following paper (Adelman and Moore, 1961).

## RESULTS

(*a*) *Variation of External Sodium Concentration* The concentration of the external sodium ion was reduced from its normal value of 430 mM by substitution of carefully purified choline chloride for sodium chloride; the activity of choline was assumed to equal that of sodium. Following an initial control period in a normal sodium sea water, the sea water with a low sodium concentration was applied. After $E_{Na}$ appeared to have stabilized at a new value, the axon was returned to normal sodium sea water. This sequence could be

repeated a number of times with axons in good condition. The results of several such experiments are given in Fig. 3 where the change in $E_{Na}$ (from its normal sodium value) is plotted as a function of the experimental to normal sodium ratio. The Nernst relation is shown as a solid line in Fig. 3 and the experimental points generally fall close to this line. The estimation of the potential at which the early current component reverses becomes increasingly difficult and uncertain as the amplitude of the current decreases with decreasing external sodium concentration. Some of the axons were in poor condition (low resting potentials) and did not fully recover to the extrapolated $E_{Na}$

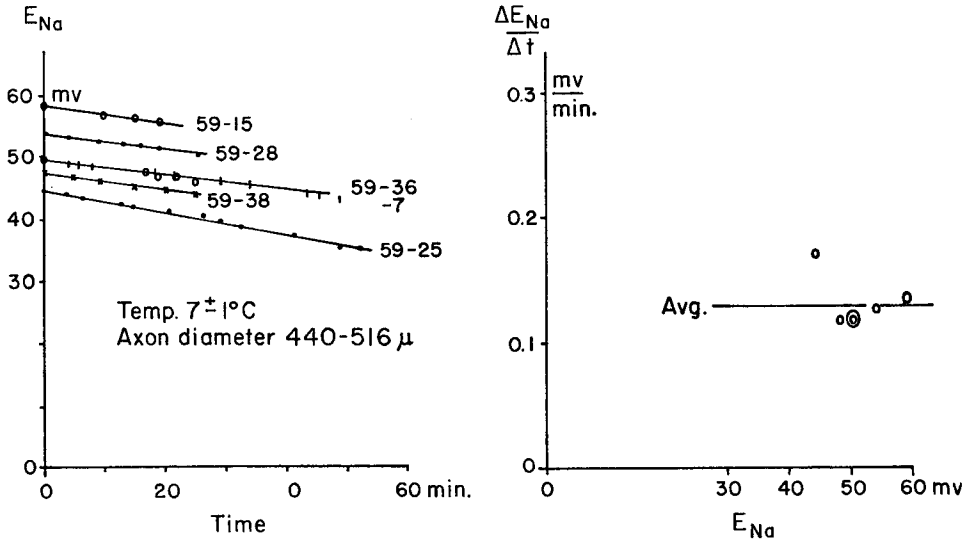


FIGURE 4*a* FIGURE 4*b*

FIGURE 4 *a*. Left, $E_{Na}$ as a function of time after initial measurement.
4 *b*. Right, the rate of change of $E_{Na}$ as a function of the initial value of $E_{Na}$

value on return to normal external sodium; this makes the estimate of the change in $E_{Na}$ less accurate. However, the experimental results agree reasonably well with the Nernst equation, substantiate the results of Hodgkin and Huxley, and establish confidence for the application of the Nernst equation as a basis for calculation of the internal sodium concentration.

(*b*) *Internal Sodium Concentration* Early measurements of the sodium potentials of a number of axons are plotted (with the 4 mv liquid junction correction) in Fig. 4 *a* as a function of time after the initial determination. All axons were held at a 7°C temperature to within ±1°C in this set of experiments. The small temperature difference between the various axons was not enough to appreciably alter the $RT/F \times 2.303$ factor from a value of 56 mv

(for a tenfold change in concentration). The variation in the axon diameter in this study was ±8 per cent about an average of 485 microns. The initial value of the internal sodium calculated by Equation 2 ranged from 38 to 68 mM with an average value of 55 mM.

Although the initial value of the sodium potential varied considerably, the rate of decay was rather uniform. This is shown more clearly in Fig. 4 *b* where the time rate of change of $E_{Na}$ has been plotted against the initial $E_{Na}$ value and a line drawn at the average of 0.125 mv/min. The time between

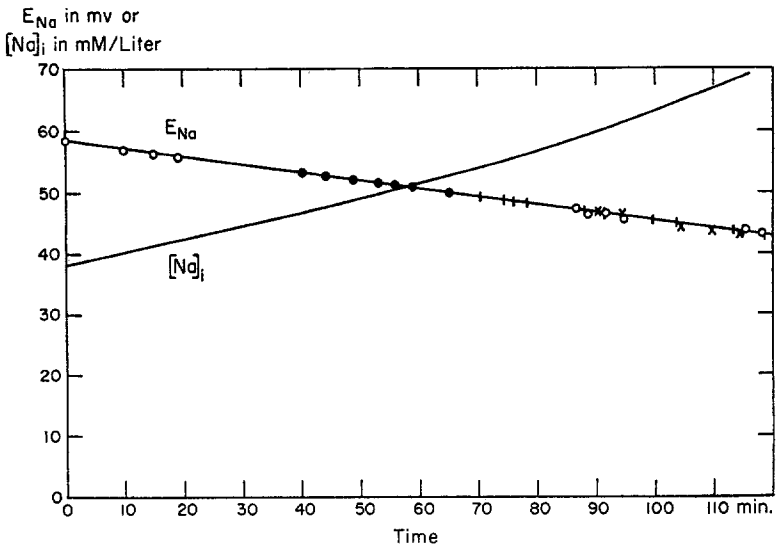


FIGURE 5. A hypothetical plot of the decline of $E_{Na}$ with time using our highest value of $E_{Na}$ from Fig. 4 *a* and the average slope of Fig. 4 *b*. Experimental points from 4 *a* (same symbols) were also marked after a temporal translation to match initial value with hypothetical line. The corresponding internal sodium concentration was calculated for a 485 $\mu$ (diameter) axon and also plotted.

decapitation of the squid and the initial value of $E_{Na}$ was not recorded in these experiments, but probably ranged up to an hour or two. The differences in the initial $E_{Na}$ values might well be simply the result of observations of a continuously declining system at different initial times. Certainly there is usually a slow continuous decline in the resting potential from the initial value.

Therefore it seemed reasonable to *assume a linear decline* of $E_{Na}$ and construct a hypothetical time course starting with our highest (and probably earliest) $E_{Na}$ determination and falling at the average rate given above (Fig. 5). Corresponding values for the internal sodium concentration, $[Na]_i$, have been calculated by Equation 2 and are also plotted. For a linear decrease

of $E_{Na}$ with time, it would be expected that $[Na]_i$ would increase exponentially. However, the time constant of the change of $[Na]_i$ is so large that the departure from linearity over a measurement period of 20 or 30 min. would not be noticeable. On the basis of this curve the internal sodium concentration would fall from 38 to 50 mM in about 50 min.

*Net Sodium Flux*

The net movement of sodium was inward and was calculated from the time rate of change of the internal sodium concentration by means of Equation 3 and the values ranged from 40 to 100 pmoles/cm²sec. for the axons shown in Fig. 4. Flux calculations were also made for the hypothetical curve given in Fig. 5, using an average diameter of 485 microns. The initial net flux of sodium was 40 pmoles/cm²sec. and after about an hour this had increased by 50 per cent to 60 pmoles/cm²sec.

*Increase of Internal Sodium with Stimulation*

We have made a few observations on the increased accumulation of sodium in the axoplasm associated with rapid stimulation on somewhat deteriorated axons (action potentials were low and the resting sodium influx, about 160 pmoles/cm²sec., was several times the value of our freshest preparation). Stimulation at 100 impulses per sec. for 4 min. produced a definite increase in $[Na]_i$ over that expected from extrapolation of the resting rate as seen in Fig. 6. An average increment of 1.5 pmoles of axoplasm sodium per impulse per cm² of membrane was observed in this experiment. Because the action potential declined appreciably during the stimulation period and the resting potential changed enough to inactivate the sodium conductance considerably, this value represents a smeared average of a large variation.

*Electronic Sodium Battery Charger*

In all normal physiological situations the axon membrane potential (inside with respect to an external ground) appears to stay between the potassium potential on the negative side and the sodium potential on the positive side. Throughout this potential range, sodium leaks into the axoplasm at a rate depending on the condition and activity of the axon as already described. The voltage clamp provides a means for exceeding this range of potential excursions, driving sodium out, and changing $E_{Na}$.

When a voltage-clamped membrane potential is changed suddenly from a value near rest to a value at which the interior is positive and in excess of the sodium potential, there is an early surge of outwardly directed sodium

current (see Fig. 2). Although the duration of this current is short because of "inactivation" of the sodium channel (Hodgkin and Huxley 1952 *b*), the sodium conductivity of the membrane can be restored by returning its potential to the resting value for a short time and the process repeated many times per second. Thus the Hodgkin and Huxley model (Hodgkin and Huxley, 1952 *b*) would predict that such treatment would reduce the internal sodium concentration and thereby increase the potential of the "sodium battery."

Such an experiment with an axon in fair condition is shown in Fig. 6. Voltage clamp pulses taking the inside of the axon to about 80 mv more positive than the sodium potential at a rate of 100 per sec. produced a marked

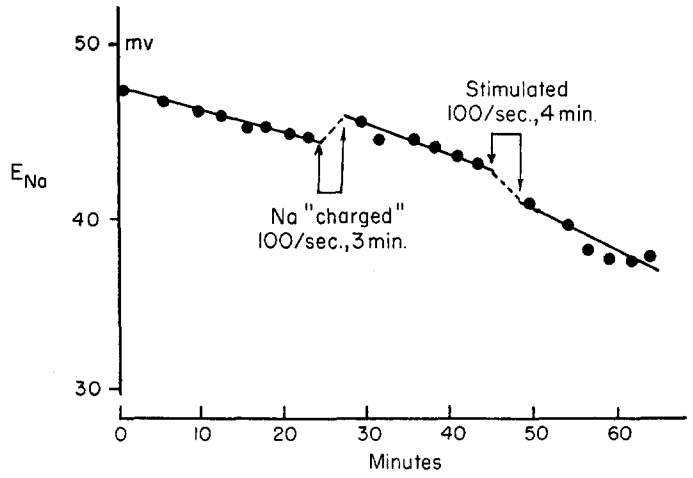


FIGURE 6. Graph of $E_{Na}$ as a function of time. An electronic sodium battery charger was run at 100 pulses per second for 3 min. and later the axon was stimulated externally at the same rate. Temperature 7.5°C.

increase in $E_{Na}$ in a period of 3 min. The width of the pulse was arbitrarily set at about 0.5 msec.; at this time the outward surge of sodium current was largely over and a relatively small amount of sodium current flowed back into the axon when the pulse was turned off. Also at the end of this 0.5 msec. period the potassium conductance had attained only a fraction of its maximum value and probably the net movement of potassium was close to zero because the outward flux during the pulse was countered by inward flux after the pulse turn off. The membrane was given a steady hyperpolarization of about 20 mv between pulses in order to completely reactivate the sodium conductance system and delay the onset of the potassium current (Cole and Moore, 1960 *c*). The change in $E_{Na}$ from before to after the pulsing is clearly shown in Fig. 6. After correction for the resting leakage, an average outward pulse of 5.2 pmoles of sodium per $cm^2$ of membrane was obtained for this battery charger.

## DISCUSSION

Although an early surge of ionic current through a membrane following a step in potential has not been identified unequivocally as sodium, all the results derived from measurements of the reversal potential are consistent with earlier measurements and with the Hodgkin and Huxley interpretation.

Steinbach and Spiegelman (1943) found the sodium content of extruded squid axoplasm from freshly dissected axons to be 44 mM/kg. Keynes and Lewis (1951) subjected axoplasm to nuclear activation in an atomic pile and measured 46 mM/kg of sodium by induced radioactivity. If these results are expressed as concentrations on the basis of 900 gm of water per kilogram of axoplasm, values of 49 and 51 mM (respectively) are obtained (Hodgkin and Katz, 1949). The values found with the present method are in good agreement, ranging from 38 to 66 mM, with an average of 55 mM.

Hodgkin and Katz (1949) have pointed out that a net entry of sodium of about 60 mM/cm²sec. may be calculated for a 500 $\mu$ axon from the average increase in internal sodium over a 3 hour period (50 mM/kg of axoplasm) as determined by Steinbach and Spiegelman (1943). Shanes and Berman (1955) reported a resting net inward flux of sodium of 30 pmoles/cm²sec. at room temperature (22–23°C) from radioactive tracer measurements. In satisfactory agreement with these findings, the electronic method gives a range of values of flux from 40 to 100 pmoles/cm²sec.

The additional fluxes of sodium and potassium associated with excitation have been measured (Shanes, 1954, Keynes, 1951, and Hodgkin and Keynes, 1955) for cephalopod axons and found to be in the order of 3 or 4 pmoles per cm² per impulse. In these terms, a *resting* fresh axon gains sodium at a rate equivalent to that for excitation at 10 to 15 impulses/sec. As the axon deteriorates, the resting rate of sodium gain increases; this may be thought of as equivalent to more rapid stimulation. Therefore, in order to obtain $E_{Na}$ changes large enough to make reasonably good estimates of the net sodium influx per impulse, very rapid stimulation of a fresh nerve is required. The period of rapid stimulation should be only a few minutes in order to avoid too much uncertainty in the extrapolated resting decline of $E_{Na}$ (which must be subtracted). The action potential amplitude should also be monitored continuously during this period because it will decrease in amplitude as $E_{Na}$ decreases. Our measurement of 1.5 pmoles/cm² impulse is certainly in as close an agreement with the other measurements (Shanes, 1954, Keynes, 1951, and Hodgkin and Keynes, 1955) as can be expected under the circumstances.

For the electronic sodium battery charger the largest changes of $E_{Na}$ can be expected on fresh axons with low sodium leakage and internal concentration, as was the case with influx with normal activity. Not only will a fresh axon with

a high resting potential give a large net movement per pulse because of its high conductivity during the pulse, but also the per cent change in a low internal sodium concentration will be larger for a given net charge movement per pulse. As the internal sodium content is reduced, the value of $E_{Na}$ should rise and the peak current associated with a potential pulse of constant amplitude should decrease. It is desirable therefore to pulse to as high potentials as possible in order that the per cent change in the sodium driving force may not change appreciably with the change in $E_{Na}$.

With the decrease in $[Na]_i$ brought about by the battery charger one might expect to find a few millivolts increase in the height of the action potential following such treatment. However, the resolution of only a few millivolts change in a spike in excess of 100 mv would be poor in contrast to the change in $E_{Na}$. Because of this and the necessity for taking other data, action potentials were recorded immediately before and after a pumping experiment in only one case. In this experiment we saw no increase in the height of the spike, but the resting potential was less afterwards and the rate of its decline indicated that the very large pulses (about +180 mv) did not help the general condition of nerve.

The finding that the influx of sodium increases with time is to be expected because the gradual decline of the resting potential would increase both sodium and potassium conductances (Hodgkin and Huxley, 1952 *b*). This net flux rate obviously cannot increase indefinitely and will have to approach zero again as the internal concentration rises and eventually equals that of the exterior solution. Therefore, the approximation that $[Na]_i$ is an exponential function of time over the short span of 1 to 2 hours should not be extrapolated further; it may be only a reflection of the gradual slowing of the metabolically driven sodium pump (Hodgkin and Keynes, 1955). It is possible that the one high point off by itself in Fig. 4 *b* represents an aberrant axon. If this is true, a straight line might be drawn through the origin and the remaining points. This would indicate a slow exponential decline of $E_{Na}$. However, because the time constant of this process would be long (6 to 7 hours), the curves of $E_{Na}$ and $[Na]_i$ would not be far different from those given in Fig. 7.

In order to obtain an idea of the magnitude of the sodium pumping capacity of the membrane, we have plotted (from the data in Table 3 of Hodgkin and Keynes's (1955) paper on *Sepia*) the $[Na]_i$ against the Na efflux. A rather linear relation is found showing that the rate of extrusion of sodium increases with the axoplasm sodium content. The sodium efflux equation for *Loligo* axons derived by Shanes and Berman (1955) is also dependent on the axoplasm concentration; values obtained from their equation 2 are also plotted. The figure of 26 pmoles/cm$^2$ sec. would seem to be an appropriate estimate of the sodium efflux at room temperature and for an internal sodium concentration of 40 mM. From the Hodgkin and Keynes (1955) study of the effect of

temperature on the sodium efflux, we should expect only about 0.2 of the above figure (5 pmoles/cm²sec.) at our temperature of 7.5°C. Therefore, the sodium pump is probably capable of handling only a small part of the sodium influx under our experimental conditions.

On the basis of this efflux value and our net inward flux of 40 pmoles/cm² sec. the initial sodium entry rate is estimated to be 45 pmoles/cm²sec. At an

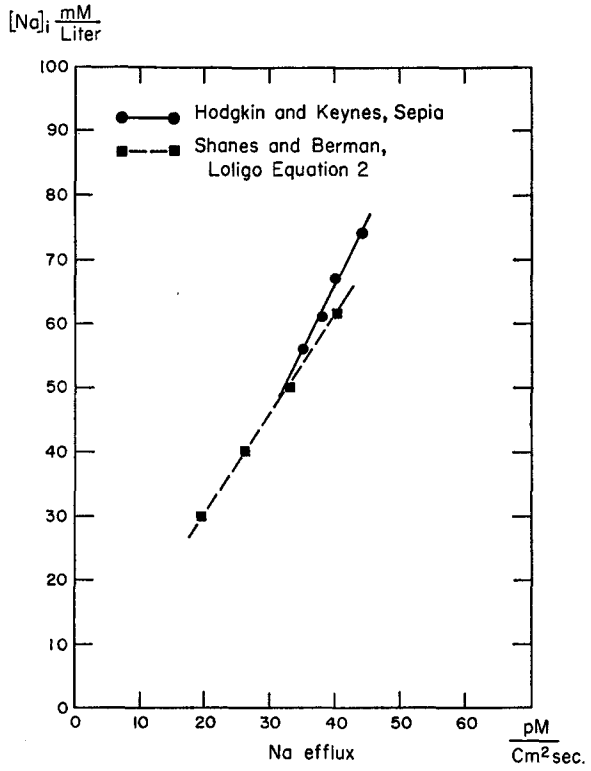


FIGURE 7. Sodium efflux as a function of the internal concentration. The circles are from Table 3 of Hodgkin and Keynes and the squares from Shanes and Berman's equation 2. Temperatures 18 and 22–23°C, respectively.

axoplasm sodium concentration of 50 mM, the influx may be estimated at 61 pmoles/cm²sec. These values are in excellent agreement with those found by Shanes and Berman (1955) and Hodgkin and Keynes (1955) for *Loligo* sodium influx.

It is unfortunate and regrettable that the length of cleaned axon required and the complexity of this experimental arrangement cause the minimum preparation time to be as long as 1 hour. Hopefully, a shorter axon chamber and simplification of the axon mounting and electrode insertion procedures would allow somewhat earlier measurements. One is tempted to extrapolate

our results to an earlier time, say an hour, to estimate the internal sodium concentration in the swimming squid's axon at 27 mM from a 67 mv value for $E_{Na}$. The corresponding net influx may also be extrapolated from the composite curve to 36 pmoles/cm²sec. However, there is some evidence from *in vivo* measurements of the resting and action potentials that the $E_{Na}$ in the living animal is not far from our initial value. In the present series of experiments, the sodium potential exceeded the peak of the membrane action potential by 3 to 9 mv. Therefore, it seems reasonable to add 7 mv to *in vivo* action potential peaks to obtain the corresponding sodium potential. From the *in vivo* experiments of Moore and Cole (1960) a sodium potential of 57 mv may be estimated (using liquid junction corrections already mentioned in Cole and Moore, 1960 *a*). The *in vivo* action potential taken by Hodgkin and Keynes (1955) on *Loligo forbesi* and published in Hodgkin's Croonian Lecture (1957) (Fig. 3) gives a somewhat lower value for $E_{Na}$.

While only a few measurements of the sodium transferred between exterior and interior with action potentials and voltage clamp charging were made, the values obtained are in quite satisfactory agreement with other measurements or calculations. For appropriate comparison with other results at other temperatures the complex effect of temperature on the flux per impulse should be considered. Hodgkin, Huxley, and Katz (1952) and Moore (1958) have observed a threefold increase in the rates of the membrane change for a temperature rise of 10°C. Because of the variability in the maximum conductance between axons at various temperatures, Hodgkin, Huxley, and Katz were uncertain as to its temperature coefficient. The results of sequential measurements on an axon as the temperature was varied showed a definite increase with temperature for both peak sodium and steady state potassium conductances by about 4 per cent per degree of the value at 15°C (Moore, 1958). FitzHugh (data to be published) has taken this into account in action potential calculations with the Hodgkin-Huxley axon equations. The computed ratio of the net potassium flux at low and high temperatures is in excellent agreement with the experimental ratio obtained by Shanes (1954). Presumably, the sodium flux per impulse would be about the same function of temperature as that for potassium, because equality usually has been found when investigated.

There are some advantages of the electronic method of determination of intracellular concentration and net flux of sodium over the more conventional methods. In the first place, the electronic measurement of the axoplasm sodium content does not completely destroy the axon as is required by chemical methods. Therefore it is possible to make successive determinations on an axon and obtain flux measurements while at the same time maintaining the capability to make a wide range of measurements of the electrical characteristics of the membrane. The effects of various chemical changes on the intra-

cellular sodium accumulation as well as the recovery from such treatment may also be observed (Adelman and Moore, 1961) during the course of normal voltage clamp experiments.

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