Current-Voltage Relations in
the Lobster Giant Axon Membrane
Under Voltage Clamp Conditions

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ABSTRACT   The sucrose-gap method introduced by Stämpfli provides a means for the application of a voltage clamp to the lobster giant axon, which responds to a variety of different experimental procedures in ways quite similar to those reported for the squid axon and frog node. This is particularly true for the behavior of the peak initial current. However, the steady state current shows some differences. It has a variable slope conductance less than that of the peak initial current. The magnitude of the steady state slope conductance is related to the length of the repolarization phase of the action potential, which does not have an undershoot in the lobster. The steady state outward current is maintained for as long as 100 msec.; this is in contrast to a decline of about 50 per cent in the squid axon. Lowering the external calcium concentration produces shifts in the current-voltage relations qualitatively similar to those obtained from the squid axon. On the basis of the data available, there is no reason to doubt that the Hodgkin and Huxley analysis for the squid giant axon in sea water can be applied to the lobster giant axon.

INTRODUCTION

Cole (1949) first showed that when the potential difference across the membrane was under control and varied in a stepwise fashion, the membrane current density of a squid giant axon varied continuously. Later, Hodgkin, Huxley, and Katz (1952) refined the technique and coined the name "voltage clamp;" and Hodgkin and Huxley (1952 a, b, c, d) interpreted a series of experiments now regarded as major contributions to the understanding of electrically excitable cells. Further, the use of the voltage clamp technique on the frog node by Dodge and Frankenhaeuser (1958) has given results similar to those obtained from the squid.

We have applied the voltage clamp to the lobster giant axon because the equations which Hodgkin and Huxley (1952 d) developed from their analysis of the squid giant axon should be tested on other axons. However, the lobster axon presents a problem since its diameter (about 100 micra) is small enough to make the longitudinal insertion of a short-circuiting wire very difficult, and the active area is not small and well delineated as in a myelinated fiber. It was apparent that it might be possible to produce a "node" in the lobster axon by bathing two sections of the axon with non-conducting solutions, which would act as artificial "myelin sheaths," leaving a narrow region between. In such an arrangement, the membrane potential inside the "node" could be measured from one end of the axon and current injection could be carried out via the other end. If the length of axon covered with insulating solution could be made short enough, the resistance of the axoplasm connecting an end to the "node" would be small compared to the "node" membrane resistance and electronic control would be possible. No internal electrodes would be needed.

These ideas developed from a consideration of the sucrose-gap technique introduced by Stämpfli (1954) for the study of the membrane potential of small bundles of frog fibers. A sucrose solution should form an insulating sheath around a single fiber too, and if two sucrose solutions could be separated by a narrow stream of sea water, a "node" could be produced in the lobster giant axon. Flowing sucrose solutions would have the advantage of forming sharply defined potential and current boundaries, which would be impossible to do with vaseline seals for example. Vaseline or mineral oil could not be used to form the insulating sheaths because they do not remove the ions in the layer of solution between the active membrane and the surrounding connective tissue sheath.

A sucrose-gap method for measurement of the lobster giant axon membrane potential was developed and is reported in the preceding paper (Julian, Moore, and Goldman, 1962). One effect shown there was a 20 to 60 mv increase in the resting potential of a lobster giant axon in the sucrose-gap arrangement. The exact cause for the hyperpolarization is unknown; however, the results so far obtained with this voltage clamp on the lobster axon are being presented since they are so similar to those obtained from the squid giant axon and frog node. A short report of some of this work has been given (Julian, Moore, and Goldman, 1961).

MATERIALS AND METHODS

The dissection technique, apparatus, and experimental procedure are described in detail in the preceding paper (Julian, Moore, and Goldman, 1962).

Single giant axons, about 100 micra in diameter and 3 cm long, were dissected

from the circumesophageal connectives of the lobster, *Homarus americanus*, and drawn through holes 300 micra in diameter in a lucite block as shown in the upper part of Fig. 1. Three pools are formed by two lucite partitions (striped areas) and two isotonic sucrose solutions (stippled areas). The central pool is filled with sea water and the two side pools, labeled I and V, are filled with isotonic potassium chloride (or other
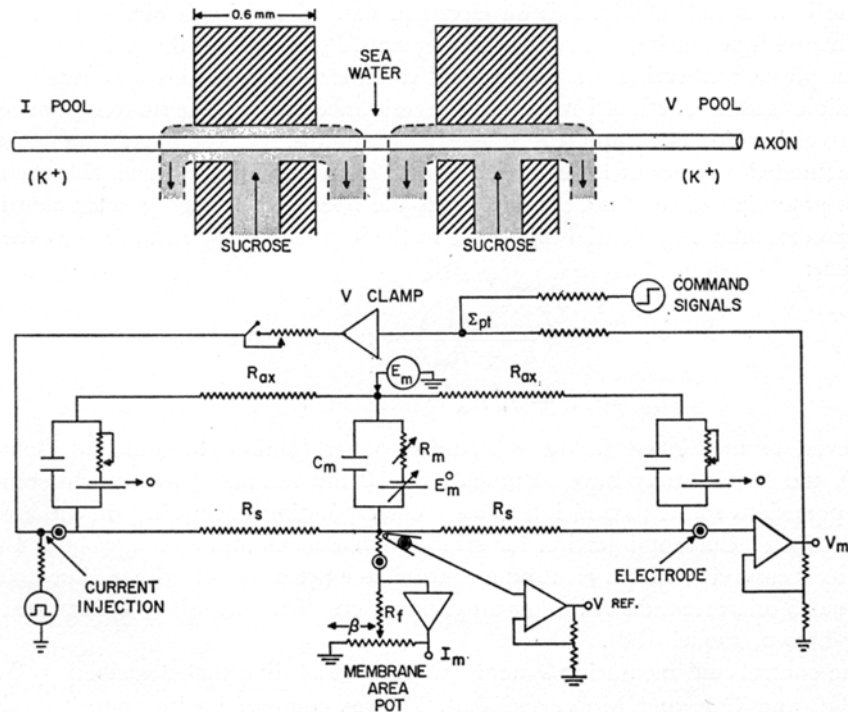

FIGURE 1.   Schematic diagram of experimental arrangement. In the upper part, striped areas represent lucite partitions, and stippled areas cover sections of axon bathed in flowing isotonic sucrose solutions. The axon extends through 300 micron diameter holes in each of the lucite partitions. Between the sucrose solutions, a small "node" of axon is exposed to a continuously moving stream of sea water. Solutions in I and V pools also continuously flowing. Notice that liquid junctions only are formed among the solutions.

Lower part of figure is electrical equivalent circuit of arrangement shown in upper part together with measurement and control apparatus. R_{ax} is resistance through axoplasm; R_s is resistance through sucrose solutions. C_m, R_m, and E_m are membrane capacitance, resistance, and potential of "node." E_m^o is the open circuit membrane potential. V_m is the potential difference between V pool and virtual ground. Current injection is *via* I pool through an isolation resistor. I_m measures current density flowing through "node" membrane. V_m and command signals form input of control amplifier labeled V clamp, whose output is connected to I pool through switch and variable series resistance. In order to clamp, switch is closed and resistance decreased to zero. V_{REF} is potential taken by low resistance micropipette placed just outside of "node"— its compensatory function is described in the text.

anion) in order to depolarize the membrane. All solutions flow continuously and liquid junctions are formed between solutions. The width of the "node" of axon membrane exposed to sea water in the central pool depends on the relative velocity of flow between the sea water and sucrose solutions, both of which are adjustable. The width was set under microscopic observation and was usually between 50 and 100 micra.

The bottom half of Fig. 1 is an electrical equivalent circuit of the arrangement together with measuring and control instruments. R_{ax} represents the resistance through the axoplasm connecting the central pool to either side pool and was usually about 100 kilohms for a length of 1 mm. R_s is the resistance through the sucrose from central pool to either side pool, and was at least 25 megohms. R_m, the membrane resistance of the "node," was about 1 megohm. C_m is the membrane capacitance, E_m, the membrane potential[1] of the "node," and E_m^o is the open circuit membrane potential. If the axon membrane potential difference in the V pool is reduced to zero by the KCl solution:

$$V_m = E_m \left(\frac{R_s}{R_s + R_{ax}} \right) \sim 95 \text{ per cent of } E_m$$

$$E_m = E_m^o + I_m R_m$$

However, as mentioned in the companion paper (Julian, Moore, and Goldman, 1962), the V pool may have as much as ± 10 mv residual potential difference in high potassium solutions and introduce a corresponding uncertainty in the absolute value of E_m. Current injection for stimulation and clamping was *via* the I pool. V_m was measured with an electrometer-transistor preamplifier with a time constant of about 5 microseconds. All other amplifiers were of the transistor operational type (Burr-Brown, model 130).

The control and measuring system was very much like that described by Moore (1959 a) and Cole and Moore (1960 a). V_m was compared with command signals (DC plus step voltage changes) at the summing point of an operational amplifier. This control amplifier applied current to the I pool in such a manner that V_m matched the command signal. The rise time of the control system to a step input was about 50 microseconds. "Node" membrane current was measured by connecting the central pool to the summing point of an operational amplifier, which holds its summing point very close to ground potential by negative feedback. The output of this amplifier was a voltage proportional to the membrane current density given by:

$$E_o = \frac{I_R R_f}{\beta}$$

where: R_f = feedback resistor
I_R = current flowing in R_f
β, $1 \rightarrow 0$, set after computing the membrane area of the "node" from measurement of its diameter and width.

[1] We use the term "membrane potential" throughout to indicate the potential of the "node" axoplasm with respect to an outside ground.

The standard artificial sea water was the same as that given by Dalton (1958). It contained (in mM):

Na	K	Ca	Mg	Cl	SO$_4$
465	10	25	8	533	4
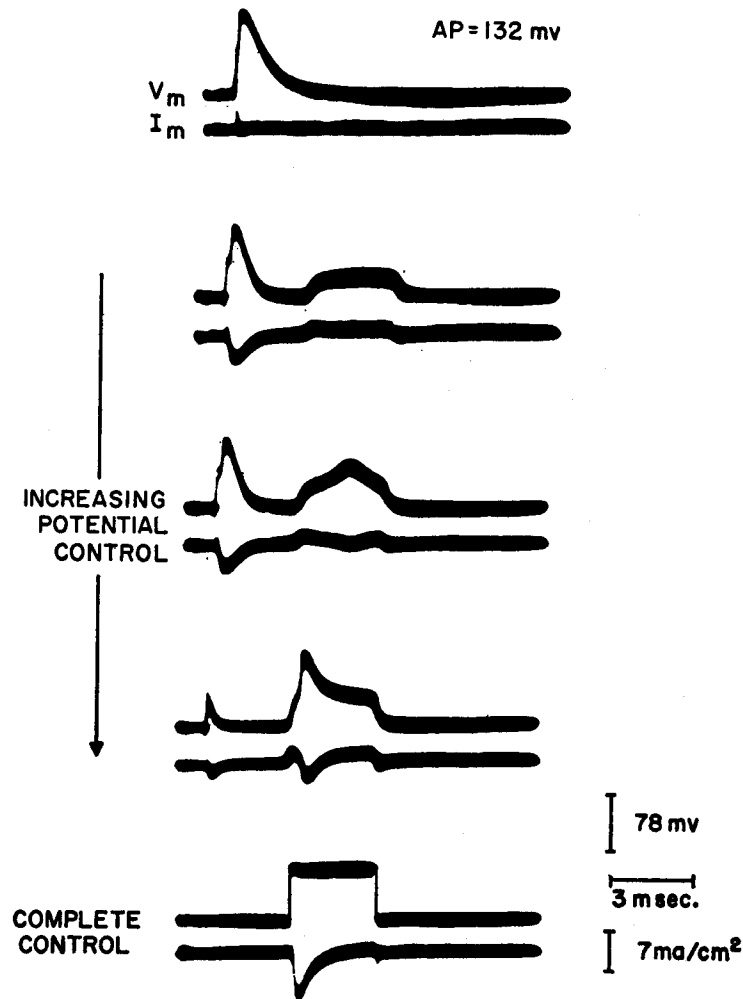
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FIGURE 2. Establishing potential control. Top record shows unclamped "node" membrane action potential. Then, switch in control loop closed and series resistance gradually decreased. Command signal consists of step changes in potential. Notice that the action potential has been completely clamped out in bottom frame even though stimulus was not turned off.

Usually, no buffer was added—the pH being slightly acidic. Choline chloride was recrystallized before use.

The electrodes were calomel half-cells connected to the pools *via* glass tubes filled with a saturated KCl-agar gel; the resistance through each half-cell and glass tube was about 750 ohms. A reference electrode was made by breaking the tip of a conventional 3 M KCl-filled glass micropipette until the electrode resistance was less than 1 megohm. Compensation for the external series resistance did not materially affect the current-voltage relation (as shown later) and the reference electrode was not used as routine.

The beginning of a typical experiment is illustrated in Fig. 2. The upper record shows an action potential initiated in an unclamped axon by a short current pulse. Then, the potential control loop was closed. Command signals consisting of step changes in potential plus a DC component to set the resting potential at the desired level were fed into the summing point of the control amplifier. A resistance in series with the output of the control amplifier was slowly decreased to zero, after which the control amplifier bandwidth was increased to maximum. In this way, gradual control of the membrane potential was achieved. Note that the action potential was clamped out because the control amplifier draws off the stimulating current pulse. In the bottom record, the membrane potential changes very nearly in a step-wise fashion; the associated membrane current pattern consisted of a large inward current followed by a small, late outward current. The command potential pulse could be varied in magnitude and duration and the resulting membrane voltage and current patterns photographed.
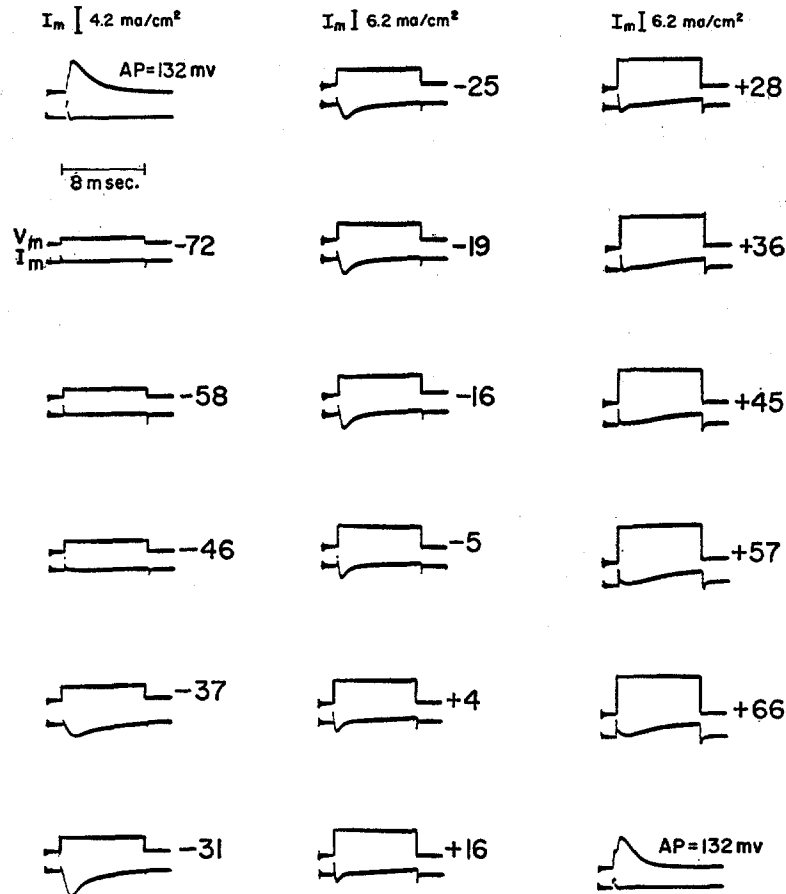
RESULTS

Membrane Current Patterns As A Function of Stepwise Changes in Membrane Potential

A series of membrane current curves produced when the membrane potential was varied over a wide range is shown in Fig. 3. The upper left hand record shows an action potential elicited before the control loop was closed. When the voltage clamp was applied, the internal potential of the "node" was pulsed in a positive direction—the value for the internal potential during a pulse is given at the right of each record. The bottom right hand record shows an action potential elicited after the clamp series had been completed and the control loop opened. The two action potentials are very similar in height, shape, and threshold so the clamp series did not have a deleterious effect on the "node."

The current patterns show striking similarity to those obtained from the squid giant axon by Cole (1949), Hodgkin, Huxley, and Katz (1952), and Cole and Moore (1960 *a*) and from the frog node by Dodge and Frankenhaeuser (1958). Membrane capacity charging and discharging current transients are visible at the beginning and end of the voltage step. Significant

current flow commenced when the membrane potential was raised to about −45 mv, or about the region of threshold in an unclamped axon. Above this point, an inward current gradually developed reaching a peak at about −25

I_m [4.2 ma/cm² I_m] 6.2 ma/cm² I_m] 6.2 ma/cm²

AP=132 mv   −25   +28

8 m sec.

V_m
I_m −72   −19   +36

−58   −16   +45

−46   −5   +57

−37   +4   +66

−31   +16   AP=132 mv

L 60-52

FIGURE 3. A number of membrane current-time curves produced as the membrane potential was varied in a stepwise fashion over a wide range. Number at right of each record gives internal potential of "node" in millivolts with respect to an outside ground. Current gain decreased in central and right hand columns. Top left and bottom right records show unclamped action potentials before and after potential control series. Inward current shown as downward deflection. Temperature, 10°C.

mv. Thereafter, it decreased until it reached +45 mv at which point the initial current, after the capacity transient, did not appear. The potential pulse required to take the membrane from the resting potential to this level was approximately equal to the height of the unclamped action potential.

At larger pulse amplitudes the initial current was outward. A point worth noting here is that under the condition of adequate control of the membrane potential, the initial inward currents are smooth, graded functions of membrane potential.

The initial current was followed by a delayed steady state outward current which did not decline. The delay in the onset of the outward current can best be seen when the initial current is flat ($+45$ mv). Cole and Moore (1960 b) and Moore et al. (1961 a) have shown that prehyperpolarization and cooling can delay the onset of the outward, or potassium ion, current in the squid giant axon. Since the resting potential of the lobster giant axon was increased in the sucrose-gap arrangement and the temperature in this case was 10°C, a marked difference with respect to onset of the outward current compared to the squid axon is not expected, although more work is needed on this point. The magnitude of the delayed outward current in the lobster giant axon is related to the duration of the repolarization phase of the action potential. That is, in axons whose action potentials showed a slow repolarization phase, there was a small outward current under voltage clamp conditions. In the squid axon (Hodgkin and Huxley, 1952 d), repolarization of the membrane from the peak of the action potential is hastened by an outward flowing potassium ion current. We have shown (Julian, Moore, and Goldman, 1962) that the decrease in membrane resistance associated with an action potential of the lobster giant axon has nearly disappeared before the repolarization phase is completed. Further, the last part of the repolarization phase is exponential; i.e., suggestive of a passive recharging of the membrane capacitance during that time. It would seem therefore, that in the lobster giant axon the falling phase of the action potential may not be associated with a large increase in the delayed outward current. In this case, repolarization would be influenced considerably by the rate at which inactivation of the increase in sodium conductance occurred. Most of this work has been done at 10°C or lower, and more data are needed over a wider temperature range. It is worth mentioning that in a few cases in which the temperature was lowered much below 10°C, the falling phase of the spike was drastically prolonged before marked changes in the rising phase were observed.

The data in Fig. 3 were plotted as membrane current density, initial peak, and steady state, as a function of membrane potential and shown in Fig. 4. Very small currents developed when the membrane potential was more negative than -50 mv and are not recorded on the graph. The inward current increased smoothly, reaching a peak amplitude of over 6 ma/cm^2 (typical values ranged from 5 to 10 ma/cm^2 at around 10°C). The resistance of the membrane in the negative slope region of the inward current was typically about -2.5 ohm cm^2; in the positive slope region, about 8.5 ohm cm^2. The value for the sodium equilibrium potential (E_{Na}) in Fig. 4 would be $+45$ mv,

assuming zero membrane potential in the V pool, which is rather low for the lobster. E_{Na} is defined as the internal potential at which the membrane current starts off with zero slope after the capacity transient. The outward current present at E_{Na} is generally taken to be a non-specific leak current, and usually had a value in the lobster similar to that shown in Fig. 4. The initial current pattern, then, is quite similar to that obtained from the squid axon.

The outward current develops with a delay, and the steady state value is a
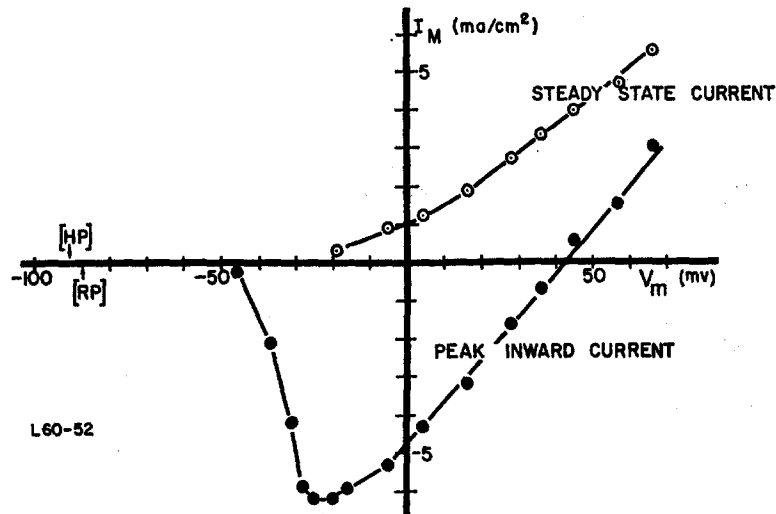
FIGURE 4. Current-voltage relation of the lobster giant axon. Points taken from data presented in Fig. 3. Black dots refer to peak current appearing after initial capacity transient; open circles with dots refer to amplitude of current in the steady state. [HP] = holding potential (−90 mv), level at which membrane potential held throughout clamp series. [RP] = resting potential (−88 mv), unclamped resting potential of "node."

less steep function of voltage than the initial peak current. In contrast to the squid axon in which the initial and steady state conductances are often about the same, the slope of the steady state current in the lobster axon varied from about equal to much less than that for the initial peak; that shown in Fig. 4 represents an intermediate value. As has been mentioned, a low slope steady state outward current characteristic in the lobster is observed when the re-polarization phase of the action potential is long. Such behavior does not necessarily imply a basic difference from the squid giant axon. Suitable adjustments in the parameters of the Hodgkin and Huxley equations should be sufficient to describe these curves and the lobster giant axon action potential, although such calculations have not yet been made.

Maintenance of the Steady State Outward Current

An obvious difference between lobster and squid giant axons in the voltage clamp is that in the lobster the steady state outward current does not decline

during potential pulses of length up to 100 msec. and of magnitude sufficient to carry the internal potential to the level of E_{Na} or beyond. In this respect, the lobster giant axon is similar to the frog node (Dodge and Frankenhaeuser, 1958). The explanation given by Frankenhaeuser and Hodgkin (1956) for the decline of outward current in the squid is that potassium accumulates during a potential pulse in a very small space between the membrane and an external diffusion barrier. This lowers the effective potassium equilibrium potential and therefore decreases the force driving potassium ions outward and causes the current to decrease. In Fig. 5, an example is given of the maintained
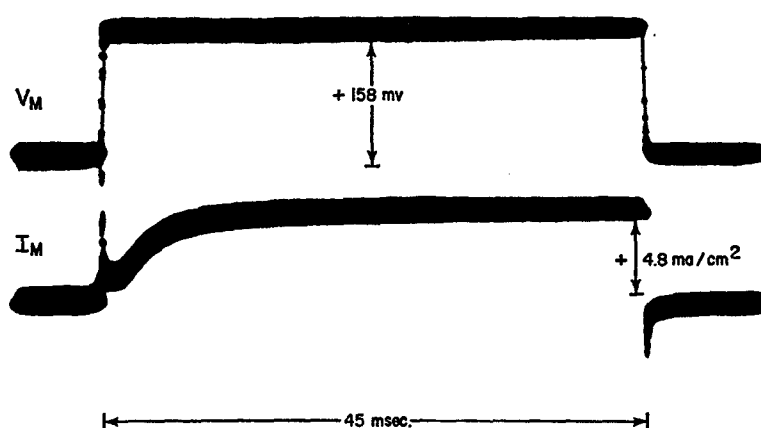

FIGURE 5. Maintenance of steady state current. V_m pulsed 158 mv in positive direction to vicinity of E_{Na} and held for 45 msec. Steady state outward current (upward deflection) does not decline at long times. Temperature, 10°C.

outward current of the lobster. The pulse duration was 45 msec. in this case, and the pulse amplitude took the internal potential to about the level of E_{Na}. In the squid axon, a tail of inward current develops at the turn off of the pulse which increases in magnitude with the duration of the pulse. This is a consequence of the progressive change in the potassium equilibrium potential, E_K, as potassium ions accumulate in the Frankenhaeuser-Hodgkin space. No such effects have been observed in the lobster even with pulses of 100 msec. duration.

The Relation between the Sodium Equilibrium Potential, E_{Na}, and the External Sodium Concentration

Hodgkin and Huxley (1952 *a*) and Moore and Adelman (1961 *b*) working with the squid giant axon and Dodge and Frankenhaeuser (1959) with the toad node found that the voltage clamp potential at which the initial current reversed varied with the external sodium concentration in a manner predicted

by the Nernst equation. Similar experiments on the lobster giant axon in the sucrose-gap are reported here.

E_{Na} has been defined as the internal potential at which, after the capacity transient, the membrane current starts off with zero slope. During this time (Hodgkin and Huxley, 1952 *a*), the internal potential is just positive enough to produce zero net sodium ion current. It is a period during which the membrane behaves as a "sodium electrode." Therefore, E_{Na} should be given by the Nernst equation:

$$E_{Na} = \frac{RT}{F} \log_e \frac{[Na^+]_o}{[Na^+]_i} \tag{1}$$

where $[Na^+]_o$ = concentration of sodium in the external solution

$[Na^+]_i$ = internal (axoplasm) concentration of sodium.[2]

At 7.5°C, equation 1 becomes:

$$E_{Na} = 56 \log_{10} \frac{[Na^+]_o}{[Na^+]_i} \, mv \tag{2}$$

The internal concentration of sodium in the lobster giant axon is not known. However, the change in E_{Na} to be expected from a change in external sodium concentration may be found as follows:—

$$E_{Na(1)} - E_{Na(2)} = 56 \log_{10} \frac{[Na^+]_o(1)}{[Na^+]_i} - 56 \log_{10} \frac{[Na^+]_o(2)}{[Na^+]_i}$$
$$= 56 \log_{10} \frac{[Na^+]_o(1)}{[Na^+]_o(2)} \, mv \tag{3}$$

Equation 3 should hold only if the internal concentration of sodium does not change appreciably during the course of the measurements.

The experiments were carried out as follows. E_{Na} was measured in sea water containing the standard concentration of sodium. Then, the external sodium concentration was decreased a certain amount by substituting choline chloride for sodium chloride, and E_{Na} was measured in the new solution. Sodium concentration was changed to 0.6 and to 0.2 of its normal value. A set of measurements in normal sea water, low sodium sea water, and after return to the normal solution could be completed within 10 minutes. The data from three axons fall close to the line drawn in Fig. 6 on the basis of the Nernst equation. Evidently, the passive movement of sodium ions down their electrochemical gradient can account for the initial current observed under voltage clamp conditions in the lobster giant axon, as in the squid axon and toad node.

[2] Activity coefficients in the two phases assumed equal.

The Current-Voltage Relation in High External Potassium Solutions

Moore (1959 *b*) has studied the current-voltage relation of the squid giant axon in 0.5 M potassium chloride. When the potential was controlled and varied, a continuous variation of the current was found, including a negative slope region. Further, when current was controlled and varied in high potassium, two stable states and even long duration "action potentials" could be obtained. Mueller (1958) has reported that a frog node can produce "action
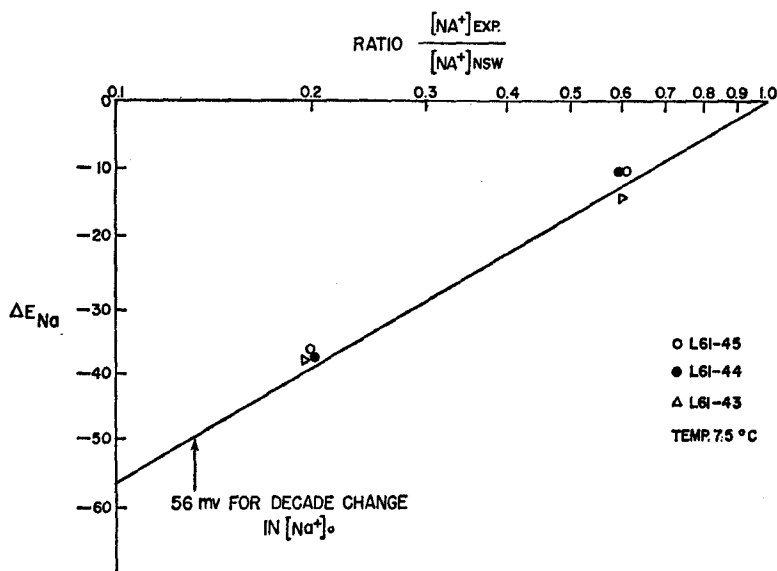

FIGURE 6. Change in E_{Na} plotted against ratio of sodium concentration in experimental solution (EXP) and in normal sea water (NSW). Line drawn on basis of Nernst equation for sodium electrode, at a temperature of 7.5°C. Circles, black dots, and triangles refer to data from three different axons.

potentials" in 0.1 M sodium-free KCl solution provided that the membrane potential is brought back to its original level by an external current. A simple explanation can be given for these findings based on the assumption that the curve relating steady state potassium conductance to membrane potential is about the same in high potassium as it is in sea water. When the potassium concentration is increased very much in the external solution, usually at the expense of sodium, the potassium equilibrium potential and the membrane potential are reduced to about zero. In this potential region, steady state potassium conductance of the membrane is high, but falls to a low value when the membrane is pulsed strongly to more negative potentials (or hyperpolarized). According to the voltage clamp analysis of the squid fiber made by Hodgkin and Huxley (1952 *b*), it would be expected that in high $[K^+]_o$ the

potassium current would be inward at potentials more negative than E_K. If these inward potassium currents are sufficiently large enough and the change in potassium conductance is strongly dependent on the membrane potential, then a region with negative slope should appear in the current-voltage plot.

It seemed worth while to test the lobster giant axon in high external potas-
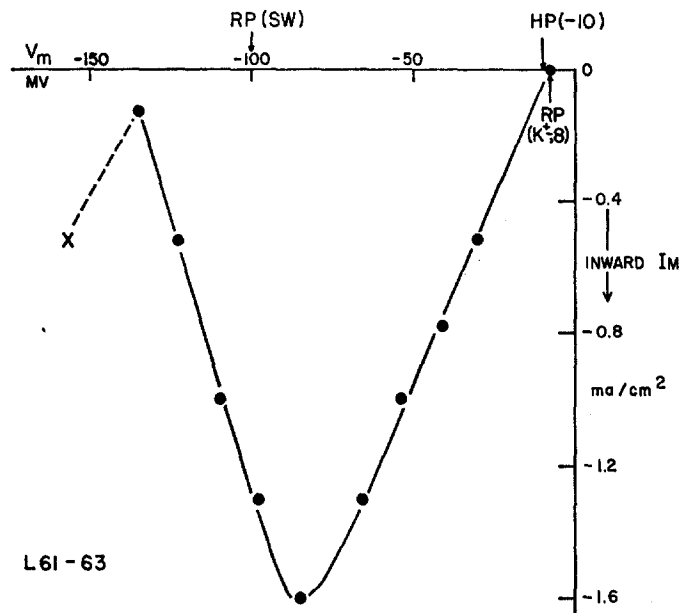

FIGURE 7.   Current-voltage relation of lobster giant axon in external solution of 0.5 M potassium thiocyanate and 25 mM calcium chloride. The resting potential of the "node" in normal sea water—RP(SW)—was −100 mv; in high potassium—RP(K+)—it was −8 mv. HP was the holding potential. The dashed line extending to the X refers to the membrane breakdown which occurred when the internal potential was pulsed more negative than 150 mv. Temperature, 10°C.

sium, particularly since a difference had been detected in the delayed outward current pattern of the lobster axon compared to that of the squid axon. The negative slope region was not always present in the lobster; this was unrelated to whether chloride, thiocyanate, or methylsulfate was used as the anion in 0.5 M solutions of potassium. However, there was a definite correlation between the amplitude of the outward current developed in normal sea water and the appearance of a negative slope region in high potassium.

The steady state current-voltage relation for a lobster axon in high potassium is shown in Fig. 7. The resting potential was shifted from −100 mv in sea water to −8 mv in high potassium. Inward current flowed as the internal potential was pulsed in a negative direction producing two positive resistance branches separated by a negative slope region. However, extension of the left

hand branch of the positive slope region was not possible because the membrane broke down rapidly with pulses forcing the axoplasm more than 150 mv negative. Consequently, "excitation" thresholds under controlled current conditions were not sought. Nevertheless, the current-voltage relation under potential control is rather similar to that observed in the squid giant axon by .Moore and expected from the Hodgkin-Huxley equations.

The Effect of Low Calcium on the Electrical Properties of Lobster Giant Axons

Using the voltage clamp technique, Frankenhaeuser and Hodgkin (1957) investigated the effect of changing the calcium concentration on the current-voltage relation of the squid giant axon. They observed that a fivefold reduction of the external calcium concentration shifted the curves relating peak sodium and potassium conductance along the voltage axis in such a way that a smaller depolarization was required to increase these conductances to a given size. The resting potential increased or showed little change when the calcium concentration was reduced. Adelman and Moore (1961) showed that a tenfold reduction of the normal external divalent ion concentration had little or no effect on the resting potential of the squid giant axon. Further, such a reduction increased the inward resting net sodium flux almost 3.5 times. There was a marked decrease in the amplitude of the action potential.

The situation seems to be somewhat different for the lobster giant axon. Dalton (1958) reported that the resting and action potential declined whenever the external calcium was reduced below normal levels. Dalton and Adelman (1960) showed that the decline in the resting potential of the lobster giant axon in low external calcium was continuous for periods of exposure up to 30 minutes. Adelman and Dalton (1960) suggested that the fall in resting potential might be the result of a continuous loss of internal potassium.

Investigation of the current-voltage relation of the lobster giant axon in low external calcium using the voltage clamp technique was carried out in order to compare the curves with those reported for the squid. However, good quantitative data were difficult to obtain because of the marked adverse effect of low external calcium on the resting properties of the lobster giant axon in a sucrose-gap arrangement. The normal concentrations of divalent ions in the artificial sea water used were 25 mM calcium and 8 mM magnesium. When calcium was reduced by a factor of 10, the resting potential fell precipitiously, the action potential declined, and the membrane resistance decreased. If the "node" was left in low calcium, the resting potential continued to fall with no sign of reaching a steady value. The effect was reversible provided that the "node" was exposed to the low calcium solution only for a few minutes. Voltage clamping under these conditions was difficult, since the "node" usually deteriorated steadily during the course of a clamp series. Especially noticeable in this respect was a large increase in the leakage current.

Nevertheless, shifts of 15 to 20 mv were observed of the peak inward current along the voltage axis in a direction such that smaller depolarizations were required to produce a given peak inward current. The magnesium concentration was increased to 53 mM in the low calcium solutions in order to test whether or not magnesium could substitute for calcium. However, this had little effect in preventing the fall in resting potential, which is in line with Dalton's finding (1958).
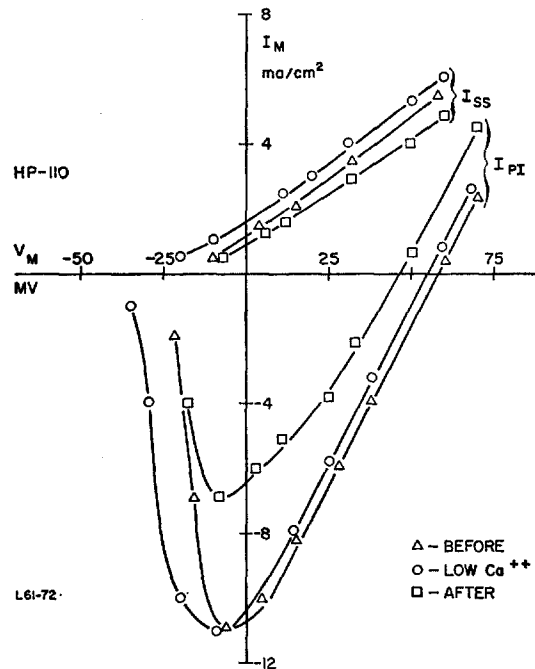

FIGURE 8. The effect of a fivefold reduction in external calcium concentration (from 25 mM to 5 mM) on the current-voltage relations of a lobster giant axon. I_{PI} refers to peak initial currents, I_{ss} to steady state currents. Tonicity maintained by adding equivalent amount of sucrose. Temperature, 10°C.

Since a .tenfold reduction in external calcium was so poorly tolerated, a fivefold reduction was tested. The effects described above were still present, though less marked. A rather large increase in the leakage current persisted and made analysis of the inward currents troublesome. Shifts of about 10 mv were observed in the inward currents in the same direction indicated above. These shifts were not prevented by increasing the magnesium concentration to 48 mM. Current-voltage curves for an axon before, during, and after exposure to low calcium are shown in Fig. 8. A shift to the left is apparent for both the peak initial and steady state current curves. E_{Na} decreased during the low calcium exposure indicating a rise in internal sodium concentration. The

decrease in E_{Na} accounts for much of the decrease in peak inward current observed after exposure to low calcium. The shifts in the current-voltage relations are at least qualitatively similar to those obtained from the squid axon.

Compensation for Error Introduced by External Series Resistance

A perfect voltage clamp requires the potential across the membrane capacity to be under control. In practice, however, there is usually some series re-
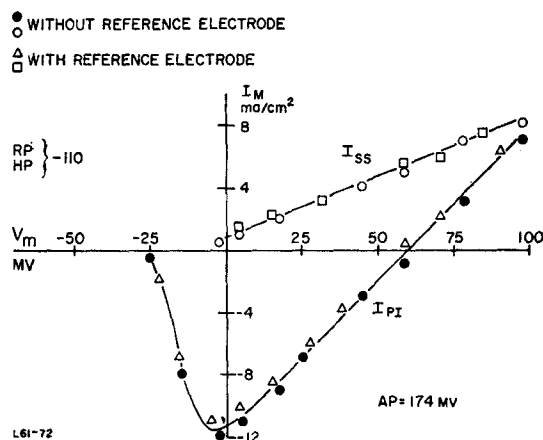

FIGURE 9. Current-voltage relations on an axon with and without compensation for the voltage drop across the series resistance between the outside of the "node" membrane and the summing point of the current-measuring amplifier. "Without reference electrode" refers to *I-V* relation taken in usual fashion; "with reference electrode" to *I-V* relation taken with compensation for the voltage drop across the series resistance. Temperature, 10°C.

sistance between the control points. In Fig. 1, the unlabeled resistance between the outside of the membrane and the summing point of the current-measuring amplifier represents series resistance. It consists mostly of sea water and electrode resistances. It may be measured as shown in Fig. 1. A micropipette was brought up from downstream until the tip was just about to make contact with the "node." A known current was injected through the membrane, and the potential developed across the series resistance was measured at the output of the amplifier labeled V_{REF}, from which a series resistance of about 1600 ohms was calculated. A representative figure for "node" area would be 2.7×10^{-4} cm^2. For a membrane current of 10 ma/cm^2, there would be an error in the membrane potential of 4.3 mv (10 ma/cm^2 $\times 2.7 \times 10^{-4}$ cm^2 $\times$ 1600 ohms). This calculation for series resistance does not take into account any resistance between the outside of the active membrane and the reference electrode—such as that of the Schwann cell layer and

connective tissue sheath. In addition, the current pattern in the axoplasm is distorted in the vicinity of the "node" thus giving rise to an additional difference of potential between E_m and V_m. However, an approximate calculation made by K. S. Cole (personal communication) shows this error to be negligible for an axon as small as the lobster.

For a membrane current of the order of 10 ma/cm^2, then, an error of 4 or 5 mv can occur in the measurement of the membrane potential. Fig. 9 shows the similarity of the current-voltage curves for a lobster axon with and without compensation for this series resistance error. The black dots and open circles refer to peak initial and steady state currents obtained in the usual way without compensation. The control system was then compensated for the voltage drop across the external series resistance by subtracting the potential of the reference electrode, V_{REF}, from the membrane potential, V_m, by an operational amplifier not shown in Fig. 1. In Fig. 9, triangles and squares refer to peak initial and steady state currents obtained with compensation. Curves were drawn as best fits through the points obtained from both procedures. It seemed that the series resistance present did not introduce a serious error as far as the purposes of this paper were concerned and, therefore, the uncompensated control system was used as routine.

Oscillations in Membrane Potential and Current

Many users of the voltage clamp technique have at some time observed notches or oscillations in the current pattern when the control of the membrane potential was inadequate. This effect usually occurs over the range of membrane potential in which the current-voltage relation of the axon has a steep negative slope. Tasaki and Spyropoulos (1958) have stated that such phenomena indicate a strong, basic tendency of the excited membrane to take one of two stable states. However, Cole and Moore (1960 *a*), and Taylor, Moore, and Cole (1960) have shown that notches in the current pattern of a squid axon can be produced by variation of a single experimental or equivalent mathematical parameter: surface impedance of the axial current supply electrode. If this impedance exceeds a certain minimal value, adequate spatial control of the membrane potential fails and notches and oscillations appear in the current record. Dodge and Frankenhaeuser (1958) report that in the frog node oscillations and all-or-none regenerative responses were seen, but were regularly traced to insufficient stabilization of the membrane potential. Dodge (1960) has shown that the presence of a very small capacitance between the outer pools in the usual gap technique for the frog node can account for the oscillations reported. Taylor, Moore, and Cole (1960) concluded that in order for voltage clamp data to have any clear meaning the potential across the membrane capacity must be uniform and constant over the entire region in which membrane current is measured.

It seemed obvious that the width of the "node" in the central pool would have to be quite small in order that the voltage gradient from one side of the "node" to the other be minimal. Furthermore, the potential gradient at the boundaries of the "node" would have to be extremely steep. For example, some experiments using very thin vaseline seals between the sucrose solutions
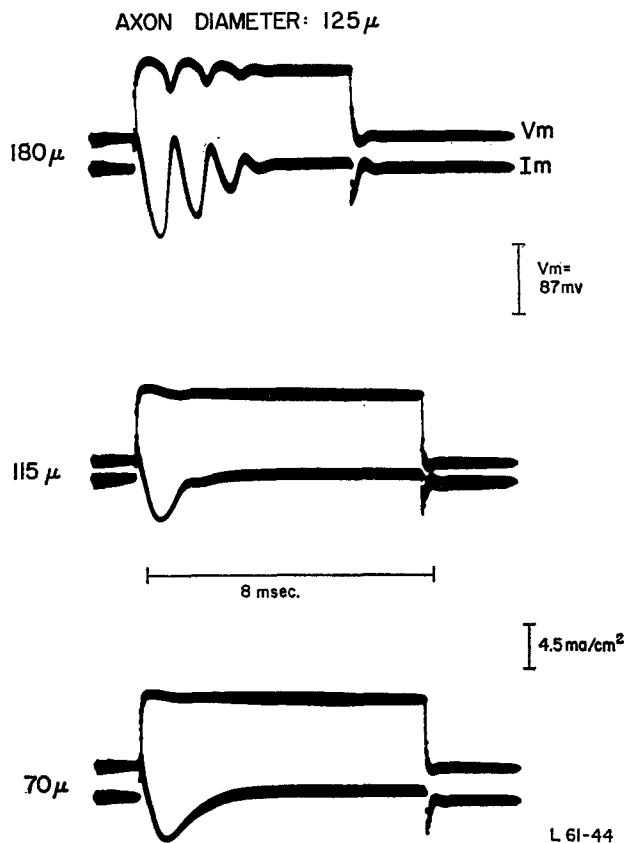

FIGURE 10. The elimination of oscillatory behavior in membrane potential, V_m, and membrane current, I_m, by progressively narrowing the width of the "node." "Node" width is given to the left of each record. The amplitude of the command potential pulse was the same in each case.

and sea water showed that in such an arrangement it was not possible to control the membrane potential. Potential control was very much improved by the use of liquid junctions and "nodes" having a width of less than an axon diameter. When oscillations in the current pattern were present, they were invariably associated with poor control of the membrane potential. On the other hand, when membrane potential control was good, as in Fig. 3, inward currents were perfectly smooth, graded functions of the membrane potential

and had no sign of any threshold. This is illustrated in Fig. 10 where an oscillatory pattern was transformed into a smooth response by merely reducing the width of the "node." The amplitude of the command signal was the same in each case, taking the membrane potential into the region of steep negative slope in the current-voltage relation. Oscillatory patterns could also be produced by an exceptionally high resistance of the axoplasm through which current was injected into the "node." For this reason, the lucite partitions were made as thin as possible in order to keep the resistance through the axoplasm path low. Finally, oscillations in potential and current appeared when the bandwidth of the control amplifier was reduced.

It is true that the appearance of a well controlled potential step, as shown in the bottom trace of V_m in Fig. 10, does not necessarily mean that the potential is uniform and constant in space throughout the "node." However, in a previous paper (Julian, Moore, and Goldman, 1962), we have shown that action potentials recorded by the sucrose-gap method and by a micropipette which has punctured the "node" are practically superposable. Though such experiments are not conclusive, they are at least reassuring that there is little spatial variation of potential in the "node." Cole (1961) has made an analysis of the membrane potential along a squid axon clamped with an internal current electrode and having external non-conducting boundaries applied in such a way that only a short segment of axon was exposed. He found that the potential was uniform over a sufficiently short length of exposed axon. As Cole points out, external sucrose seals could serve as the non-conducting boundaries. Such an analysis fits in with our finding in the lobster giant axon that the width of the "node" had to be short before potential control could be gained.

In summary, adequate control of the membrane potential could be achieved in the lobster if the following conditions were fulfilled: a short "node" width— less than an axon diameter (50 to 100 micra); non-conducting solution present at the boundaries of the "node;" low access resistance pathway for current injection; and the presence of sufficient gain and bandwidth in the control amplifier. Under such adequate potential control, the membrane currents were smoothly graded functions of membrane potential. Oscillations could be produced by variation of any of these parameters.

DISCUSSION

The voltage-clamped lobster giant axon responds to a variety of different experimental procedures in ways quite similar to those reported for the squid axon and frog node. On the basis of the data available, there is no reason to doubt that the analysis Hodgkin and Huxley (1952 *d*) made for the squid giant axon in sea water can be applied to the lobster. The behavior of the

peak initial current and the dependence of E_{Na} on the logarithm of the concentration difference of sodium follow quite closely the results obtained from the squid axon and frog and toad node; the region of turn on, magnitude, and values for negative and positive slope conductances are about the same as those reported by Cole and Moore (1960 a) for the squid giant axon. The peak initial current does not appear to deviate from a straight line relationship as Dodge and Frankenhaeuser (1958) reported for the frog node.

However, the steady state current of the lobster axon shows some differences. It has a variable slope conductance less than that of the peak initial current; the magnitude of the steady state slope conductance appears to be related to the duration of the repolarization phase of the action potential. The steady state outward current is maintained for as long as 100 msec. in the lobster axon, as in the frog node; this is in contrast to a decline of about 50 per cent in the squid axon. An explanation of this difference between lobster and squid giant axons may be that the lobster fiber does not have a Frankenhaeuser-Hodgkin space. According to Geren and Schmitt (1954), both the squid giant axon and nerve fibers from the walking legs of the lobster have Schwann cell layers about 0.5 micron thick. In both types of fiber the interface between axon and Schwann cell is formed by a pair of membranes. However, tortuous channels passing from the axolemma to the outer surface of the Schwann cell were rarely present in the lobster fibers. An electron microscope study of the lobster giant axon would be very helpful, particularly with respect to the question of whether or not any channels were present between axolemma and outer surface of the Schwann cell.

From the evidence at hand, it appears likely that the lobster giant axon differs from the squid axon in that the resting potential of the lobster axon decreases markedly, perhaps continuously, in low external calcium, and in the fact that magnesium does not seem to substitute for calcium. It remains to be investigated whether this is an effect of the use of sucrose. The fact that E_{Na} decreased after exposure to low calcium solutions is in line with the finding of Adelman and Moore (1961) of an increased rate of sodium accumulation by a squid giant axon bathed in sea water having 0.1 times its normal calcium and magnesium concentrations.

In evaluating the data, the following errors and uncertainties must be kept in mind. Resistance through the external sucrose barriers was finite but should have made the measured value of V_m low by only about 5 per cent. The membrane potential of the section of axon lying in the V_m pool could have differed from zero potential by as much as ± 10 mv, although this uncertainty was probably within ± 5 mv of zero for most of our experiments. Measurement of "node" area had an uncertainty of about 10 per cent and may have varied by a similar amount over the time for an experiment. At high membrane current densities, the presence of a small series resistance

introduced an error of 4 or 5 mv in membrane potential measurements, but was neglected for the purposes of these survey experiments. None of the results or interpretations presented in this paper needs to be significantly revised on the basis of errors and uncertainties of this magnitude.

The study of the permeability properties of electrically active membranes is very greatly advanced by the use of membrane potential as the independent variable, since the mechanisms which allow ionic currents to flow in the membrane are primarily voltage-dependent. The establishment of potential control over a known area of membrane through which current flow may be measured presents a severe technical problem in most cells. The value of the sucrose-gap method on a long, cylindrical cell like the lobster giant axon is somewhat weakened by the effects of the sucrose on the electrical properties of the "node" membrane. Obviously, such effects need further study before a final decision on the usefulness of the technique can be made.

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