

Ionic Current Measurements in the Squid Giant Axon Membrane

KENNETH S. COLE and JOHN W. MOORE

From the National Institutes of Health, Bethesda, Maryland, and the Marine Biological Laboratory, Woods Hole, Massachusetts

ABSTRACT The concepts, experiments, and interpretations of ionic current measurements after a step change of the squid axon membrane potential require the potential to be constant for the duration and the membrane area measured. An experimental approach to this ideal has been developed.

Electrometer, operational, and control amplifiers produce the step potential between internal micropipette and external potential electrodes within 40 microseconds and a few millivolts. With an internal current electrode effective resistance of 2 ohm cm.², the membrane potential and current may be constant within a few millivolts and 10 per cent out to near the electrode ends.

The maximum membrane current patterns of the best axons are several times larger but of the type described by Cole and analyzed by Hodgkin and Huxley when the change of potential is adequately controlled. The occasional obvious distortions are attributed to the marginal adequacy of potential control to be expected from the characteristics of the current electrodes and the axon.

Improvements are expected only to increase stability and accuracy. No reason has been found either to question the qualitative characteristics of the early measurements or to so discredit the analyses made of them.

INTRODUCTION

Measurements of the ion current across the squid axon membrane, after a sudden change of the membrane potential difference, are of considerable interest in studies of the structure and function of excitable membranes. There does not, however, seem to have been an appropriate consideration of the degree to which the experimental conditions should and do approximate those assumed. Except as this can be done, the extent to which the current measurements represent the desired properties of the membrane is difficult to estimate.

The interrelations between the axon membrane and the equipment for

A summary of this paper and one in manuscript (Taylor, Moore, and Cole, TMC) has been presented to the National Academy of Sciences (Cole, Moore, and Taylor (1959)).

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investigating it are complicated, at best. Historical background, criteria of performance, and descriptions of equipment and procedures are given to help understand and evaluate the difficulties, defects, and accomplishments in these and other tests and in the results. They also serve as an introduction to consideration of the even more involved critical phenomena to follow (TMC).

This paper develops requirements for reasonably adequate measurements of the axon properties, describes the performance of the experimental system designed to meet these requirements, presents data to indicate that the system has been developed to a reasonably satisfactory stage, and points to some improvements that seem advisable.

History

Many quantitative analyses of the electrical properties of a uniform nerve fiber have relied heavily upon the partial differential equation used most successfully by Lord Kelvin a hundred years ago to describe the characteristics and to predict the performance of the submarine cable. Many subsequent investigations, such as those of Weber, Hermann, Cremer, and Adrian, showed that communication by means of a nerve fiber is quite different from that provided by a cable. Nevertheless, this equation has continued to serve as a help and a guide towards an understanding not only of the nerve but also of its most successful analog—the passivated iron wire.

The cable equation describes a nerve as an external longitudinal resistance, r_1 ohms/cm., and internal longitudinal resistance, r_2 ohms/cm., and a surface membrane with a capacity, C farad/cm., and leakage resistance, r_3 ohms cm., and, in terms of the potential difference across the membrane, V volts, distance, x cm., and time, t sec., is

$$\frac{1}{r_1 + r_2} \cdot \frac{\partial^2 V}{\partial x^2} = C \frac{\partial V}{\partial t} + \frac{V}{r_3} \quad (1)$$

But this cable equation could not be used with any real confidence before Höber (1912, 1913) had demonstrated the interior of an intact cell to have an electrical conductance and to be comparable in this respect to its environment, or before Fricke (1925) had measured the electrical capacity of a cell membrane, or before Blinks (1930) had found a membrane conductance. Even as more and more different types of cells were found to have rather similar internal conductances and membrane capacities (Cole, and Curtis (1950)), the possibility still remained that nerve, and perhaps other irritable cells, might have quite different characteristics. The squid giant axon, however, was found to conform with an internal resistivity of about 30 ohm cm. and a membrane capacity near 1 μ f./cm.² (Curtis and Cole (1938)). It also first provided the figure for the resting membrane resistance of 1000 ohm cm.² (Cole and Hodgkin (1939)) that also seems to be not far from typical for many other cells. Using these elementary physical properties the cable equation quite adequately described the *passive* performance of the axon as an impossibly slow and inefficient means of communication.

By analogy with the iron wire model, it had seemed reasonable to think that a nerve membrane might all but disappear during excitation if an ion permeability increase took place as suggested by Bernstein. This ion permeability of the membrane—as measured by its electrical conductance—did increase many fold during the passage of a propagating impulse along a squid axon but the capacity of the membrane was relatively unaltered (Cole and Curtis (1939)). Since this capacity also changed but little by comparison with the variations of the conductance that accompanied a number of other experimental situations, it became reasonably safe to expect the cable equation to apply to an *active*, as well as to a passive axon, in the more general form,

$$i_m = \frac{1}{r_1 + r_2} \cdot \frac{\partial^2 V}{\partial x^2} = C \frac{\partial V}{\partial t} + i_i \quad (2)$$

in which the total and the conductive currents, i_m and i_i in amp/cm., appear in place of r_3 as illustrated by Fig. 1 a. It was thus possible to describe the ionic current in

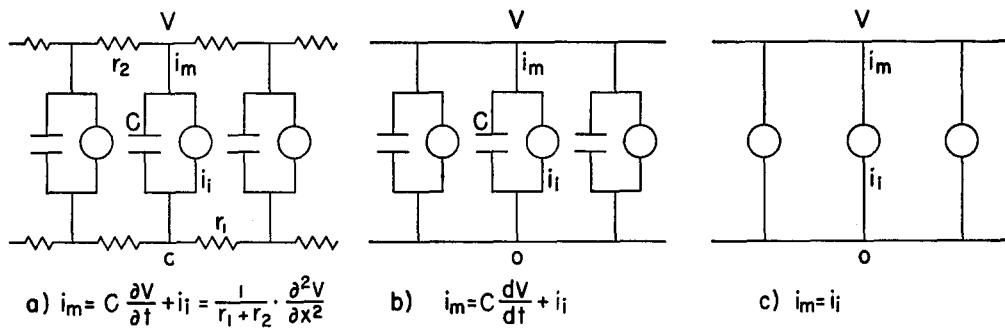


FIGURE 1. Axon circuits and equations. r_1 and r_2 are the external and internal longitudinal resistances and C is the membrane capacity. V and i_m are the membrane potential and current and i_i is the ionic current flow around the capacity. x and t are distance and time.

- (a) The usual cable approximation for an unrestrained axon with a non-linear membrane.
- (b) An axon with perfect internal and external electrodes to eliminate spatial variations of potential and propagation.
- (c) After the application of a constant potential between the electrodes only an ionic current flows across the membrane without a threshold or other instability.

the membrane as it changed with the potential difference during an impulse. In electrical terms it could be seen that the membrane resistance was not only non-linear but indeed so non-linear as to become negative for a while and to force the current to flow against the potential difference across the membrane. But without more specific information as to the relation between V and i_i as a function of time, the spatial variation of V could not be predicted by the cable equation and under conditions of spatial variation this information could not be obtained.

Although the capacity characteristics of a passive iron surface were rather less definite than those of the squid axon, the model seemed to have a very considerable

advantage in that a small surface area could be used under conditions of relatively uniform current densities and potential differences. Thus, on a microscopic scale at least, $\partial^2V/\partial x^2$ was rather small by comparison with its contribution to the phenomena of an extended wire. Since it is this term that is responsible for the propagation of excitability, the promise in this approach lay in the simplification of analyses to be made possible by the *prevention of propagation*. A number of early investigations had demonstrated an instability of the potential difference across the passive iron surface of the type to be expected from a system having negative resistance characteristics and a high resistance in the external measuring circuit. Since it was generally recognized that such a system could not be stable if the applied potential difference were not nearly constant, Bartlett (1945) was persuaded to use the alternative approach of constant potential. Although his results with a quite low resistance external circuit were more complicated than was expected or could then be interpreted, the concept of *potential control* which was later to be applied to the squid axon—and again to the passive iron wire with similar results by Franck (Bonhoeffer (1953))—had been developed and used for an irritable system.

Although the work on the iron wire model had not been particularly productive, the similar approach to the characteristics of a living axon seemed no less attractive and important. The prevention of propagation by elimination of the space derivative and the consequent reduction of the cable equation from a partial to an ordinary differential equation shown in Fig. 1 b were achieved with a long, large, current-carrying electrode inserted along the axis of the squid axon and an external, concentric measuring electrode with a similar guard at each end as described by Marmont (1949). Rather extensive and mostly unpublished investigations were made of the effect of membrane current patterns, imposed by electronic feedback control, upon the membrane potential. These measurements confirmed many of the conclusions from less direct experiments and gave absolute values for some characteristics, such as threshold, that had not been otherwise inferred with satisfactory certainty under less drastically controlled conditions.

In many ways the behavior of the membrane potential was disappointingly similar to that of an axon without spatial and current control and emphasized even more strongly than before the amazing power of the membrane to function in its normal all-or-nothing manner. Since this occurred without a net current flow across the membrane, it finally seemed obvious that the ionic current flowing against the potential difference could come only from the charge on the membrane capacity and so change the potential difference in the direction to require a yet larger ionic current in the early stages of the action potential. This process was, however, much too complicated for a satisfactory analysis and a control of the membrane potential again suggested itself. This was undertaken in the hope of achieving stability in the negative part of the ionic resistance characteristic, and so to avoid the all-or-none response. This control of the potential at a constant value after a sudden change would also eliminate the capacity displacement current since $i_c = dV/dt = 0$ as shown in Fig. 1 c. The remaining ionic current showed *no indication of any discontinuous behavior* as either a function of the time after a displacement of the potential from its resting value or as a function of the size of the potential step (Cole (1949a)). For

potential steps from the resting potential to values lying between the threshold and the maximum of the all-or-none response, the ionic current first rose to a maximum in the inward direction—and so against the applied change of potential—before it reversed to a maintained flow in the direction of the applied step, while outside this range there was no “reverse current”—as it was then described. Even this statement of the experimental results was seen to be adequate to account for excitation and its

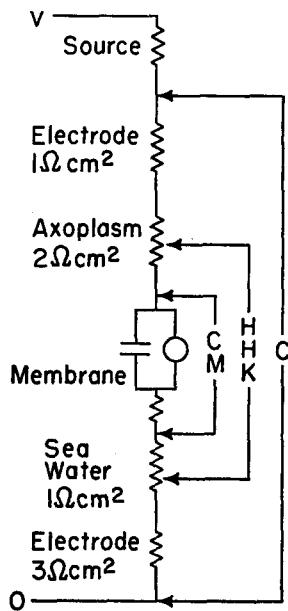


FIGURE 2. Development of the membrane potential control: C , between the current electrodes by Cole (1949a); HHK , between one potential electrode inside near the axis of the axon and another outside by Hodgkin, Huxley, and Katz (1952); CM , between two reversible potential electrodes close to the membrane, a micropipette inside and a small pipette outside, by Cole and Moore (this paper).

propagation in an unrestrained axon—giving a threshold for the all-or-none response, and its maximum amplitude and recovery.

There were rather few of these experiments, the axon survivals were not long, and the potential control which relied upon the current-carrying internal and external electrodes as shown in Fig. 2 by C , was far from ideal. Also the potential difference between these electrodes could be directly affected by events occurring in and beyond the guards as well as by current flow through the impedances between the electrodes and the membrane capacity in the central measuring region.

In confirming, extending, and interpreting these observations so spectacularly, Hodgkin, Huxley, and Katz (1952) and Hodgkin and Huxley (1952)¹ christened the potential control a “voltage clamp” and restricted it to the measuring region. They used separate current and potential electrode wires both inside and outside the axon to avoid the effects of electrode polarization, indicated in Fig. 2 by HHK , and they made some use of a compensated feedback that essentially neutralized part of the resistance between the potential electrodes and the membrane capacity.

The particularly useful and probably highly significant contribution, which they

¹ These five papers will be referred to as HHK, HH I, II, III, IV.

might not have been able to make without potential step data, is that the ionic current can be well described in terms of but two independent variables, membrane potential, V , and time, t . It then was technically feasible to compute, very successfully, far more of the important electrophysiology (HH IV; Cole, Antosiewicz, and Rabinowitz (1955, 1958); Huxley (1959); FitzHugh (1960)) than had been apparent in the original data. But this unique role of the membrane potential also strongly suggests that this potential provides the fundamental force by which the mechanisms of ion permeability are controlled.

Subsequent Developments

In starting to build upon these advances soon after their publication, it seemed that only a few further modifications should be needed to make a critical examination of the data and the analyses and to extend the application of them towards a better understanding of the physiology and pharmacology of nerve in particular and of the membrane permeability processes in general.

A careful examination of the HH data suggested that even for their peak inward currents of about 1 ma./cm.², the resulting 3 to 4 mv. error in the membrane potential, as indicated in Fig. 2 by HHK, might be significant. Consequently one of the first steps (Moore (1958a)) was to move the internal potential electrode as close as possible to the membrane capacity by replacing the axial potential measuring wire with the micro salt bridge that just penetrated the membrane and a calomel half-cell (Ling and Gerard, 1949, often referred to as a micropipette electrode, microelectrode, or microtip). Subsequently a larger tip with a similar reversible potential electrode, was placed close to the outside of the axon as indicated in Fig. 2 by CM and Fig. 12. Continuing experience with these potential electrodes showed that the axons with the higher resting and action potentials and the longer survivals produced inward and outward membrane currents of up to 5 or 10 ma./cm.² (Cole (1958a)). For such axons the membrane potential, if controlled by an axial electrode, would have varied by 20 mv. or more in both directions and could not have been assumed to be even approximately constant. In addition to drastically reducing such errors, the pipette potential electrodes have allowed the use of reversible and stable half-cells. This has become increasingly important as evidence has accumulated to show that, as the resting potential changes, the ionic currents are much more dependent upon the actual, or "absolute," value of the potential difference across a membrane than they are upon the departures from the resting potential that were used previously by Cole and HH.

Along with its advantages, the use of the microelectrode brought the need for radical improvements in the fidelity and stability of the preamplifier output. These have been met by compensation for the capacity of the tip of the microelectrode and input leads and by chopper stabilization of the amplifier itself.

As more of these axons were found to give the higher membrane currents that have since come to be considered as normal, the deviation, called a "notch" (Fig. 11), in the usual ionic current pattern first made its appearance. Both the existence

and the amplitude of this erratic notch in the otherwise smooth transition from an inward to an outward current were soon found to be at least roughly correlated with the surface impedance of the axial current electrode. Others have explained this and similar patterns as an effect of action potentials arising at the ends of the electroded regions (Frankenhaeuser and Hodgkin (1957)) or as basic membrane phenomena (Tasaki and Bak (1958); Tasaki and Spyropoulos (1958)). Continuing and somewhat successful efforts to decrease the internal and external current electrode impedances have considerably reduced the incidence of the notch even for the hyperpolarized, high temperature axons that are the most difficult in this respect. Conversely both unintentional and deliberate use of higher impedance electrodes has practically guaranteed the production of a notch by a good axon (TMC; see also Taylor and FitzHugh (1959)).

These observations also emphasized the need for a low impedance output from the control amplifier while the highest ionic currents also required a larger output from it. But these properties along with the use of as high a control amplification as possible in turn tended both to make stability more difficult and the consequences of instability more disastrous. As modifications and additions to the original technique became important, the increasing complexity of the system (Fig. 13) led to the use of the operational amplifier, as developed for electronic analog computation, for a basic unit to give flexibility and stability while the considerable use of automatic methods provided rapid and accurate recording of much of the experimental data.

These continuing and various developments of more than a decade have strengthened, elaborated, and refined the original idea of measuring the ionic membrane current caused by a sudden change of the potential difference across the capacity of the membrane. But we believe that this progress has not yet modified, exhausted, or replaced this concept as a uniquely useful path towards the understanding of the excitability and permeability of the squid axon membrane.

Criterion for a Voltage Clamp

The results and interpretations of voltage clamp data—whether constructive, or critical, or both—are significant only to the extent that the experimental methods conform to the fundamental concept. As the various aspects and consequences of this concept have been developed it has become clear that when the measured current is the result of a potential difference that is either unknown or variable, any descriptions of the current and any suggested permeability theory based on the assumption of a constant potential may be wrong. Thus the general, formal, experimental criterion is both simple and obvious:

The potential difference across the membrane capacity shall have a known and constant

value during the time and over the area of the membrane in which the current flow is measured.

This criterion can probably be met only by a constant potential applied to perfect electrodes in direct contact with the membrane capacity. Until such an ideal experiment is possible, the effects of practical concessions to axon survival and equipment capabilities upon the experimental results need to be examined.

Conventions

In an attempt to avoid confusion, the present discussion will be limited to the voltage clamp concept and measurements. No matter what the beliefs of the authors may be, we will here refrain from any implications as to the interpretations of the concept and measurement. We do, however, find it convenient to use some of the rather well known terms of interpretation that are experimentally well defined. Thus for present purposes, ionic current means only the current that may be assumed to flow in parallel to the measurable membrane capacity, peak sodium current is close to the maximum inward current flow, sodium potential is the potential at which the fast component of the ionic current reverses its direction, and so on. Similarly the membrane potential is considered as an independent variable along with time.

The conventions of sign are those used recently by Hodgkin (1958), Tasaki and Bak (1958), and Taylor (1959). The membrane potential differences are referred to the external solutions as measured by reversible half-cells but are without liquid junction correction. The usual resting potential is thus negative and the action potential starts from this value to become somewhat positive before returning. An outward flow of positive charges is a positive current, as the first potential control membrane currents were presented.

The resistances used here are usually "variational" or "steady state" rather than the zero time or infinite frequency conductances used by HH as discussed in Appendix A. The nominal axon and electrode characteristics used in this paper are presented in Appendix B.

REQUIREMENTS, TESTS, AND RESULTS OF POTENTIAL CONTROL

It has not yet been possible to apply perfect electrodes directly onto an area of axon membrane and observe the current flow for the chosen differences of potential between the electrodes. The capabilities and limitations of the less direct experimental means that have had to be used are to be examined and understood as well as possible. Estimates of the departures of the membrane

potential from ideal constancy then form the basis upon which the resulting membrane currents are to be considered as valid experimental results.

The use of a microelectrode inside and a small nearby or "reference" electrode outside the axon has resulted in a measurement of membrane potential difference that emphasizes the properties of the membrane in their immediate vicinity and is less affected by the more distant portions of the membrane. Because of this localized measurement, the control of the membrane potential is similarly restricted. It therefore becomes necessary to differentiate between the membrane phenomena at the control point or in the control region and those—which may or may not be similar—occurring at points remote enough to have little if any influence upon the measured potential. Thus both the phenomena and the requirements are to be separated into those of the controlled and the uncontrolled regions. The extent and interaction of these regions are not simple to define nor are they easy to understand, but it will be seen that the control region may be of an essentially indefinite extent with near to ideal conditions or may correspond to less than a millimeter length of a squid axon under some conditions.

The phenomena at the control region are of basic importance and they will be presented first. The less definite or more difficult problems of the behavior outside of this control region will be considered up to the limits of stability in this paper and beyond those limits in a subsequent paper (TMC). The forms of the membrane current that are to be found in regions under good potential control will then be presented.

In each of the following sections, estimates will be made of the desirable approximations to the ideal requirements and, along with them, such evidence as is available on the experimental performance will be given.

Control of the Membrane Potential

The experimental arrangement indicated in Figs. 3 and 12 has potential electrodes, i and r , close to the membrane, which control the current supplied less directly to the membrane by electrodes a and e . The control system of Figs. 13 and 20 provides this current by an amplification, μ , of the difference between the membrane potential and the value desired, E . Equation (6), derived in Appendix C, shows that the system produces a potential difference between the potential electrodes that is the same as would be produced by an equivalent E.M.F., $E/(1 + 2/\mu)$, in series with the equivalent resistance, $R/(1 + \mu/2)$. So for an amplification of $\mu > 200$, the control is quite good as this E.M.F. is within 1 per cent of E and the resistance is less than 1 per cent of R , the sum of the electrode, axoplasm, and sea water resistances.

The operation and the effectiveness of the potential control are demonstrated in Fig. 3 by the records of the potentials, potential differences, and

current in the control circuit. The command to change the membrane potential from -70 to -20 mv. was obeyed quite promptly and accurately—as shown in the record of V_m obtained between the potential electrodes, i and r . In order to do this, the control amplifier had to provide a potential to the axial electrode, a , that ranged between -60 and -5 mv. as shown in the top

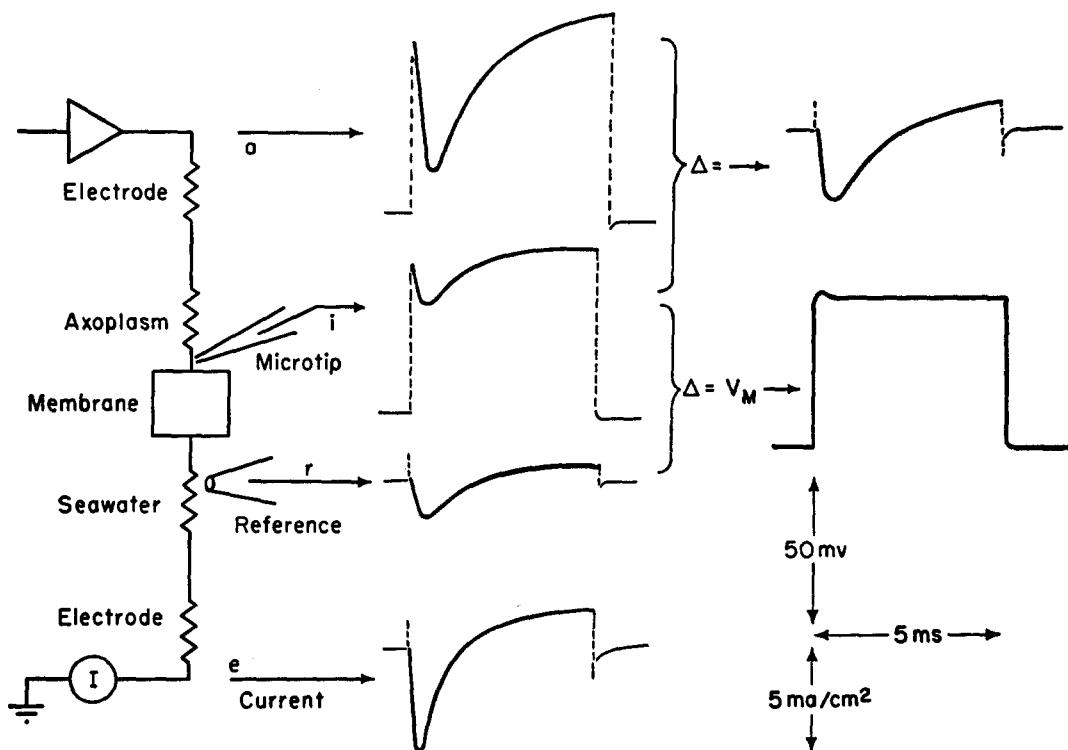


FIGURE 3. Control of the membrane potential. At the command to change the membrane potential between the internal and external potential electrodes, i and r , the control amplifier at the top has first to discharge the membrane capacity as indicated by dotted lines on records. The amplifier has then to produce the potential and the current, shown at a and e , to maintain the membrane potential shown by the difference record, V_m . The internal potential difference across the axial current electrode and axoplasm is shown in the differential record, Δ , and that across the external current electrode and sea water is given directly at r .

record and the appropriate ionic current, with an inward peak of 5 ma./cm.², that is shown by the bottom record.

ACCURACY OF POTENTIAL CONTROL Extensive experience has shown that the peak inward current may change very considerably for small differences of the applied membrane potential in the range from -20 to -50 mv. In

Fig. 6, for example, a difference of 1 mv. in the applied potential resulted in a change of 0.5 ma./cm.² for a peak current of 2.5 ma./cm.². Consequently an error of less than 1 mv. in V_m is certainly desirable. This, in turn, would require the equivalent resistance in series with the membrane capacity to be as little as 0.1 ohm cm.² for a membrane current of 10 ma./cm.².

The average effective resistance of the better axial electrodes has been 1 to 2 ohms for a cm.² of axon membrane and that of the axoplasm also about 2 ohms while the external sea water and electrodes amount to an additional 4 ohms. An important and encouraging check upon these estimates of the internal and external resistances is obtained under experimental conditions from data as shown in Fig. 3. The internal resistance, as given by the ratio of the potential difference between the axial electrode and the microtip to the current density, averages 3.6 ohm cm.² during the pulse and, similarly, the external resistance, between the reference electrode and ground, averages 2.2 ohm cm.².

However, the total of 6 to 8 ohm cm.² is entirely within the feedback loop and can be made negligible by a control amplification of more than 200. Practical tests of the equivalent resistance have been made on axons and on a resistance-capacity model of the axon and its electrodes. The change of the membrane potentials upon the introduction of a known current at the axial electrode showed that the equivalent impedance external to the potential electrodes was less than 0.1 ohm for a cm.² of membrane with a control amplification of $3 \cdot 10^4$ and demonstrated that the potential electrode control was entirely adequate.

There is, however, another and less obvious source of error in the potential control. In order to obtain stability of the control system it has been necessary to reduce the high frequency cutoff of the control amplifier. A result of this is to introduce an error into the control potential which is proportional to the time derivative of the membrane current, as can be seen in the record of V_m of Fig. 3. This effect may reach a value of 5 mv. in extreme cases.

The control of membrane potential at the potential electrodes, as indicated in Fig. 3, was reasonably accurate and constant but it was found advisable to independently confirm such evidence with a potential probe introduced into the axon from the end opposite to that at which the axial current electrode was inserted. The typical records of Fig. 4, for a good axial electrode, show the accuracy and constancy of the membrane potential near the potential electrodes in following the command pulses. It is interesting and also somewhat ominous to find that the largest deviation occurs for the potential at which control is the most difficult and notches are most frequently apparent. This may, however, be somewhat exaggerated because the probe usually was not as close to the membrane as was the micropipette electrode

and consequently should be expected to show some of the potential drop across the axoplasm. However, the conclusion may be drawn from these and similar other measurements that the membrane potential difference was known and constant to within a few millivolts in the immediate vicinity of the control electrodes. This conclusion applies not only to the better axial electrodes but also to those that were inferior or even bad to the extent of having an effective resistance of 5 or more ohms for a square centimeter of axon membrane. Even in the face of the serious failures, considered by TMC,

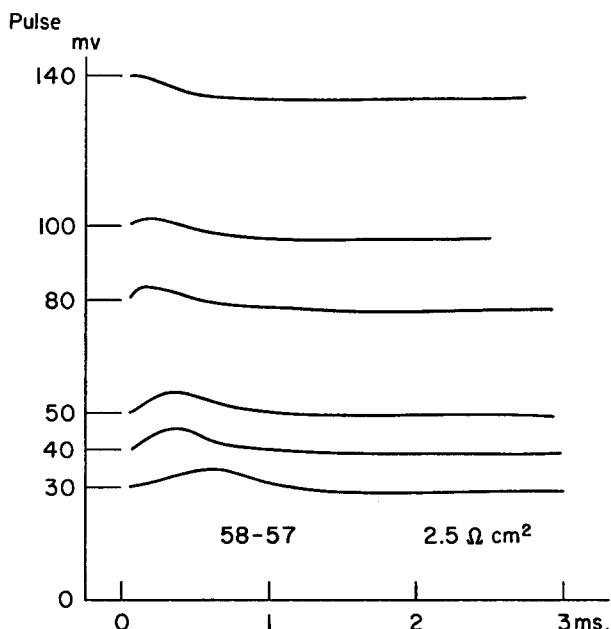


FIGURE 4. Differences of potential between the internal micropipette electrode and a nearby internal probe, introduced as shown in Fig. 10. The traces show the variations of this potential after application of the depolarizing pulses given as ordinates for a good axial current electrode, 2.5 ohm cm^2 .

on one, or both sides, the potential near the control point as measured by the potential probe was found to be accurate to within a few millivolts.

The accuracy of the potential difference across the membrane capacity is less certain than that between the potential electrodes. The bulk axoplasm and sea water between the potential electrodes may contribute 0.4 ohm cm^2 but the evidence of a larger membrane resistance in series with the capacity, shown as R_s in Fig. 20, raises a more serious and as yet unsolved problem. With the original current clamp Cole and Marmont calculated an average of 4.8 ohm cm^2 (unpublished) and the data given by HHK lead to 3.4 ohm cm^2 while current incomplete work suggests a somewhat lower value. Since

the feedback control does not operate in the current path between the potential electrodes, a series membrane resistance of 1 ohm cm.², for example, could result in an error of up to 10 mv. in the potential difference applied to the membrane capacity and its parallel ionic current path.

It may be that this error can be corrected by the compensated feedback introduced by HHK and incorporated in the control system as a "negative resistance" (Fig. 13), but the procedure has not yet been fully investigated. The error could be computed if the resistance and the ionic characteristics at constant potential were known but it has not been found possible to reverse the process and compute these characteristics from experimental data. However, it seems reasonable to expect that the slope of the negative limb of Fig. 6, for example, should not be as steep because the capacity potentials in this region are more positive than those between the potential electrodes that have been used for abscissae. An improvement in the control would then be expected to somewhat reduce the requirements for its accuracy.

However, without some form of correction even more serious errors of the potential control are to be expected for potential electrodes farther away from the membrane. With internal potential electrodes near the axis of the axon, Cole and Marmont had an average series resistance of 9.1 ohm cm.², including the electrode impedances, HHK had an average of 7.6 ohm cm.², and presumably Tasaki *et al.* had somewhat similar values. Consequently the errors of potential could well have been some five to twenty times as large as for potential electrodes close to the membrane.

Noise, drift, and hum in any part of the entire circuit may have the same effect upon the measured current as a change of the membrane potential, and their cumulative effects should also be equivalent to as much less than 1 mv. at the membrane as is reasonably possible. Careful attention to the design and construction has usually brought the noise well below a millivolt although some had to be tolerated in order to obtain speed of response in some amplifiers. Chopper stabilization of all amplifiers and stable potential electrodes have reduced the drift to a matter of a millivolt over several hour periods. The effect of hum has been practically eliminated by careful grounding and by synchronizing the experimental pulses with the sixty cycle power supply.

SPEED OF MEASUREMENT AND CONTROL The potential across the membrane capacity should be changed from a normal, or "holding" potential, to an experimental level and from one experimental potential to another quite rapidly in order that the transient capacity charging current be over early enough to allow for a good measurement of the initial ionic current before significant ionic conductance changes take place. At the higher temperatures and above the Na potential an appreciable conductance change occurs in

less than 50 μ sec. so that the initial capacity transient should be over within 20 μ sec. It is also generally desirable to have the potential move from one value to another with only an optimum overshoot.

This goal has not been reached consistently and over-all response times² from 30 to 50 μ sec. without overshoot have been more usual. In Fig. 5, the records of the membrane potential after a step command potential have response times of 27 and 66 μ sec. but the capacity transients in the membrane current are of nearly equal durations. The initial transients have usually been made shorter than those at the return to the holding potential or to other experimental levels. Although all amplifiers in the control circuit contribute to this delay, the most important single component has been the microelectrode and its preamplifier which has not had a response time much less than 10 μ sec. Preliminary experiments using a bridge circuit, shown in

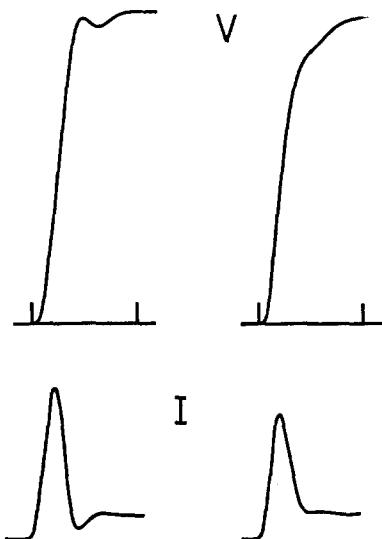


FIGURE 5. Records of the membrane potential (upper) and membrane current (lower) to show the response time of the control system and the capacity transient in an artificial axon circuit. The abscissae, time, units are 100 μ sec. The left and right hand pairs of records were obtained with different control characteristics.

Fig. 13, to balance out the capacity transient have been very promising and without the interference of the recorded transient a 40 μ sec. time for the change of potential is probably relatively unimportant. The peak condenser current of about 5 ma. required for a 50 μ sec. response has not been a difficult amplifier load but would become more of a problem for increased speeds.

STABILITY The control system should not only respond rapidly, but it must also be stable in face of the low negative resistance region of the membrane characteristic in the range from -10 to -50 mv. The conditions for stability in this region are not simple except for those cases in which it is reasonable to

² For present purposes, the response time is that from the start of a step input until the response remains within 5 per cent of the final value.

assume the membrane characteristic to be steady state, as given in Appendix D. It has not yet been possible to test the validity of such an assumption in general—either by analysis or by the extensive numerical computations now in progress. For the present the peak inward current *vs.* potential characteristic, as is shown in Figs. 6 and 21, will be used as if it were a steady state characteristic. Another approach is to consider the current at a fixed time after the start of the potential pulse. The negative resistance limb of this isochronal current *vs.* potential characteristic is found to be rather constant

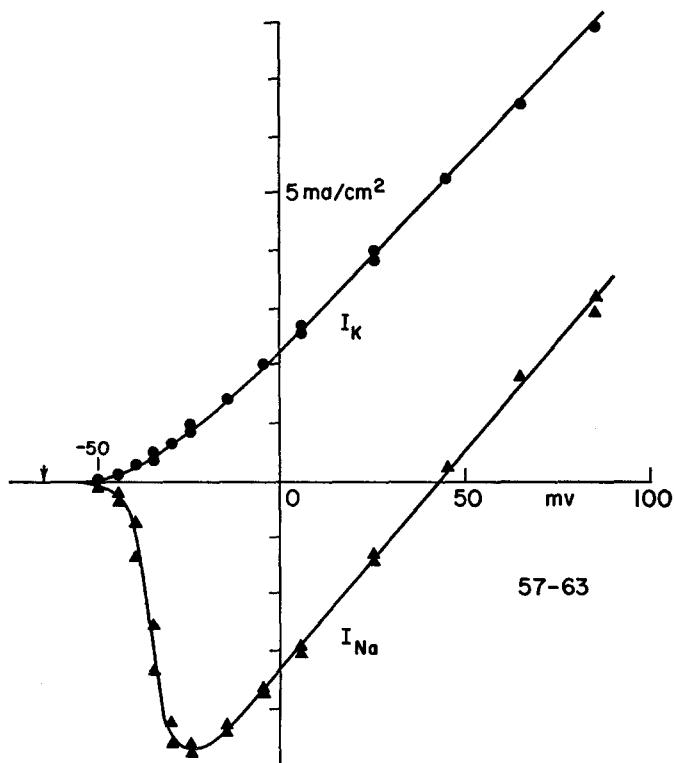


FIGURE 6. Typical results for the peak inward current, I_{Na} , and steady state outward current, I_K , after changes from a holding potential of -65 mv. to the potential given as abscissae. The scatter of points in the negative resistance limb of the I_{Na} characteristic indicates the sensitivity of this current peak to small changes of potential.

between 0.5 and 1.5 msec. and to give about the same negative resistance value as that of the peak current characteristic. The negative resistance limb of the peak current characteristic between -20 and -40 mv. has averaged about -4 ohms cm^2 with a limit of about -2 ohms cm^2 . On the assumption of a steady state, even the most difficult axons should then be stable according to Appendix D with an equivalent external resistance of less than 2 ohm cm^2 .

Any series membrane resistance, R_s , should, however, not enter into this consideration because the data from which the figure of -2 ohm cm.^2 was obtained already contain the effect of this resistance. On the basis of the earlier guess, an improved potential control, excluding R_s , would increase the numerical values of the negative resistances and so increase the margin for the stability of the system.

The system can thus be expected to be stable at the control point because the equivalent external resistance was measured to be less than 0.1 ohm cm.^2 and practically negligible as far as stability is concerned. On the other hand it seems quite possible that the control of axons such as these by the earlier axial potential electrodes would have been not only grossly inaccurate but also unstable.

Spatial Uniformity of Membrane Potential

The current density at the membrane should be measured over a well defined area in which the membrane potential is uniform. It may be interesting and significant to confine the current measurement to an area in which the current density is also uniform rather than to have it extend over an area in which current densities vary considerably. This is not, however, a necessary condition for perfect potential control and is not even desirable in the many cases for which an average current density is more useful.

However, under experimental conditions, the control region has a limited distribution which is determined in part by a characteristic length, or space constant. Similarly both the range of effect of a non-uniformity in or near the measuring region and the penetration of action potentials into the ends of the electrode regions may also be thought of in terms of the appropriate characteristic length. Although it seems obvious that the ranges of influence of non-uniformity and of impulse invasion should be as restricted as possible, it would be highly desirable to have a control region of considerable extent, if such were possible.

CABLE THEORY An approach to an understanding of the factors involved in the steady state distribution of membrane potential along an axon is obtained by extending the elementary cable theory, from equation (1), to include the effects of axial and external electrodes. In Fig. 7, r_1 , r_3 , and r_5 represent the radial resistances of the axial electrode plus nearby axoplasm, the membrane plus nearby axoplasm and sea water, and external electrode plus nearby sea water, respectively, in ohm cm. while r_2 and r_4 represent the longitudinal resistances of the axoplasm and sea water in ohms/cm. The usual applications of Kirchhoff's laws give

$$\frac{d^2V_2}{dx^2} = -\alpha(V_0 - V_2) + \beta_1(V_2 - V_4); \quad \frac{d^2V_4}{dx^2} = -\beta_2(V_2 - V_4) + \gamma V_4 \quad (3)$$

where

$$\alpha = r_2/r_1, \beta_1 = r_2/r_3, \beta_2 = r_4/r_3, \gamma = r_4/r_5$$

are the squared attenuation factors in cm.^{-2} , contributed by the axial electrode, the inside and outside of the membrane, and the external electrode, respectively.

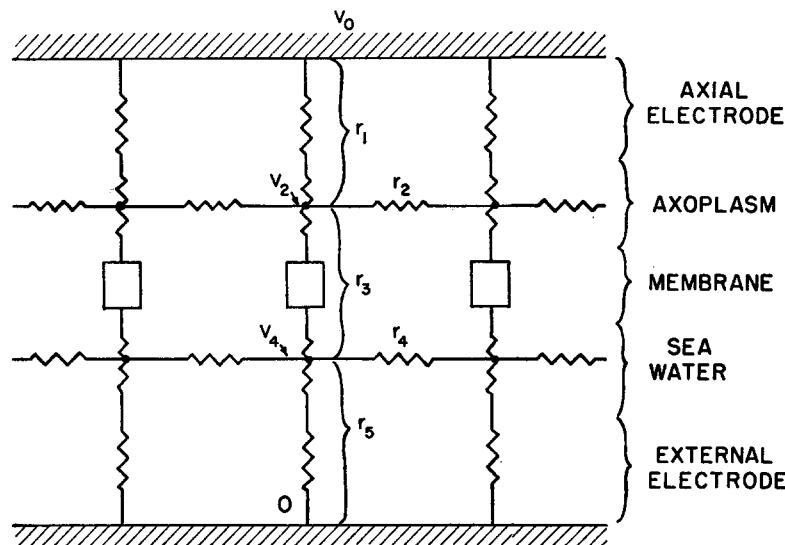


FIGURE 7. Equivalent circuit for an approximate analysis of the steady state distribution of potential along an axon membrane with a potential difference V_0 between the internal and external current electrodes.

The membrane potential satisfying these equations is

$$V_m(x) = V_2(x) - V_4(x) = Ae^{-x/\lambda_1} + Be^{-x/\lambda_2} + V_2(\infty) - V_4(\infty) \quad (4)$$

where

$$1/\lambda_1^2 = (\alpha + \beta_1 + \beta_2 + \gamma + D)/2, 1/\lambda_2^2 = (\alpha + \beta_1 + \beta_2 + \gamma - D)/2$$

and

$$D^2 = (\alpha + \beta_1 + \beta_2 + \gamma)^2 - 4(\alpha + \beta_1)(\beta_2 + \gamma) + 4\beta_1\beta_2$$

The evaluation of the arbitrary constants, A , B , from the boundary conditions in a particular case may be rather involved. For the present experimental conditions it has been found (TMC) that the effect of the external sea water

is small and

$$V_m(x) \simeq Ae^{-x/\lambda} + V_m(\infty) \quad (5)$$

is correct within 10 per cent for $\lambda = 1/\sqrt{\alpha + \beta_1}$.

Without this simplification the spatial variation of V_m will not extend beyond that determined by the larger of λ_1 and λ_2 . When the electrode resistances are high, so that both β_1 and β_2 are much larger than α and γ , $\lambda \simeq 1/\sqrt{\beta_1 + \beta_2}$ which is the usual characteristic length. If, on the other hand, α and γ are much larger than β_1 and β_2 , $\lambda_1 \simeq 1/\sqrt{\alpha}$ and $\lambda_2 \simeq 1/\sqrt{\gamma}$ —as can be derived directly on the assumption of a negligible membrane conductance. In general it can be said that the characteristic lengths λ_1 , λ_2 will be dominated by the largest of the attenuation factors α , β_1 , β_2 , γ . Thus it is found with values given in Appendix B, that at the lower membrane resistances, such as 7.5 ohm cm.², the membrane characteristic length is less than 0.6 mm. and the current electrodes can only decrease it. Conversely, with a non-conducting membrane a good axial electrode gives an internal space constant $\lambda_1 = 1/\sqrt{\alpha} = 0.4$ mm. and an experiment on such a model axon gave an external space constant $\lambda_2 = 1/\sqrt{\gamma} = 0.6$ mm. Positive membrane resistances can only decrease these values and consequently a characteristic length of more than a half millimeter is not expected.³

Calculations of the interaction between any two points along an axon can become quite complicated but on the basis of equation (5) the effects of longitudinal current flow and longitudinal conductance are negligible between regions separated by more than two or three times the characteristic length. Then, since even the more general space constants are near 0.5 mm. or less, it is to be expected that regions 0.5 mm. away from the control point will be under rather weak control and that those more than a millimeter away are practically beyond the control region.

EFFECT OF AXON AND ELECTRODE VARIATIONS With variations of axon diameter, membrane current density, or current electrode resistances, portions of the membrane outside the control region may not be subjected to the same potential difference as that at the control point. At the control point the potential difference between the current electrodes, V_0 , must vary at each time approximately according to $V_0 = V_m + RI_m$, where R is the resistance between the electrodes and the membrane, ohm cm., and I_m is the membrane current, ma./cm. or $V_0 = V_m + R \cdot 2\pi a \sigma$ for an axon radius a , cm., and

³ Although this analysis gives characteristic lengths comparable to the axon diameter and more complete calculations have not been made, it is not expected that the usual one dimensional cable theory assumptions of uniform internal and external longitudinal current densities cause serious errors (*cf.* Taylor (1960)).

membrane current density σ , ma./cm.². If then outside the control region these quantities vary by δV_m , δR , δa , and $\delta\sigma$ from their values at the control point,

$$\delta V_0 = \delta V_m + 2\pi Ra\sigma \left(\frac{\delta R}{R} + \frac{\delta a}{a} + \frac{\delta\sigma}{\sigma} \right) = 0,$$

since the current electrode potential difference, V_0 , is the same everywhere. For $R = 45$ ohm cm., $a = 0.025$ cm., and $\sigma = 5$ ma./cm.²,

$$-\delta V_m = 35 \left(\frac{\delta R}{R} + \frac{\delta a}{a} + \frac{\delta\sigma}{\sigma} \right) \text{ mv.}$$

If, as before, the membrane potential is to be known and constant to within 1 mv. in the measuring region, the sum of the variations in electrode resistance, diameter, and current density should be less than 3 per cent. These variations should not be expected to cancel. Therefore the value of each of them should be kept to within 5 or 6 per cent of that at the control point. The spatial uniformity of our axial electrodes has not been thoroughly investigated but there are some indications that the variation in the effective resistance was not more than 10 per cent along the length of the best electrodes. For the size of squid and the axon cell usually used, the region of the axon at the "crossover" of the stellar and fin nerves often came into the center chamber and had variations of diameter of as much as 20 per cent. Occasional measurements with differential electrodes showed a few axons to have a membrane current density that was uniform to within a few per cent while others had variations of up to a factor of two, particularly in a negative resistance region. Although the membrane potential may thus be expected to vary over a 5 mm. length of axon by considerably more than the desired amount in extreme cases, the power and convenience of the average measurement have much to recommend it.

STABILITY At the control point, any small deviations of the membrane potential are so corrected by the feedback control that the system is stable in the negative resistance region as shown in Appendix D (Fig. 21a). However, without the control, the small variations are not corrected and the system may be unstable as shown in Fig. 21b. Regions of the membrane close to the potential electrodes are somewhat restrained by "local circuit" currents that flow longitudinally inside and outside the axon. But as these currents decrease with distance from the control point, the membrane potential approaches one of the two stable points, B and C of Fig. 21b.

This instability outside the control region is to be feared with the most powerful axons. The axoplasm and sea water resistance of 4 ohm cm.², with-

out even the 1 ohm cm.² or less for the best axial electrode, should not be expected to be adequate for an axon showing a region in which the resistance is -2 ohm cm.².

TESTS OF SPATIAL POTENTIAL CONTROL Rather extensive tests of the control of the membrane potential have been carried out under a variety of experimental conditions (Moore and del Castillo (1959)). One group of experiments

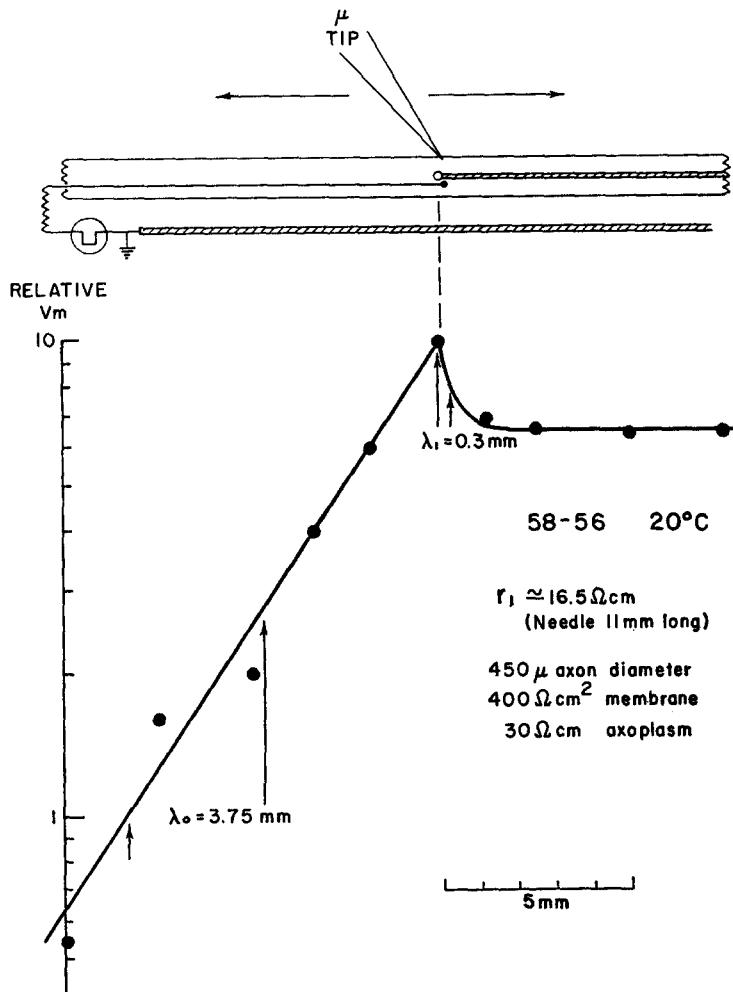


FIGURE 8. Distribution of the change of membrane potential caused by the introduction of a small current near the end of an axial electrode. The potentials were recorded from the micropipette electrode inserted at points along the axon. The current was applied through the probe electrode from the left with the rather good axial electrode at the right left unconnected or "floating." The parameters listed above are reasonable values chosen to give the calculated solid line.

was designed to test the degree and extent of control under conditions to which the elementary linear theory might be expected to apply. In this a small constant current was introduced into the axoplasm by a probe electrode close to one end of an axial electrode. The internal potential was measured with a micropipette electrode at points on both sides of the current electrode and end of the axial electrode with the results for one experiment given in Fig. 8. The solid curve, which rather well fits the experimental data, is that given by a particular solution of equation (5) which it is not appropriate to derive or discuss here. The axial electrode properties used are approximately those found for the electrode before and after the experiment while the axon membrane properties have been chosen to best fit the behavior beyond the end of the axial electrode where they are most important. Although the membrane resistance is lower than that usually found for the best axons, it is quite evident, first, that the theory is entirely adequate to express the variation of the potential in this somewhat complicated situation and, second, that this theory and this experiment agree in demonstrating that one of the better axial electrodes, such as was used in this experiment, is quite adequate to control the membrane potential except within the expected short distance from its end.

Another group of experiments was made to determine the penetration of an action potential into the electrode region. The axon was stimulated beyond the end of the axial electrode and the internal potential measured with a probe electrode. In the first case, shown in Fig. 9 by the dotted line, the axial electrode was left unconnected and so served only to equalize the potential over its length as required by the membrane properties at and between its ends. Although the axon was obviously in poor condition between the stimulating electrodes and the end of the axial electrode, the action potential rose rapidly to a reasonable value as the impulse came into the axial electrode region and remained expectedly constant for as far as it was measured. Although not done in this experiment, it has several times been found that after the introduction of a low impedance axial electrode a rather poor axon—showing a considerable variation in the amplitude and form of the action potential propagated without an axial electrode—was able to produce an essentially constant action potential of nearly the previous maximum over the electrode length. However, a constancy of amplitude and form over the length of an axial electrode is only a necessary and not a sufficient indication of a satisfactorily uniform potential. A rather more dramatic test is that of the simultaneity of potentials as well as the equality of amplitude and form at points near the two ends of the axial electrode. Results reported elsewhere (del Castillo and Moore, 1959) show that action potentials simultaneously recorded by two micropipette electrodes more than a centimeter apart in the

axial electrode region were identical to within a millivolt and a few microseconds.

The second series of measurements in Fig. 9 shows the invasion of an externally initiated impulse allowed by a comparatively poor axial electrode held at the resting potential. The effective resistance of 10 ohms cm^2 and calculated characteristic length of 0.7 mm . are seen to be reasonable where the action potential—or probably the passive spread—is finally blocked. Along

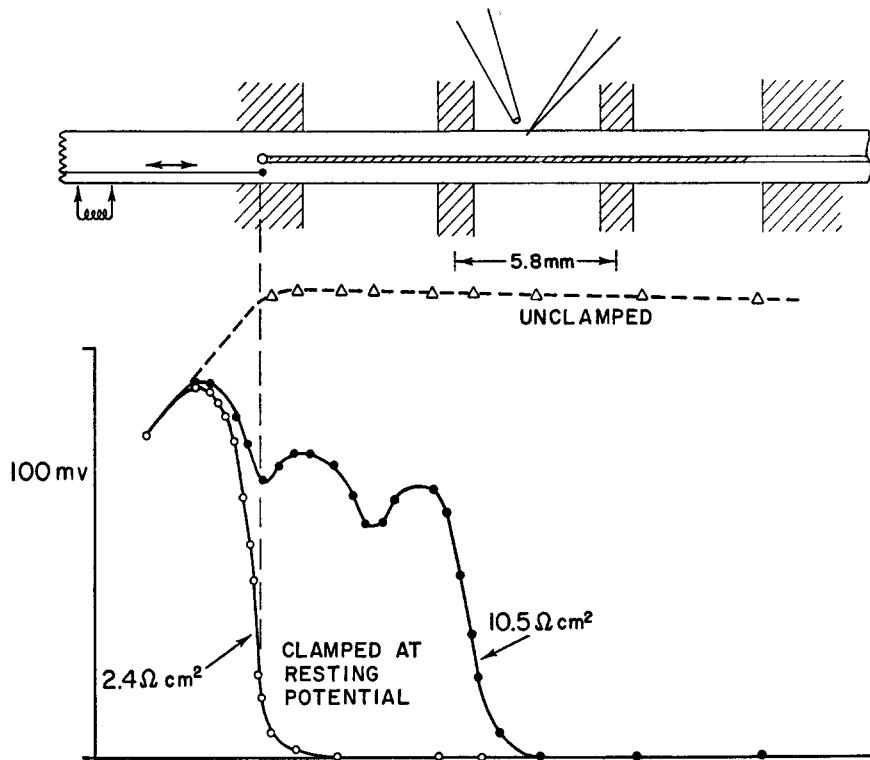


FIGURE 9. Measurements of the height of action potentials in the axial electrode region. The axon was stimulated at the left and the membrane potential measured with the internal probe inserted at the left. The triangles and dotted curve show the near uniformity of potential with the axial electrode not connected. The solid circles show the extensive invasion of activity allowed by a poor axial electrode, 10 ohm cm^2 , held at the resting potential while the open circles show the blockade presented by a good electrode, 2.4 ohm cm^2 , also at the resting potential.

with its inability to block an action potential rapidly this axial electrode and others with similar electrical characteristics have been found both by experiment and by analysis to be inadequate for potential control of any but the rather weak axons.

The third series of measurements in Fig. 9 was made with one of the best

axial electrodes then available. Its effective resistance was 2.4 ohm cm.² and its calculated characteristic length of 0.33 mm. is in general agreement with the action potential attenuation that it produced.

This group of experiments has produced evidence that axial electrodes are about as effective in producing the desired uniformity of membrane potential over their length as could be predicted and that in the face of so powerful a disturbance as a propagated action potential even mediocre electrodes are somewhat effective while the better ones constitute an almost impenetrable barrier.

On the other hand, it has not been found possible to calculate the effect of an action potential, initiated at an end of the axial electrode, upon the potential control at potentials other than those near the resting potential and particularly at potentials somewhat above the threshold for excitation of the uncontrolled axon.

However encouraging the above tests may be, they still are as yet not certain proof of the adequacy of the potential control over a measuring region. This most direct and crucial evidence has been obtained by measuring the potential with a probe electrode along the interior of an axon under control at various potentials in the range routinely used. At each point time records of the probe potential, similar to those of Fig. 4, were made. The extent of the maximum variations of the potential at each point and at each control potential is given in Fig. 10, for one experiment.

These results are representative of those obtained with the best available axial electrodes and good axons. As seen in the earlier time record, the variation is several millivolts from the mean in the critical region. Although this is not much more than that to be expected if the probe were close to the axial electrode, as shown at the side of Fig. 10, there are not yet sufficient observations to establish this as a satisfactory explanation particularly since the position of the probe sometimes varied from near the axial electrode to close to the membrane as the probe was moved along the interior of the axon. It is, however, somewhat reassuring to find that in this, as in other comparable experiments, there are no marked changes of the potential perturbations along the length of the measuring chamber.

Such evidence as this supports a conclusion that the membrane potentials of all but the most powerful of uniform axons can be controlled over a length of several millimeters to within a few millivolts.

It is to be anticipated that the more difficult, and presumably the better, axons will be unstable outside of the control region, even under the best of present conditions. The current measurement should then be confined to an area of membrane near the potential electrodes in which the potential control is adequate. The behavior of the membrane potential along the length of such an axon is not immediately apparent and in its entirety is one of considerable

complexity. With the previous assumption of a negative steady state membrane resistance, equation (5) has an imaginary space constant for $r_1 > -r_3$. The membrane potential is then a sinusoidal function of distance in this region. Although the boundary conditions are not obvious, a simple solution has been used to estimate the potential in the vicinity of the control point. Deviations of 1 mv. for the potential and 20 per cent for the current density

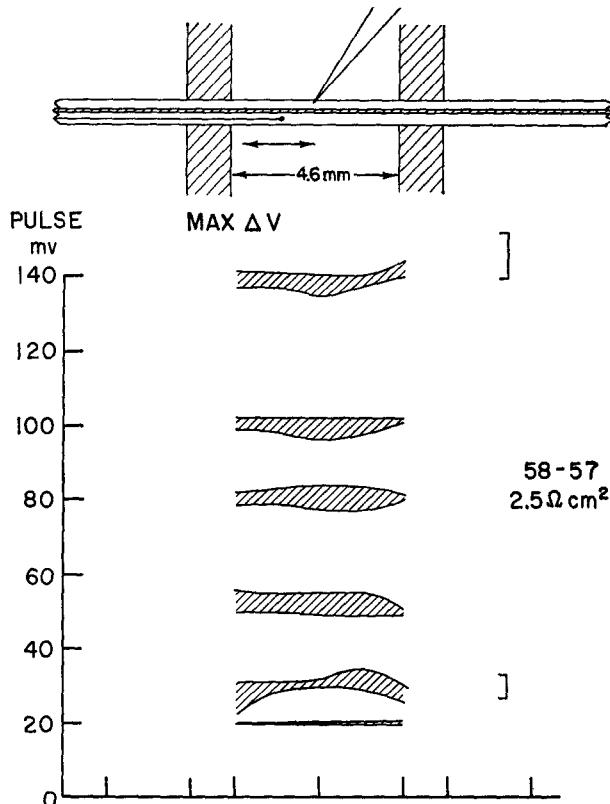


FIGURE 10. Maximum variations of potential inside an axon over the center electrode region. The maximum potential differences between the micropipette control electrode and the internal probe were recorded during the depolarizing pulses indicated as ordinates and are shown by the width of the shaded band at the probe position. The axial current electrode had a low effective resistance, 2.5 ohm cm^2 , and the axon had a peak inward current of 5 ma./cm^2 . The vertical lines at the right show the maximum potential differences to be expected in the axoplasm.

are then calculated at 0.3 mm. from the control point with values of r_1 and r_3 corresponding to 6 ohm cm^2 and -2 ohm cm^2 respectively. However, the experimental data are so complicated, as will be shown (TMC), that there is no evidence as to the validity of this prediction. Further, this prediction may not permit use of either the analyses or the data upon which it is based.

The simple cable approximation comes into question because this distance is again close to an axon radius. The routine procedure has been to insert the micropipette electrode at the top of the axon and to place the external reference at one side and the differential electrode at the other. The control point is therefore not well defined but is probably almost an axon radius away from the area of membrane to which the differential electrode is most sensitive.

It can then only be assumed that the membrane current measurement should be made both as close to the potential electrodes and over as small an area as possible.

Membrane Current Measurement

Our primary objective at present must be to determine the characteristics of the membrane ion current as accurately as possible under as nearly perfect control of the membrane potential as may be necessary. The current measurement should be restricted to a few millimeters length of axon at most in order that it not include areas of membrane too far from the control electrodes nor demand too long axons. The measuring technique should also not modify the membrane potential distribution by more than a millivolt. Since current densities from a microampere to 10 milliamperes per square centimeter are important, considerable flexibility is required.

ACCURACY AND SPEED For most purposes an error of a few per cent in the current measurement is tolerable and this accuracy has usually been possible. A response time of a few microseconds is desirable for an accurate record of the membrane capacity transient. This has been done with the operational ammeter but has not been achieved with differential recording.

With the series resistance, R_s , between the potential electrodes and the membrane capacity, some current will flow through the capacity. But for a series resistance of 1 ohm cm.² the capacity current would be at the most a few per cent of the total and can safely be ignored in comparison to the direct effects of the potential variation on the ionic current.

Practically all the work to date has been done in cells having a central measuring chamber with guards at each end as first described by Marmont (1949). Equal resistors of 10 ohms between each chamber electrode and ground gave adequate sensitivity with an axon length of 5 mm. in each chamber. These were used until the invention of the operational ammeter, shown in Fig. 13, made it possible to keep all chamber electrodes at actual or virtual ground potential. Also the effect of end currents was eliminated except as these currents disturbed the potential distribution in flowing through the external sea water resistance and electrode impedances.

The more logical and elegant measurement of current density by the potential difference between a pair of closely spaced electrodes was recalled to us by Dr. Walter Pitts and Dr. J. Y. Lettvin and is a very considerable refinement of the arrangement used by HHK. Such a differential electrode, close to and with its axis perpendicular to the membrane can be used to restrict the current measurement to less than a half millimeter length of axon and in particular to emphasize the membrane current density close to the potential electrodes. Problems of sensitivity and resolution and of noise, stability, and speed have delayed the routine use of this measurement.

EFFECT OF POTENTIAL CONTROL Considerable evidence has been presented to show both the possibilities for and the limitations of the control of the squid axon membrane potential. Perfect control seems certainly to be unobtainable as yet and the departures from it are difficult both to estimate and to measure. Too many approximations have had to be made in the attempts at analysis both because of the complexity of the problem and because of our ignorance of the answer. And too few measurements can be made on a single axon or on identical axons that are in the good condition for which the problems to be resolved arise. There is, however, sufficient information to lead to characteristics of the membrane ion current that seem reasonably certain to be correct.

No attempt is made at this stage to go into the detailed quantitative kinetic description of the membrane current. Our present and preliminary problem is entirely qualitative. It is essentially confined to an investigation of the rather objective single question:

After a perfectly controlled potential change must the membrane current be expected always to show a smooth, continuous transition from the inward peak of sodium current to the steady state outward potassium current or must it show a notch in some range of the applied potential?

Ninety-four axons in good to excellent condition were investigated with low resistance axial electrodes during the 1958 and 1959 experimental seasons. Of these, eighteen showed clear cut notches in the total center chamber membrane current records for low potential depolarization pulses and about half as many more gave slight, but suspicious, indications of this type of behavior.

It has been found that when the axoplasm potentials, as measured by a probe, were constant to within a few millivolts across the center chamber, the membrane current measured in this chamber never showed a notch.

Conversely, the axoplasm potential, when measured, always showed variations of 10 mv. or more in time and distance if a notch was present in the center chamber membrane current.

In view of the conclusions that the control region may be less than a 2 mm. length of axon and that accurate or even stable control should not be expected

in the remaining 3 mm. of axon under measurement, such a considerable majority of membrane current patterns without notches is very impressive. Impressive also is the high degree of uniformity of axoplasm potential and the limited range of membrane current density to be found over such an extended length of axon that is outside of a reasonable probability of influence by the control point and so beyond the region of control.

Although rather less extensive, the evidence obtained with the differential electrode measurement of a comparatively restricted membrane current density is more compelling. Of the thirty-one axons investigated with the differential electrodes in the past two seasons, six produced a notch in the membrane current that was measured over the entire center chamber.

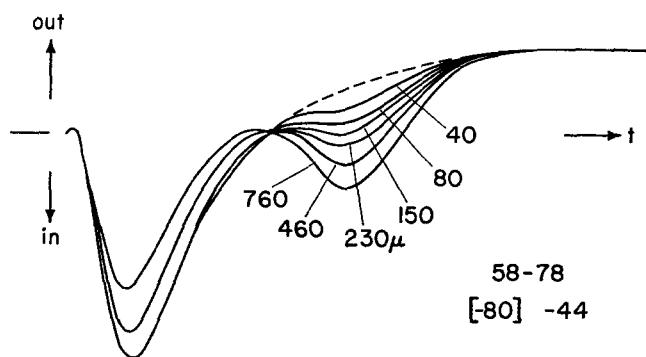


FIGURE 11. Current densities (ordinates) after a change of membrane potential from -80 to -44 mv. with a poor axial electrode, 8 ohm cm^2 , and guard electrodes not connected. Solid lines are densities recorded with the near electrode of the differential pair at the indicated distance from the axon surface; the dotted line is an extrapolated current density at the axon surface.

It has been found that when the center chamber current showed a notch, an exploration of the local current density with a differential electrode always encountered a region in which the current contained a notch.

On the other hand, with two exceptions, a notch has never been found in a differential electrode measurement made as close as possible to the potential control electrodes—whether or not there was a notch in the current from the axon in nearby regions or in the center chamber as a whole.

One example of a notch near the control point is shown in the differential records of Fig. 11, made opposite the control point at several distances away from the axon. However, these records can be extrapolated back to a notch-free membrane current density at the axon surface. Records made in both directions along the axon showed increasingly violent notches and the notch in the center chamber current was very severe. In this experiment the axial current electrode had the rather high resistance of 8 ohm cm^2 and the guard

electrodes were not connected! The other exception appeared in the first differential experiment of early last season, in which the potential control was shown to be unsatisfactory. These two exceptions are not considered adequate to invalidate the conclusion and the statement that the membrane current pattern does not contain a notch at the control point.

By analysis it is expected and, by experiment, demonstrated that the accuracy of potential is good and stability is reasonably certain at the control point. Since nothing equally as certain is otherwise available, the best control of membrane potential is to be correlated with the virtually complete absence of notches in the membrane current density records made at the control point. Indeed, because of deviations of membrane potential and current density predicted near the control point for poor electrodes in good axons, it is quite remarkable that no more indications of notches have been found with differential electrodes of the comparatively poor resolutions that have been used.

A notchless region has been found in every carefully examined axon, and in enough good enough axons that it should be a characteristic of a good axon. If then a notch is also a normal characteristic of a good axon it can only appear as a separate region in an axon that must also have a notchless region. But if good axons have both notch and notchless regions it is highly improbable that the axon should have always been so placed in the cell that the potential electrodes subjected a notchless region to control. It is so much more reasonable—as well as easier—to accept the conclusion that a notchless pattern at the control point is not fortuitous and appears because it is the characteristic of a membrane under good control. Consequently one arrives at the conclusion that the notch is an indication of bad control and that a notch does not appear in a good axon under good control.

A natural objection to this line of reasoning and to this conclusion is the possibility that the axons were mistreated at the control point. Thus a notch, as a postulated highly sensitive sign of the superior axon, was never able to make its appearance and so could never be found by this technique. This type of objection is not usually an easy one to answer but can at least be discussed.

It is certainly true that the micropipette electrode can and has produced visible injuries but these have only been seen in axons that have deteriorated rapidly and not in the axons that have been usable. It is also true that a micropipette electrode is usually found to record a highly stable action potential before the insertion of the axial electrode and that the start of the usual steady deterioration is closely correlated with the insertion of the axial electrode. In the presence of the axial electrode, the action potential is, as has been mentioned, not spatially significant and it has usually not been possible to remove the axial electrode without starting a precipitous decline of the axon performance. Consequently the action potential at and near the site of the micropipette electrode has not been explored. But the deterioration associated with

the axial electrode should extend over at least its length. When the axon gives a notch elsewhere it seems a little far fetched to require that the axial electrode injury shall always occur at the control point for all electrodes and all axons. It has been mentioned that considerable variation of membrane current density may be found along the length of axon in the center measuring chamber. On occasion this density has been found to have a suspicious minimum near the point of insertion of the micropipette electrode and so close to the control point. But it has much more often been true that the density showed a continuous change of amplitude along the axon without any irregular behavior at the control point and indeed it has, if anything, as often been found that the control point was one of maximum membrane current density. Yet under all these conditions no notch has been found at the location of the best control of the membrane potential whether or not a notch appeared elsewhere.

On such bases it is concluded that although a notch in the membrane current pattern may be evidence of a good axon but poor control, all axons—from the poor to the best—show a smooth, notchless current pattern under a good control of the membrane potential.

Summary

It appears from analysis and experiment that the requirements of a 1 mv. accuracy can be approximated and of stability can be achieved at the potential electrodes as the outside resistance, R , of the current electrodes, axoplasm, and sea water, is reduced to $R/(1 + \mu/2)$ by a feedback control amplification, μ . The known and unknown resistances, R_s , between the potential electrodes and the membrane capacity probably make the potential across the capacity considerably less accurately constant. The contribution of these resistances towards instability is already included in the measurements so far available, and experimental evidence indicates that there is a margin for stability of 2 ohm cm.² or more at the control point. Improved control may decrease the accuracy requirements and increase the stability margin.

The control becomes less effective at points along the axon away from the control point as the longitudinal coupling current decreases. The equivalent resistance then increases from $R/(1 + \mu/2)$ to nearly R at two or three characteristic lengths—which may be little more than a millimeter.

The accuracy of the potential in an uncontrolled stable region of a uniform axon will be the same as at the control point. But an accuracy of 1 mv. for an axon that is not uniform requires that the sum of the deviations of the actual electrode resistance, the axon diameter, and the membrane current density from their values at the control point be less than 3 per cent. This severe limitation practically eliminates the possibility of a 1 mv. accuracy of potential anywhere except near the control point. It also strongly suggests

that for extended potential electrodes the desired accuracy of the membrane potential can only be accidentally achieved anywhere.

It is predicted that with even the best axial electrodes, the outside resistance, R , will exceed the maximum value for stability of the most powerful axons in an uncontrolled region. In this situation the deviation of the membrane potential near the control point is further predicted to be so large that the current measurement cannot now be restricted to an area of membrane under control to within 1 mv.

In the face of these requirements and predictions it is not only understandable that the older technique should fail to control the more powerful axons but indeed it is difficult to believe that as many of such axons have been as well controlled by the present procedures as the experimental evidence indicates. It may well be that the assumed criterion of stability will be shown to be too stringent and that other approximations may not be justified, but we believe the evidence indicates that all but the most difficult axons have been controlled to within a few millivolts over the extended current measuring area and that all axons are adequately controlled over a measuring region confined to a fraction of a millimeter from the control electrodes. Correspondingly, it has been found that the membrane current density at regions under good potential control has a form similar to that originally obtained by Cole and HHK, but several times larger. A variation from these patterns, a notch in particular, is correlated with poor potential control.

It may be concluded that a membrane current with a notch indicates a good axon under poor control and that for axons under a good enough control to qualify as voltage clamps the membrane currents are free from such anomalies.

EXPERIMENTAL

As may have been inferred, considerable experience has been built into the equipment and procedures. These have been described briefly (Moore (1958a, 1959b); Taylor (1959)) and a more extensive account will be presented elsewhere (Moore and Cole (1960b)). They are outlined here only in terms of function. A few experimental results are given for their own sake as well as to give illustrations of the rather extensive work that has paralleled the development of the experimental means.

Axon Cell and Electrode System The type of axon cell shown schematically in Fig. 12 has been used for most of the work to date. The horizontal design was not only familiar since the first experiments on the squid axon (Cole and Curtis (1939)) but also was made necessary by the early decision to use a micropipette for the internal potential electrode. The central measuring electrode chamber was almost 6 mm. long and had a guard at each end, somewhat as usually has been used since its introduction by Marmont (1949). Oxygenated artificial sea water or other ex-

ternal solutions were circulated through the chambers in parallel and the temperature controlled at the inflow and measured at the outflow. The axial electrodes were guided by V grooves at either end of the cell into a small opening at a bend near an end of the axon.

The internal micropipette potential electrodes were pulled mechanically from uniform pyrex hematocrit tubing 0.8 mm. in diameter with a rather steep taper to the tip in order to penetrate the axon connective tissue without undue breakage. Tip diameters from 1 to 3μ and resistances of about 1 megohm have been found to be the most satisfactory compromise between the distortion of the recorded potential given by smaller electrodes and the difficult penetration and subsequent injury encountered with larger electrodes (Moore and Cole (1960a)). These electrodes were filled with 3 M KCl through a hypodermic needle immediately after drawing and were inserted into a holder to make connection with a 3 M KCl calomel electrode.

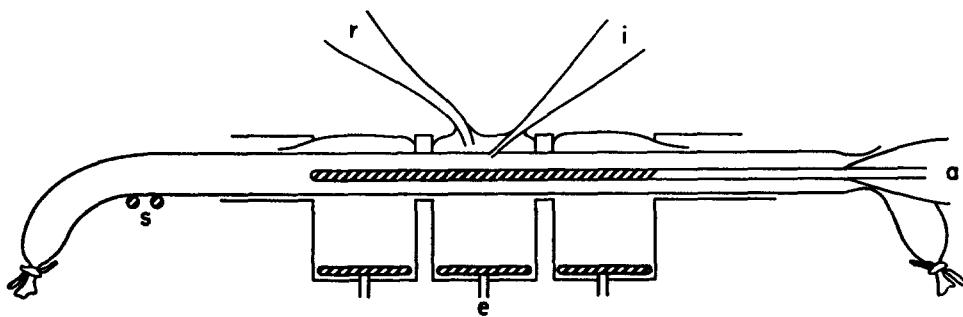


FIGURE 12. Schematic drawing of axon cell with axon and electrodes in place. The membrane potential is measured and controlled between the micropipette internal, and the small nearby external reference electrodes, *i* and *r*, introduced through the sea water surface. The axial current electrode, *a*, is inserted through a cut at the right end of the axon until the conical shank makes a tight seal. The external current electrodes, *e*, are in sea water at the bottom of the center and guard chambers. The axon may be stimulated at the electrodes, *s*.

The external potential electrodes were 3 M KCl, or sea water, agar-filled tubes with an opening of about 200μ and calomel or Ag-AgCl electrodes.

The axial electrodes have all been made of wire, usually 75μ in diameter, and sealed into discarded micropipettes with wax or cement forming a cone to make a tight seal when forced into a small opening in the axon. Exposed electrode surfaces of silver-silver chloride have been found to have a usually high and variable impedance and to be toxic when fresh. The most generally satisfactory electrodes have been those of conventionally platinized platinum which have an average measured impedance of 20 ohm cm. or 3 ohms for a cm^2 of membrane in the first millisecond of an applied current. The latter figure includes an estimated 1 to 2 ohm cm^2 of nearby electrolyte. The probe electrodes were similarly made of 50μ wire with an exposed platinized tip 100μ long.

Differential electrodes for a relatively restricted measurement of current density have been pairs of electrodes 200 to 300μ apart. 50μ enamelled silver wires platinized

at the exposed tips and glass micropipettes broken off to about the same diameter and containing sea water and Ag-AgCl electrodes have been used. The former were oriented to measure radial, longitudinal, or circumferential components and the latter have been used radially for more routine membrane current density measurements. One of the differential electrodes used could resolve two narrow current "stripes" around an axon and 250μ apart. The limitations of stability, noise and amplifier sensitivity, and speed are still serious.

Measurement and Control System The control and measuring system, as shown in Fig. 13, makes considerable use of the operational amplifier concept (Appendix E). This system has first to produce a measure of the potential difference across

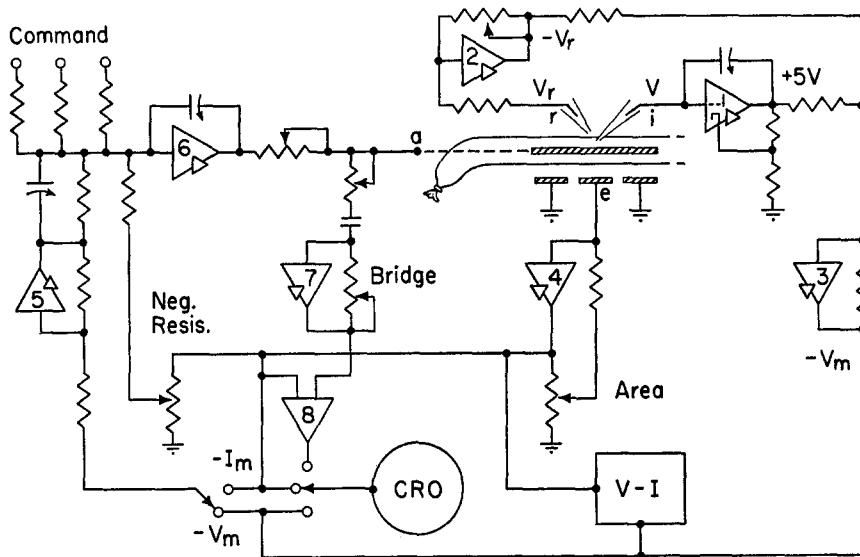


FIGURE 13. Schematic circuit of the measurement and control system. Amplifier 1 is the special micropipette preamplifier; 2, 3, 4, 5, and 7 are stabilized operational amplifiers; 6 is the control amplifier; and 8 is a differential input amplifier to the cathode ray oscillosograph, CRO. The functions, V_m or I_m , to be controlled and recorded are chosen with the left and right hand switches. $V-I$ is an X-Y plotter for recording potential-current characteristics at high sensitivity.

the axon membrane, V_m . The potential of the microelectrode, V , is amplified by the stabilized wide band electrometer 1 (Moore and Gebhart, to be published) and corrected to give an output of 5 V. The input capacities are compensated by the indicated capacity feedback, the grid current is made less than 10^{-11} amp by tube selection, and the impedance of the grid is considerably increased by making the cathode and screen follow its potential. With internal feedback, a resistor network determines a stable amplification while a novel chopper stabilization reduces drift ten- to a hundredfold. The potential inside the axon membrane is measured—except for liquid junction correction—within a millivolt and within 10 μ sec.

The negative of the potential near the outside of the membrane, V_r , appears as the output of operational amplifier 2 when the feedback resistor is adjusted to equal the resistance of the reference electrode circuit. This output, $-V_r$, and the output of the preamplifier, 5 V, are summed with the proper weighting resistors by operational amplifier 3 to give the output $-V_m$ which is now available for use throughout the rest of the system.

The system has also to produce a measure of the membrane current. The center chamber electrode is maintained at the ground potential of the guards by operational amplifier 4 and its feedback resistor (Moore (1960); Moore and Cole (1960b)). The auxiliary potentiometer is set, according to the axon diameter, so that the amplifier output is conveniently proportional to the membrane current density, I_m , and available for the rest of the system.

Either the membrane potential or the current density is observed and recorded by cathode ray oscilloscopes; ink records of the average values of both are made throughout an experiment and one may be presented as a function of the other on an X-Y plotter.

The control of the membrane potential or current density is selected by the operation switch, shown in the former position in Fig. 13, where the sign of the membrane potential is changed by operational amplifier 5 for comparison with the command potentials at the input of the control amplifier 6. Any error in the membrane potential will be amplified at the output of amplifier 6 and in the direction, when connected to the axial electrode, to produce the current needed for correction of the error. The series resistance allows a gradual application of this correction. The system as a whole is stabilized by an "error derivative" correction and by reducing the high frequency cutoff of the control amplifier with a shunt capacitance.

The accuracy of the error correction has been indicated in Figs. 3 and 4 and the speed of the operation was shown in Fig. 5.

The auxiliary operation of membrane current density control, as chosen by the operation switch, is essentially the same as for the major function of potential control. Another potential, proportional to the membrane current density, may be obtained from the "negative resistance" potentiometer and added to V to compensate for resistance between the potential electrodes and membrane capacity as was done by HHK. In the bridge circuit, the current flow from V_0 through a resistance-capacity network may be subtracted from the membrane current to balance out a transient or the response to a steady state sinusoid command and so give measures of the membrane capacity and conductance.

Axon Preparation The giant axon in the hindmost stellar nerve of *Loligo pealeii* has been used for all experiments. The whole nerve was tied and removed from the mantle under running sea water. The axon was more or less freed of small fibers and connective tissue by one of a variety of techniques. The ends of the axon were bent down and held by ligatures in end chambers of the cell, the bend at one end was dried, punctured, or cut on its axis, and the axial electrode inserted until its cone made a tight seal.

The axon was then stimulated near one end or through the axial electrode. An action potential of 105 mv. on the axial electrode was considered satisfactory and

one below 100 mv. was usually not acceptable. After the micropipette and external potential electrodes were in place, the action and resting potentials from them gave further indices of the axon condition.

Membrane Clamping While the axon was being prepared, the measurement and control system was stabilized, tested on an equivalent axon circuit, and calibrated. After the micropipette electrode was in place, the preamplifier was compen-

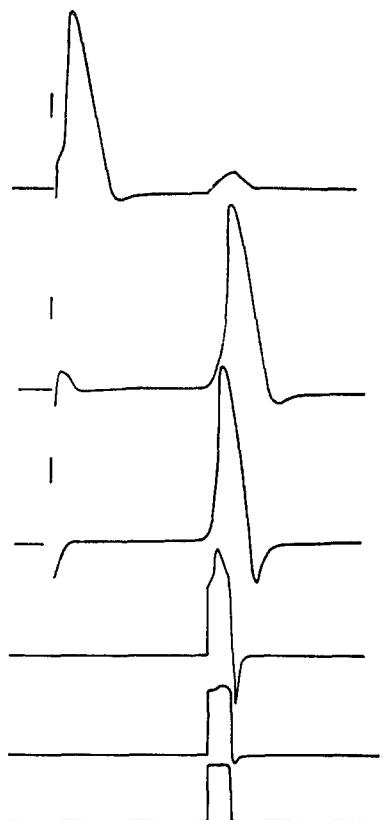


FIGURE 14. Records of the membrane potential as the potential control is increased from top to bottom. The externally stimulated action potential at the left is blocked and turned off in the last 3 records. The control pulse at the right excites the membrane in the second and third records but is gradually forced to take on the desired form in the bottom record as the control is increased.

sated for a test pulse, as seen in Fig. 18, and the axon was ready for clamping. The usual procedure is to gradually increase the potential control, or "tighten the clamp," by decreasing the resistor between the control amplifier and the axial current electrode while operating parameters are checked and adjusted, rather than to abruptly apply conditions that might cause oscillating or other excessive membrane currents. Fig. 14 gives a sequence of records of V_m as the clamping was increased from a very slight amount at the top to a full clamp at the bottom. At the top left is seen the action potential initiated at end external electrodes and propagated into the measuring region; to the right is a small subthreshold response to the clamp pulse. As the clamp is tightened the externally stimulated action potential is blocked and the clamping pulse fires off a spike. This spike is gradually brought under control (external stimulus

off in last three records) and the membrane potential finally follows the prescribed rectangular pulse when the clamp has been fully applied.

Results Reports on the effects of isoosmotic external potassium chloride (Moore (1959a)), on the effects of external procaine (Taylor (1959)), and on the apparent velocity of impulse propagation (del Castillo and Moore (1959)) obtained with these experimental procedures have been published. Preliminary reports have been given

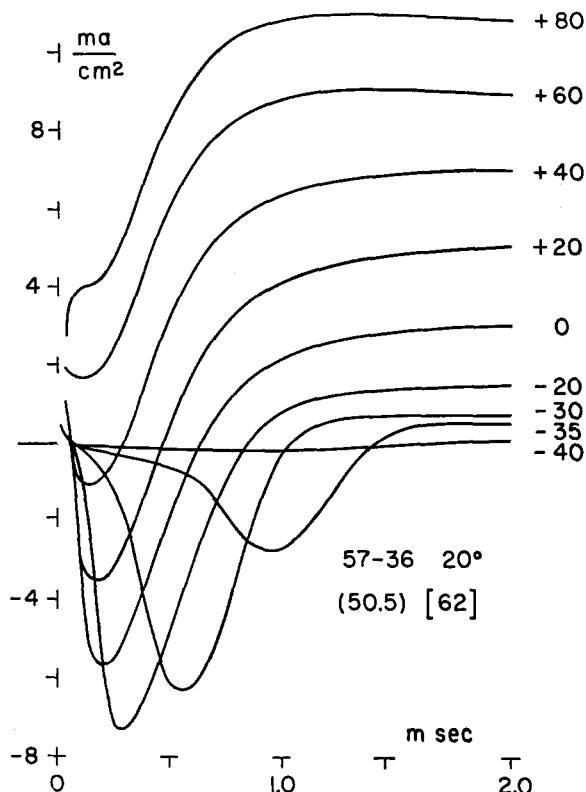


FIGURE 15. Records of membrane current density (ordinates) as function of time (abscissae) after changes of membrane potential from -62 mv . to the selected values given at the right side of each curve in millivolts. The final steady state current, attributed to potassium ions, increases steadily as the membrane potential is made increasingly positive. The early currents, attributed to sodium ions, change from inward to outward at the sodium potential of about $+60 \text{ mv}$. The initial or leakage current is unusually high in this example.

on the effects of hyperpolarization (Cole (1958a)), on temperature, lithium, and some drug effects (Moore (1958b)), and on characteristics of the potassium ion current (Cole (1958b)), and it is expected that this work will be made available in detail.

As the membrane is increasingly hyperpolarized between pulses, or the steady membrane potential made more negative, the maximum value of the peak inward

current increases until it reaches saturation at a potential usually between -70 and -90 mv. (Cole (1958a); Taylor (1959)). For acceptable axons this current is more than 3 ma./cm. 2 at low temperature.

Most experiments have been carried out under this condition of saturation hyperpolarization and a family of currents for various membrane potentials during the pulse is shown in Fig. 15. Some irregularity in the rise of the smallest inward current is a rather usual, but unexplained, characteristic. The increase of the peak sodium current is quite rapid between -40 and -30 mv. in this series. The superposition of the initial phases of the sodium current between 0 and $+40$ mv. is quite characteristic.

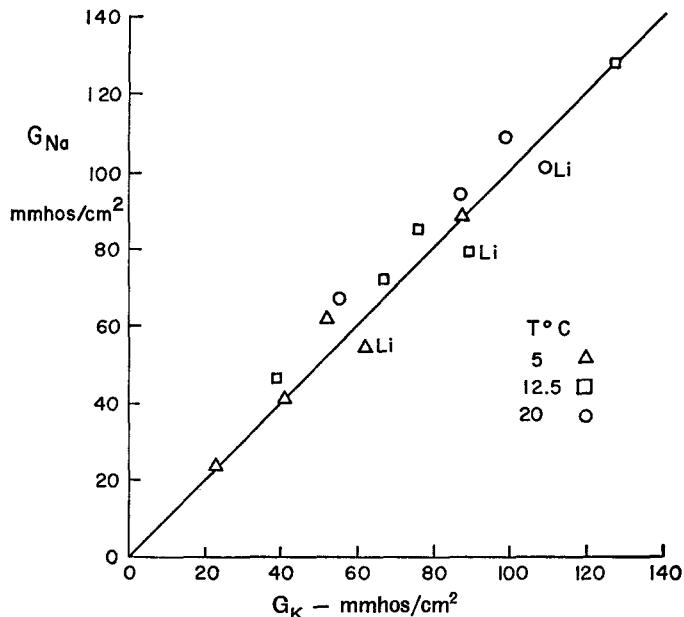


FIGURE 16. Regression of the maximum conductances at high depolarizations for peak sodium current, G_{Na} , and steady state potassium current, G_K , for several axons at three temperatures and in lithium sea water.

The sodium potential of HH, at which the sodium current reverses in direction, is here about $+60$ mv. With the liquid junction correction (Cole and Moore (1960)), this potential has been used to estimate the internal sodium concentration and the net sodium flux under several experimental conditions (Moore and Adelman (1960); Adelman and Moore (1960)).

An example of the behavior of the peak inward, sodium, current and the steady state, potassium, current as functions of the potential has been given in Fig. 6. Both these curves regularly become linear for the higher depolarizations. The corresponding conductances, G_{Na} and G_K , are usually nearly equal in a fresh axon. Such data for several axons are given in Fig. 16 at three temperatures and in an artificial sea water in which the sodium was replaced by lithium.

The steady membrane rectification characteristics near the resting potential can

be recorded directly with an X-Y plotter as the "command" potential is slowly varied. One of these records shown in Fig. 17 is typical for artificial sea water. The trace separation suggests that the time to complete the cycle—less than a minute—was too short to approximate a steady state and is evidence for the presence of quite slow components in the ionic processes. The characteristic is apparently not far from linearity at the hyperpolarization end of the cycle and the mean slope of the traces here gives a membrane resistance of 6,300 ohm cm.². The mean is 1400 ohm cm.² at the resting potential and 275 ohm cm.² at the depolarization end of the cycle.

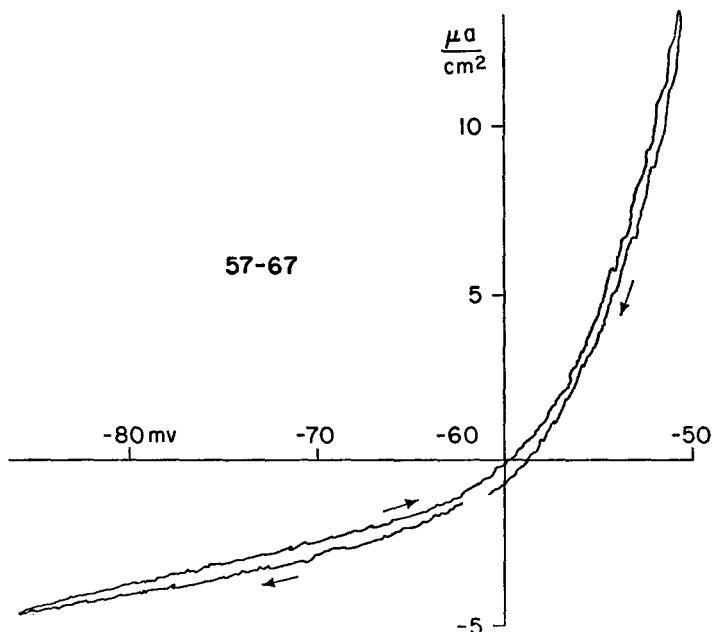


FIGURE 17. Membrane rectification characteristic near the resting potential as recorded on an X-Y plotter during a 45 second potential cycle.

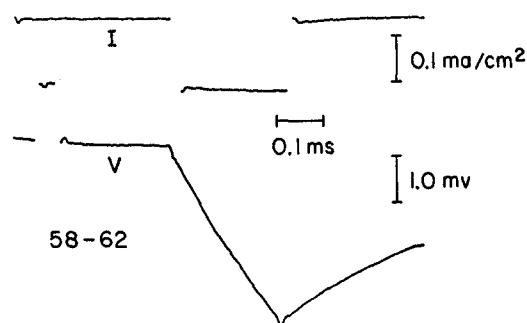


FIGURE 18. Membrane potential under current control. The controlled membrane current pulse is shown above and the resulting change of membrane potential below and preceded by the preamplifier calibration with a current pulse.

Further depolarization gave a limiting value of 20 ohm cm^2 for this axon. Other axons have given limiting values for hyperpolarization up to $8,000 \text{ ohm cm}^2$ and for depolarization as low as 5 ohm cm^2 .

Measurements of the membrane capacity and series resistance may be obtained with a "current clamp." As a step of current is applied, the membrane potential will jump by an amount proportional to the series resistance and then start to change at a uniform rate determined by the capacity. While the capacity is relatively easy to obtain, the speeds of application of the current step and of the membrane potential recording system limit the accuracy of series resistance determinations. Fig. 18 shows a record of a current step and the corresponding membrane potential changes along with a preamplifier test pulse. The capacity measured in this manner was $0.92 \mu\text{f./cm}^2$ in good agreement with earlier measurements; estimates of the series resistance values varying around 2 ohm cm^2 have been obtained with approximate correction of these records.

RESUME

The initial plan to build directly upon the progress made by Hodgkin and Huxley has been seriously delayed by the belated but necessary examination of the fundamental concepts and measurements underlying the far-reaching and obviously important interpretations. In this process, the concepts have been clarified and the experimental criteria recognized. The initial experiments of Cole and of Hodgkin, Huxley, and Katz fell far short of the ideal but it was not until our own and Frankenhaeuser and Hodgkin's work that any difficulties became apparent or until the work of Tasaki *et al.* and TMC that the anomalies took on extreme form.

Continuing and somewhat successful efforts to meet the criteria have all but eliminated such obvious anomalies and shown the probable way to avoid them completely but have not yet been able to establish the amount of a remaining recognized source of error or to correct for it. Under the best recent conditions, the most powerful axons in condition to produce their maximum responses usually give current patterns several times larger but of the type originally described by Cole and analyzed by Hodgkin and Huxley. The restriction of the current measurement to the region of best potential control and the reduction of the variation of potential across the membrane capacity are only expected to reduce the tendency to instability while improving the accuracy. Consequently, there is reason to believe that the early measurements are qualitatively correct and that interpretations, such as those of Hodgkin and Huxley, are not necessarily wrong because of having been based upon misleading data.

Our obligations for support, assistance, and encouragement are deep and extensive. Work on potential control of the squid axon was resumed with the support of the Naval Medical Research Institute

in 1952 and has been continued under our present auspices since 1954. All experimental work has been done at the Marine Biological Laboratory. Our indebtedness and gratitude to the many friends, colleagues, and collaborators who have contributed time, effort, and ideas to this work are not to be estimated quantitatively. We appreciate the help of those who have been with us at various stages of the work, Dr. H. W. Shirer, Dr. J. C. Dalton, Professor U. F. Franck, Mr. J. H. Gebhart, Dr. J. J. Chang, Mr. E. R. Whitcomb, and Dr. W. J. Adelman. We are particularly indebted to Dr. R. FitzHugh, Dr. R. E. Taylor, and Dr. J. del Castillo for their extensive and intensive participation in much of the more recent developments. We have been greatly assisted by the staffs of the Technical Development Section, NIMH, the Division of Research Services, NIH, and especially the Marine Biological Laboratory.

Figs. 1, 2, 3, 5, 13, and 18 are essentially as published by Moore (1959b) and are presented here with the permission of the *Proceedings of the Institute of Radio Engineers*.

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A P P E N D I X A

Non-Linear Parameters. A point on the current-potential characteristic of a non-linear conducting system, in a steady or quasi-steady state, may be expressed for convenience of analysis in terms of the relations between small changes of current and potential as a resistance, $r = \delta V / \delta I$, or a conductance, $g = \delta I / \delta V$. When these changes are made slowly enough, $r \rightarrow r_\infty = \partial V / \partial I$, as given by the slope of the steady state characteristic or its reciprocal, and the subscript indicates an indefinitely long time. This parameter has been termed the variational, slope, or zero frequency resistance or conductance. At the other extreme, when the changes are made too rapidly for the mechanism of the conduction process to alter appreciably, there is perfect linearity and $r \rightarrow r_o = (V - E) / I$, with the subscript indicating a time shorter than that for the onset of non-linearity. The terms chord and infinite frequency have been used to describe this resistance or conductance. The behavior at intermediate times or frequencies was described formally (Cole, 1947, 1949b), in terms of the time constant of the non-linear process, as inductive or capacitative. The two parameters, such as r_∞ and r_o , are both descriptions of a steady state characteristic such as Fig. 19 and the use of one or the other is a matter of convenience since they are formally related to each other.

Hodgkin and Huxley have used conductances exclusively, which is appropriate for elements in parallel and with the potential as the independent variable, and they have dealt only with chord, or infinite frequency conductances which are more closely related to short time phenomena. These are always positive and therefore are simpler to use in analysis and graphs. In the present work the variational characteristics are much more useful, particularly because they show the negative steady state values directly. The importance of elements in series makes the use of resistances simpler and more convenient.

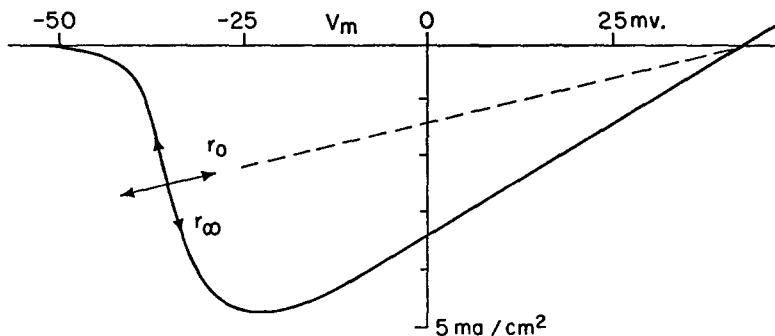


FIGURE 19. Non-linear parameters of the peak inward current considered as a steady state characteristic. Sufficiently rapid changes of potential and current are along the zero time or infinite frequency line, r_0 . Slow small changes are along the steady characteristic giving the variational or zero frequency resistance, r_∞ , which is negative in this example.

APPENDIX B

Axon and Electrode Characteristics. The nominal values used for the electrical characteristics of 75μ axial current electrodes and an average 480μ axon are as follows:

"Good" and "bad" axial electrodes have been measured to have effective surface resistances of 20 to 100 ohm cm. or 3 and 16 ohm for a cm^2 of axon membrane, respectively. This routine measurement at about a millisecond after the application of a current pulse includes a potential drop in adjacent 0.5 M KCl solution that is estimated at 1 to 2 ohm cm².

The specific resistance of axoplasm has been taken as twice that of sea water (Cole and Moore, 1960). The radial resistance between the axial electrode and the axon membrane is then 12 ohm cm. and the longitudinal resistance is 15,000 ohm/cm.

The resting, active, and excited membrane resistance values are 5000, 50, and -15 ohm cm. or 750, 7.5, and -2.5 ohm cm² respectively. The excited, negative, resistances varied between -2 and -8 ohm cm² with an average of -4 ohm cm² in 1958.

Outside an axon model, the characteristic length was less than 1 mm. and the current density was nearly uniform at 1.5 mm. from the axon axis. Using a value of 20 ohm cm. for the specific resistance of sea water, the radial and longitudinal resistances within this layer are taken as 6 ohm cm. and 300 ohm/cm. respectively.

The sea water and external electrode resistance beyond this layer is 20 ohm cm. or 3 ohm cm².

APPENDIX C

Control of the Membrane Potential. The essential circuit for the control of the membrane potential is shown in schematic form in Fig. 20.

For simplicity those parts of the circuit not included between the potential electrodes—the impedances of the amplifier, axial electrode, and axoplasm and those

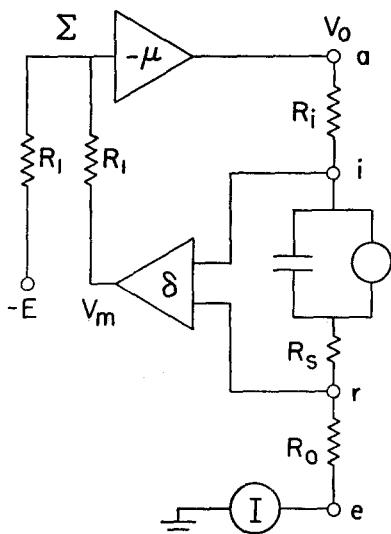


FIGURE 20. Schematic circuit for control of the membrane potential. A difference of the membrane potential, V_m , between the internal and external potential electrodes, i and r , is amplified by $-\mu$ to supply the correcting potential, V_0 , and current, I , between the axial and external current electrodes, a and e .

of the external solution and electrode—are represented by R_i and by R_o respectively. The potential difference across the membrane—consisting of its capacity C , parallel ionic current path, and series resistance R_s —appears as the potential V_m after the electrode potentials have been amplified and corrected and their difference taken in the complex designated by δ . The control amplifier, with an amplification of $-\mu$, then provides the current necessary to make V_m nearly equal and opposite to the imposed, or “command,” potential, $-E$. On the assumptions that $-\mu$ and δ are ideal and draw negligible currents and by Ohm's and Kirchoff's laws,

$$\frac{-E - \Sigma}{R_i} + \frac{V_m - \Sigma}{R_i} = 0; \quad V_0 - V_m = RI; \quad V_0 = -\mu\Sigma;$$

where $R = R_i + R_o$ and Σ is the potential at the indicated “summing point.” Eliminating V_0 and Σ

$$V_m = \frac{\mu}{\mu + 2} E - \frac{2}{\mu + 2} RI \simeq E. \quad (6)$$

In order to achieve this control, the amplifier output required is

$$V_0 = \frac{\mu}{\mu + 2} (E + RI) \simeq E + RI.$$

APPENDIX D

Stability Condition. The conditions for stability of a system can usually be approached in several ways depending to a considerable extent upon the nature of the problem and the preferences of the analyst. The Hodgkin-Huxley equations, considered as a description of the properties of a squid axon, certainly contain information sufficient to determine the stability in the situations considered here. The complete

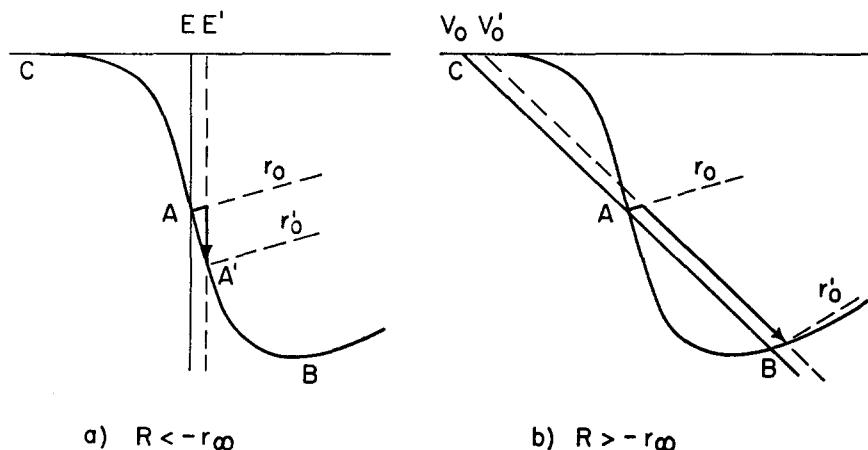


FIGURE 21. Condition for stability of a system with the negative resistance steady state characteristic, CAB from Figs. 6 and 19. In case (a), with the equivalent external resistance $R < -r_\infty$, the system is stable since a small disturbance at A will change the potential and current a correspondingly small amount. In case (b), with the equivalent external resistance $R > -r_\infty$, the system is unstable because any disturbance at A will displace the system to one of the stable points, B , C .

analytical investigations have so far appeared too formidable for serious consideration while the available analog computations may not have shown the onset of instability. Detailed digital computations which are under way should be more satisfactory and these should give an estimate of the direction and the amount by which the following approximate analysis is in error.

The locus of the peak inward currents as a function of the control potential step which requires them (Fig. 21) is used as a convenient quasi-steady state characteristic of the ionic conductance, $I(V)$. For a source electromotive force, E , and a positive series resistance, R , $E - V(I) = RI$, as given by the "load line" EA . In Fig. 21a, where $R < -r_\infty$, the only possible solution is the intersection of the load line with the characteristic at A and it will be stable if small variations in the system only

move it a correspondingly small amount. A sudden small change of E to E' at the peak of inward current requires the new, dotted, load line and the operating point at first moves towards it along the line given by r_o —as allowed by the membrane capacity. In this process the membrane potential is made more positive and the operating point path turns towards the higher peak current, A' , as r_o decreases towards the value r_o' appropriate for the new potential. Thus A is a stable point since the displacement of it is continuous with that of E . In the second case, Fig. 21b, in which $R > -r_\infty$, there are three possible combinations of potential and current at A , B , and C . Changing from V_0 to V_0' as before, V will again move first toward and then along the new load line, as allowed by the membrane capacity and the time constants of the ionic conductance. But, instead of being able to stop at a nearby peak current, V and r_o can only go to a remote point, near B . The point A is thus

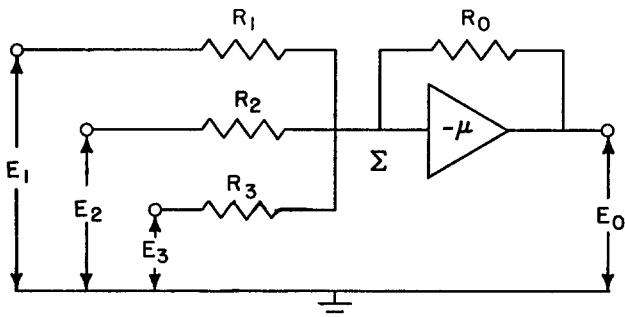


FIGURE 22. An operational amplifier with an output, E_0 , determined by the feedback resistor, R_0 , and the sum of the inputs, E_1, E_2, E_3 , as weighted by the resistors, R_1, R_2, R_3 , for a large amplification, μ . The potential of input summing point is Σ .

unstable while a similar procedure shows B and C to be stable. Consequently, the system is stable for $R < -r_\infty$ and unstable for $R > -r_\infty$ in so far as such a quasi-steady state approximation is valid.

APPENDIX E

Operational Amplifier. The various uses of operational amplifiers depend upon high amplification with feedback to maintain the input at nearly ground potential. In the circuit of Fig. 22 the currents at the summing point, Σ , are

$$I_0 + I_1 + I_2 + I_3 = 0$$

for negligible grid current. Then, since $I_0 = (E_0 - \Sigma)/R_0$, etc., by rearrangement

$$\frac{E_0}{R_0} + \frac{E_1}{R_1} + \frac{E_2}{R_2} + \frac{E_3}{R_3} = \left[\frac{1}{R_0} + \frac{1}{R_1} + \frac{1}{R_2} + \frac{1}{R_3} \right] \Sigma$$

For $E_0 = -\mu\Sigma$ the term on the right becomes negligible as μ becomes large to give

$$-\frac{E_0}{R_0} = \frac{E_1}{R_1} + \frac{E_2}{R_2} + \frac{E_3}{R_3}$$

and the output potential, E_0 , is the weighted sum of the input potentials and, for equal resistors, the actual sum. The low amplifier output impedance allows easy switching with direct connections between units and to one or more of the usual oscilloscope, recorder, or meter units. The amplifier units are stabilized with an auxiliary chopper amplifier to eliminate drift within a fraction of a millivolt. The operational amplifier is not, however, directly useful with potential sources incapable of supplying the necessary input current for usual operation.