ANALYSIS OF CERTAIN ERRORS IN SQUID AXON VOLTAGE CLAMP MEASUREMENTS

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ABSTRACT Localized membrane current and potential measurements were made on the squid giant axon in voltage clamp experiments. Spatial control of potential was impaired by the use of axial current supplying electrodes with surface resistance greater than 20 ohms for a centimeter length of axon. No region of membrane which was indeed subjected to a potential step showed more than one inward current peak. Other patterns were results of space clamp failure. Membrane current and potential patterns during space clamp failure were approximately reproduced in computations on a model containing two membrane patches obeying the equations of Hodgkin and Huxley. Non-uniformities in the axon or electrodes are not necessary for non-uniform electrical behavior. An extension of the core conductor model which includes the axial wire and external solution has been analyzed. The space constant of electrotonic spread is less than 0.5 mm with a usable electrode. Errors of about 5 per cent are introduced by ignoring the external solution. Resistance between the membrane and the control electrodes reduces the control and a few ohm cm^2 could lead to serious errors in interpretation.

The principles and ideals underlying the use of the voltage clamp concept for the study of the electrical properties of the squid giant axon, and the degree of success which has been obtained in their realization are discussed in another paper from this laboratory (Cole and Moore, 1960).

Current patterns which differed qualitatively from those reported by Cole (1949) and by Hodgkin, Huxley, and Katz (1949) were observed by Cole and Moore (unpublished) in 1954. They sometimes found that a "notch" of inward current flow occurred (as in Fig. 2*b*) between the usual peak inward current and the plateau of outward current, following a depolarizing step of 20 to 50 mv from the resting potential. These notches were not systematically investigated at that time, but appeared to be associated with the use of comparatively high resistance axial current supplying electrodes, and were thought to be largely caused by leakage of action potentials produced in the regions beyond one or both of the guard sections. This type of behavior closely resembles that reported by Frankenhaeuser and Hodgkin (1957) and, in those cases in which they used a point control system, by

Tasaki *et al.* (Tasaki and Spyropoulos, 1958). Frankenhaeuser and Hodgkin presented convincing evidence that their extra, delayed, inward current peaks were due to action potential invasion, but their discussion does not apply to the point control system used in this laboratory. Notched patterns of this type have been reported when action potential invasion was eliminated with the use of urethane in the guard regions (Tasaki and Bak, 1958*a*) or with the use of the "double clamp" (Tasaki and Spyropoulos, 1958).

Experimental and theoretical work has been in progress in this laboratory for some time concerning the interpretation of these anomalous results. It appeared at the outset that the notches, and the trains of oscillations reported by Tasaki and coworkers, would have to be explained either by a drastic revision of the concepts which emerged from the earlier work, or on the basis of non-uniform potential control and/or non-uniform membrane properties.

In this paper we shall discuss several ways in which the measured membrane current under attempted voltage clamp conditions fails to represent what the average would be over an area which was in fact subjected to a step of constant potential. It will be assumed that, for a membrane area of more than a few square microns, the accuracy with which the membrane potential may be forced to be spatially constant is limited only by the realizability of low resistance electrodes placed close enough to the membrane. Experimental results are presented which strongly support the conclusion that in any voltage clamped region, notches or oscillations will not appear, and that no new fundamental interpretations of the voltage clamp data are required at this time.

METHODS

The methods used in the experimental work presented here have been described (Cole and Moore, 1960). A schematic representation of the experimental arrangement is shown in Fig. 1 and includes all the kinds of electrodes which were used. The external differential electrodes and the internal probe were not used simultaneously. In some experiments an additional pipette electrode was placed outside the axon near the internal probe in order to increase the accuracy of the measurement of the membrane potential by this probe. Further clarification will be included as the occasion arises.

ANOMALOUS CURRENT PATTERNS

Following the observations of Cole and Moore in 1954 of notches in the pattern of membrane current during a depolarizing pulse such results have been observed from time to time. Some representative patterns are shown in Fig. 2. The notches appear only over a range of membrane potentials during the pulse which corresponds approximately to the region of apparent negative resistance in the peak inward current *versus* potential curve; they are sometimes, but not typically, associated with a "threshold;" using a point control system there are never more than

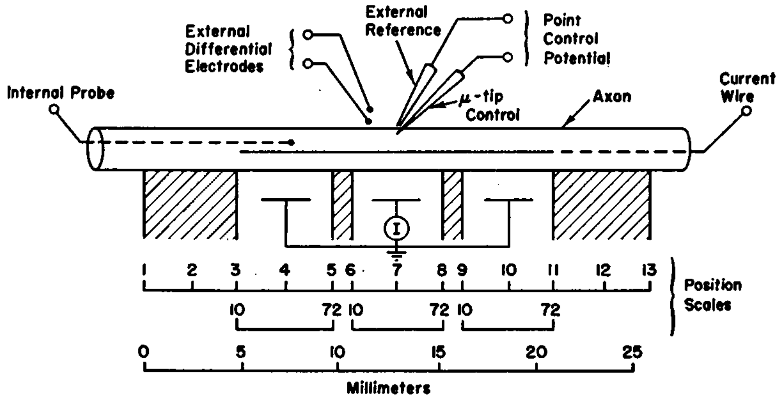

FIGURE 1 Schematic representation of experimental arrangement for point control voltage clamp experiments. The position scales refer to the positions of the external differential electrodes and the internal probe. The total current through the center chamber electrode is referred to as I_e in the text. The axial current electrode was a 75 micron diameter platinized platinum wire. Dashed lines indicate insulated wires.

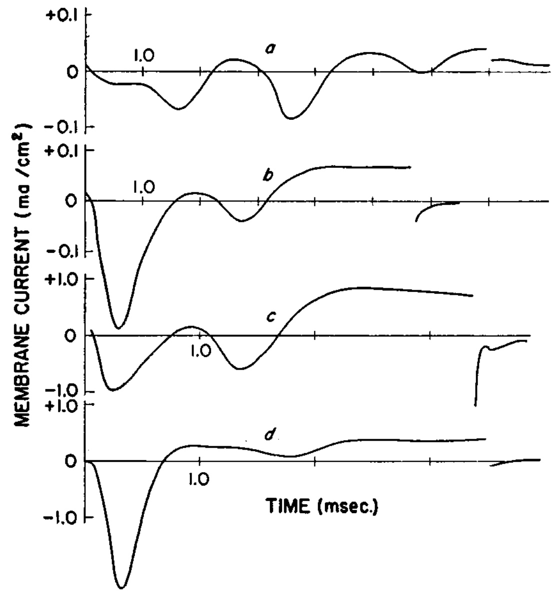

FIGURE 2 Representative center chamber current patterns having the additional peaks of inward (negative) current referred to as notches. These curves were obtained by the deliberate use of high surface resistance axial electrodes. Axons in normal artificial sea water, except *a*, which contained 2 mM K^+. *a*, membrane potential step from —55 mv to —35 mv. *b*, potential step from —75 mv to —37 mv. *c*, 40 mv depolarizing step, initial potential not recorded. *d*, Potential step from —70 mv to —40 mv. Membrane potentials with respect to external solution; *i.e.*, control microtip minus external reference.

two additional inward peaks. When axial current supplying electrodes of very low surface resistance are used the notches seldom occur. Our interest here is not in the notches *per se,* but in the implications with respect to the adequacy of the clamping and the concepts underlying the interpretation of the results. In order to investigate these matters, axial electrodes of deliberately high surface resistance were prepared and it was with the use of these electrodes that the curves of Fig. 2 were obtained.

CORRELATION OF NOTCHES WITH AXIAL ELECTRODE RESISTANCE AND MAXIMUM INWARD CURRENT

There are very good reasons (see below) why an increase in the surface resistance of the axial current supplying electrode would be expected to favor the appearance of anomalies in the current patterns. An analysis was made of all the experiments from the summer of 1958 for which the relevant data were recorded to determine how good the correlation was between the appearance of notches, the axial electrode resistance, and the maximum peak inward current obtainable. Most of the electrodes were 75 micron diameter platinized platinum wires. The surface resistance was measured before and after each experiment by applying a step of constant current and observing (through a reference electrode) the potential at the beginning of the step and at 10 milliseconds later. The ratio of potential to current obtained at zero time will be referred to as R_0; at 10 msec. as R_{10}. These values contain a certain amount of resistance due to the solution (about 12 ohm cm; *cf.* Cole and Moore, 1960, Appendix B) so that they are maximum values. No corrections were made. The electrodes used fall into two groups—those for which the resistance was as low as possible, and those which had a deliberately high resistance. In the high resistance group (R_0 greater than 30 ohm cm; R_{10} greater than 100 ohm cm) notches occurred in every case. For the routinely used electrodes R_0 varied from 12 to 23 ohm cm (average 18.3); R_{10} from 27 to 59 ohm cm (average 40.6). Notches occurred in 37 per cent of 47 experiments with no correlation between notches and resistance within the group. There is thus only a rough correlation of notches with axial electrode resistance. Attempts to correlate notches with the maximum peak inward current for any nerve were completely unsuccessful.

On almost any theory, the effect of the electrode surface resistance should be closely related to the potential drop from the inside of the metal wire to the interior surface of the axon membrane (*cf.* two patch theory below). To the extent that the radial current flow was uniform this potential drop would have a maximum given by the product of the total radial resistance times the maximum peak inward current. The radial resistance of the axoplasm for a 75 micron diameter electrode and a 500 micron diameter axon is about 12 ohm cm. This value is close to that added to the measured electrode resistance by the solution in which they were measured, so that the measured values are close to the total radial resistance with the electrode in the axon. With these approximations, the results are shown in Fig. 3. The coordinates (on logarithmic scales) are the maximum peak inward current and the average of the zero time axial electrode resistance as measured before and after each experiment. The values for the axial electrode resistance have been converted to units of ohm cm^2 (ohms for a square centimeter of membrane, not electrode surface) so that the product of the coordinates of any point is in millivolts. Any 45° line represents some approximate maximum value for the radial potential drop. Axon-electrode combinations which were capable of pro-

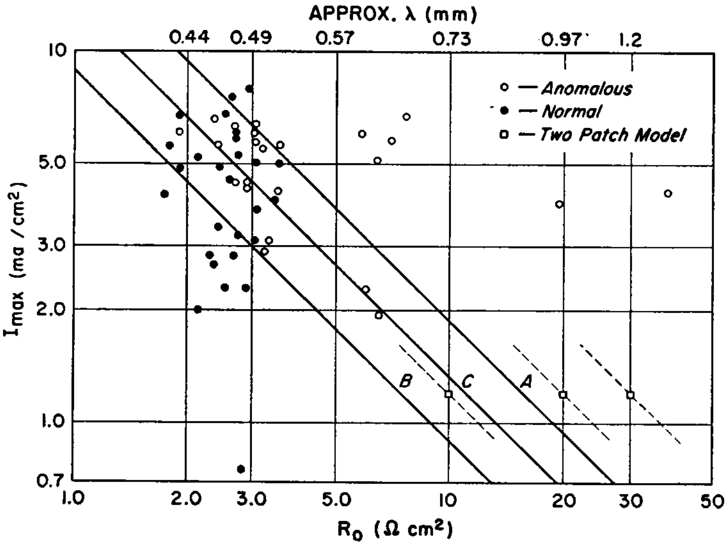

Figure 3 Appearance of notches (open circles) in membrane current during some depolarizing membrane potential step compared to normal, simple patterns (solid circles) in which no notches occurred for any value of potential during the step, for all 1958 experiments for which the relevant data were obtained. Coordinates are the maximum value for the peak inward current (I_{max}) for each particular axon, at the time of the observations, and the value of the axial electrode high frequency surface resistance (R_0). R_0 was measured as described in the text and converted here to units of ohms per square centimeter of axon membrane. Diagonal lines thus represent loci of constant potential which approximate that between the inside of the axial wire and the inside surface of the membrane at the time of the maximum peak inward current. Along the top of the graph are the values of the space constant (λ) calculated for a 500 micron diameter axon with external isopotentiality assumption and nominal values of axon parameters given in text for a resting membrane. Two patch model results (squares) are for uniform case with series resistance of 10, 20, and 30 ohms (see later text). Maximum peak inward current for the two patch model is plotted for comparison as 1.24 ma/cm^2 after multiplication by a scale factor of 0.516. This scale factor is the ratio of the peak inward current for the H-H axon as used in the computations (2.4 ma/cm^2) to that of the average of the experiments shown here (4.65 ma/cm^2).

ducing a notch in the current pattern are represented by the open circles, those which were not capable of producing a notch by solid circles.

The ten experiments for which the product of resistance and maximum inward current was the largest (greater than line A, about 20 mv) showed 80 per cent notches; the ten least products (less than line B, about 9 mv) showed no notches. The remaining thirty points are divided by line C at the median. In this group there were 53 per cent notches above the median, 47 per cent below. The lack of correlation in this group indicates that there are other variables involved. There is little doubt but that these were non-uniformities in axon diameter, axon membrane properties, and electrode surface impedance. The important conclusion is that no anomalous current patterns were observed under conditions when the maximum radial potential drop internally was less than about 9 mv but in almost every case when this potential drop was greater than about 20 mv. There is no indication whatever that the axons which were weak differed in any qualitative manner from those which could produce large inward currents. The conclusion is that the latter are merely more difficult to control. These remarks are in no way meant to imply that it is the magnitude of the inward current itself which makes control difficult.

MEMBRANE CURRENT AND POTENTIAL DISTRIBUTION —EXPERIMENTAL

Localized Membrane Current Measurements. A powerful method for investigating the spatial distribution of membrane current (originating in discussions with J. Lettvin; and see Cole and Moore, 1960) is to measure the external radial potential gradient in the immediate vicinity of the membrane. Measurements were made with a pair of 50 micron diameter electrodes spaced 200 to 300 microns apart (Cole and Moore, 1960) and oriented perpendicular to the membrane surface. It is plausible that the membrane current in the surrounding region will influence the potential between these electrodes to an extent which decays rapidly with distance to a negligible value at a few times the electrode separation.

A resolving power of about 225 microns was calculated (from measurements with a 500 micron diameter wire) as the separation between two narrow stripes on a 500 micron axon which would just give a dip in the observed apparent current density at a point midway between the two stripes. The calibration of these electrodes was only approximate and we will employ an arbitrary scale factor and consider only the time course and distribution of membrane current measured in this manner.

The kinds of results which were obtained are illustrated in Figs. 4 to 8. Figs. 7 and 8 are isometric plots of the data shown in Figs. 5 and 6 respectively. Fig. 4 is from an axon with relatively high maximum inward current (5.0 ma/cm^2) and relatively low resistance axial electrode (R_0 = 20 ohm cm). There was no obvious deviation of the pattern of membrane current averaged over the center chamber region from those described earlier (Cole, 1949; Hodgkin, Huxley, and Katz,

1949) and used by Hodgkin and Huxley (1952) in their empirical formulations. However, for a depolarizing pulse of + 50 mv (from a holding potential of −74 mv) the inward current at 0.5 msec. was about 30 per cent greater near the ends of the center chamber than in the middle and there is a large reduction in the center of the guard region. The much smaller variation in the steady state current at 5.0 msec. indicates that this is not the result of a gross injury.

Localized membrane current measurements on two nerves which did show a notch in the average center chamber current are shown in Figs. 5 and 6 and 7 and 8 in which several important points are illustrated. The first, and the most important result of the present investigation, is that the second hump of inward current becomes smaller as the point control region is approached (position 7, Fig. 5; position 41, Fig. 6). This result was found in every case. Because the electronic feed-back apparatus was capable of maintaining constant membrane potential at the control point during the pulse, and that it did so was known from direct measurement, the conclusion is inescapable that the anomalous current patterns seen as the current electrode pair is moved away from the control point are directly associated with a failure of the "space clamp." It is, of course, highly desirable to understand the

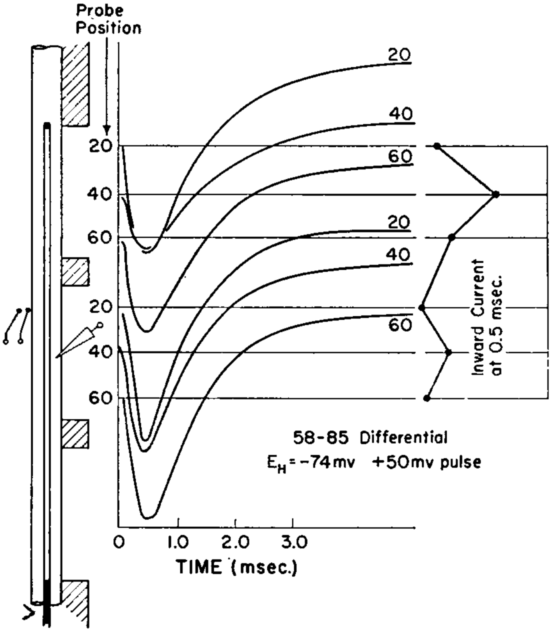

FIGURE 4 Distribution of current (in left guard and center chamber) as measured with external differential current measuring electrodes at the positions indicated on the schematic diagram at the left. This axon did not show a notch under any conditions. Profile at right shows value of inward current at each position at 0.5 msec. after beginning of potential step. E_H is the "holding potential" at which the control point was held prior to the initiation of the depolarizing pulse.

reasons behind the space clamp failure in these cases—and it is necessary to do so in order to deal with the problem effectively—but it is stressed that neither a theoretical explanation nor measurements of membrane potential variations are required in order for the above experimental results to demonstrate with almost complete certainty, for the squid axon in sea water, that regardless of what kind of experimental arrangement is employed no "notches" or "oscillations" in the current pattern occur during an applied potential step.

Although a kind of action potential invasion might be responsible for the patterns seen in Fig. 6, positions 71, 61, 51, it is difficult to see how this could be the case for any of the other patterns in Fig. 5 or 6. The patterns in Fig. 5, position 2, show that a repetitive response was occurring beyond the end of the guard region (three separate traces) but there is no indication that these action potentials actually invaded into the center chamber region. Action potential invasion will be further discussed below.

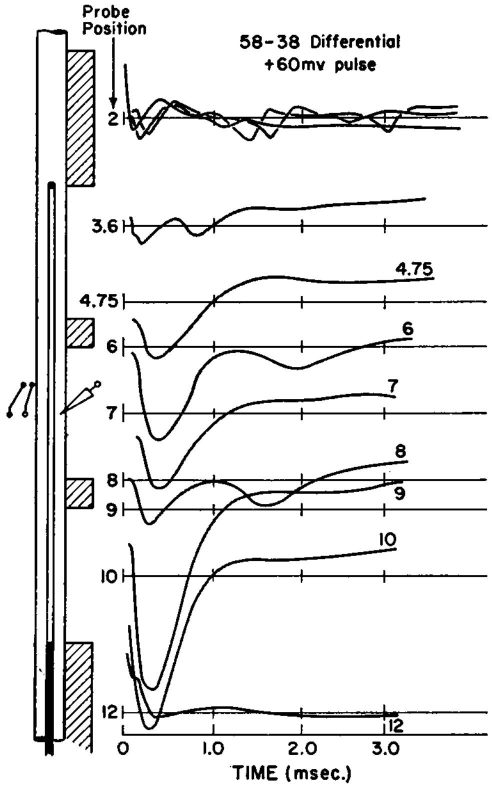

FIGURE 5 Distribution of external differential electrode current measurements for an axon, electrode, and depolarizing pulse which resulted in a notch in the center chamber current. Note the absence of the notch at position 7, near the control microelectrode. Curves at position 2 are three superimposed traces showing action potentials which did not invade the center chamber. These data are shown in isometric projection in Fig. 7.

Localized Membrane Potential Measurements. A large number of measurements were made of the distribution of potential in axons under a variety of conditions, with the use of internal and external micropipette electrodes and longitudinal insertion of a platinum wire insulated except at the tip (*cf.* Cole and Moore, 1960). We are particularly concerned with gross effects resulting from space clamp failure using high resistance axial current electrodes, but even under apparently moderately good conditions with no apparent anomalies in the total membrane current measured over the center chamber (4.6 mm), the uniformity of membrane potential may be far from ideal. This situation is illustrated in Fig. 9 in which the axial electrode resistance was $R_0 = 14$ ohm cm (2.12 ohm cm^2 of membrane),

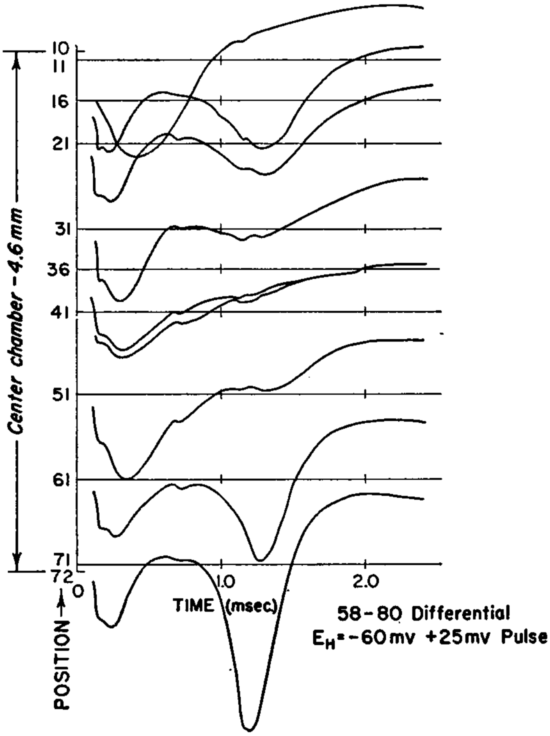

FIGURE 6 Distribution of membrane current as in Fig. 5, but for center chamber only. A notch appeared in the center chamber current under these conditions. Microelectrode potential control was close to position 41 (double trace shows current at beginning and end of this series of measurements). The pronounced notches away from the control point do not occur at position 41.

$R_{10} = 59$ ohm cm (8.95 ohm cm^2) and no indication of a notch in the membrane current. Membrane potential measurements at three positions in the center chamber were made with the internal wire probe and an external pipette; the maximum variation at various values of potential during the pulse (E_p) is plotted as delta V_m in Fig. 9. Values at the time of the peak inward current are denoted by the diamonds, at the steady state by the open triangles. These have been corrected for deterioration by a linear interpolation based on the curves of peak inward current, also shown on Fig. 9, taken before and after the period (about 20 minutes) of membrane potential measurements. The maximum variation of membrane potential over the center chamber clearly occurs at the value of potential during the pulse which is in the region where the peak inward current is changing most rapidly. The

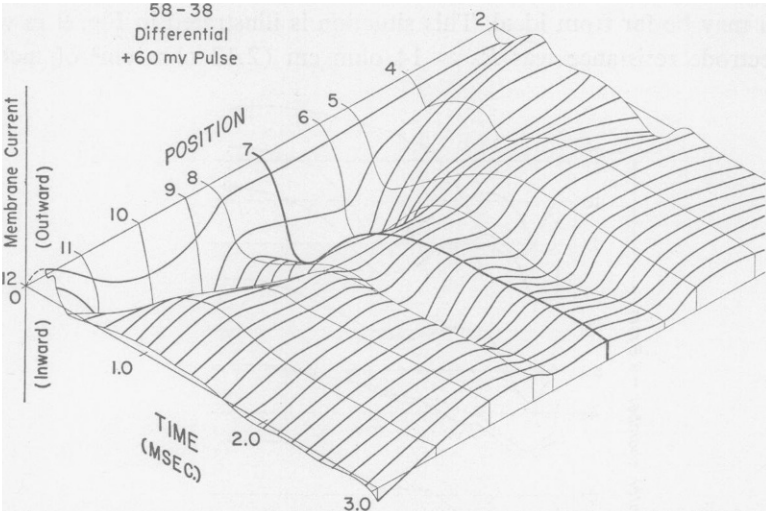

FIGURE 7 Membrane current distribution. Isometric projection of data shown in Fig. 5. Lines roughly paralleling position axis are lines of constant time, drawn freehand.

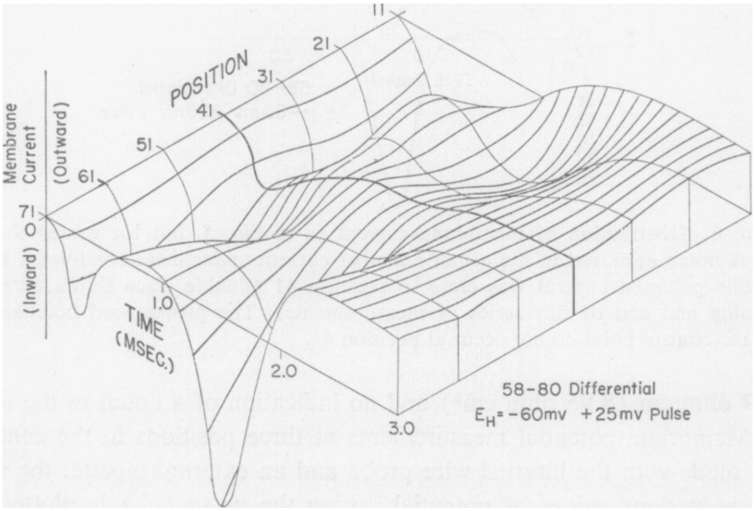

FIGURE 8 Membrane current distribution. Isometric projection of data shown in Fig. 6. Notch at positions 51, 61, and 71 might be the result of an action potential arising beyond the end of the cell and interacting with the potential-current behavior in the cell. The notches at positions 21 and 31 do not appear to be the result of an external action potential.

variation during the steady state (3.3 msec. after the beginning of the step) increases with the amount of depolarization; within the limits of error it could be proportional to the pulse magnitude. Although many significant results might be obtained with an axon such as this one a membrane potential variation reaching 16.5 mv in the center chamber cannot be considered an ideal space clamp. Presumably this axon was very non-uniform—perhaps an injury had occurred somewhere—but in the past there has been no simple, practical procedure by which it could have been eliminated from an experimental series.

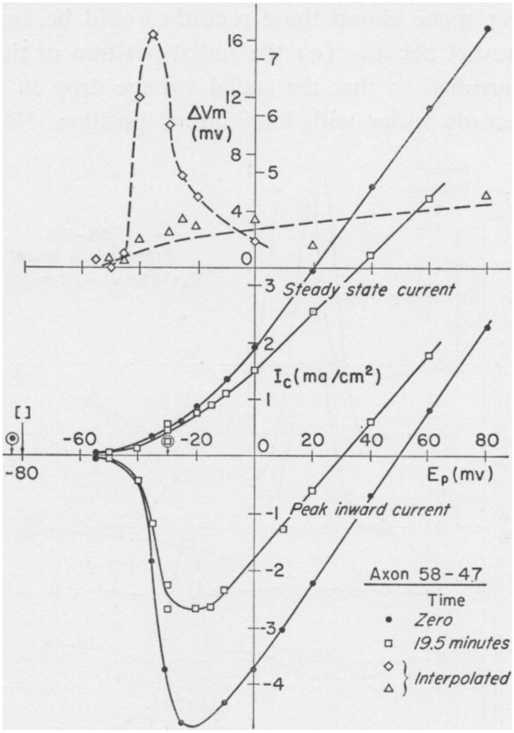

FIGURE 9 Upper graph, the variation in membrane potential (V_m) among three positions in the center chamber during a depolarizing pulse. Membrane at control microelectrode was subjected to a potential step from an initially hyperpolarized value of —80 mv (square brackets) to the value as indicated (E_p). Diamonds, variation at time of total center chamber peak inward current. Open triangles, variation at the time of the steady state outward current.

No notch occurred in the center chamber current for any value of depolarizing pulse.

Lower graph, peak inward and steady state outward center chamber (I_c) for comparison with upper graph. Two sets of data taken before potential measurements (solid circles) and after (open squares, average of 19.5 minutes later). Values given in upper graph were corrected for the deterioration of the axon by a linear interpolation, based on the data in the lower graph. The circled points on the potential axis are the resting potentials of the axon measured before and after the two current curves.

The distribution of membrane potential under conditions of extreme space clamp failure resulting from the use of very high resistance axial current supplying electrodes is illustrated in Figs. 10 to 13 (the isometric projection of Fig. 11 is from the same data as those of Fig. 10). These data were obtained by taking the difference in potential between a longitudinally inserted probe and the microtip electrode used for potential control. Adequate controls were made in each case to insure that the potential between the microtip control and the external reference electrode was indeed a step. Thus the inner probe minus microtip curves approximate the variation in the membrane potential, as a function of time, from the ideal space clamp. For a perfect space clamp these records would be zero at all times. The approximation is inexact because (*a*) the radial position of the inner probe could not be precisely controlled so that the radial voltage drop in the axoplasm which is present in the records varies with longitudinal position. Neither the magnitude

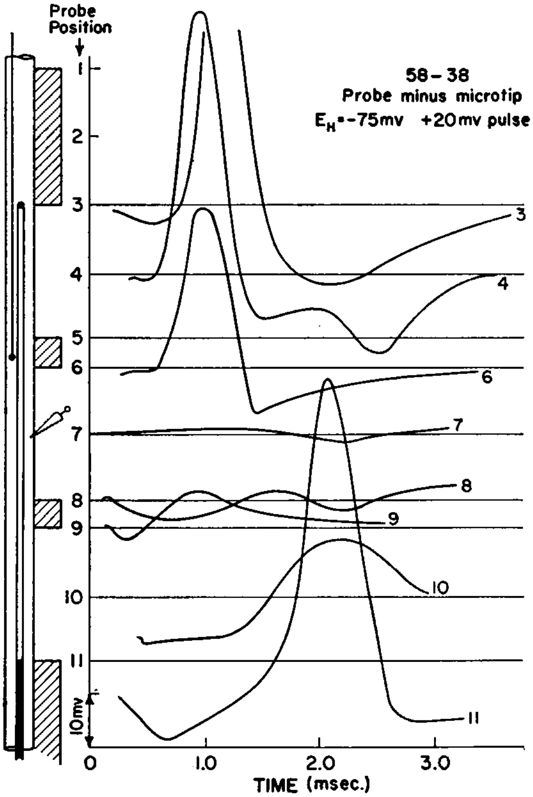

Figure 10 Space clamp failure. Deviation from base line is the potential of internal probe minus the control microtip potential. This is approximately the difference between the membrane potential and the potential at which the clamping was attempted. Deliberately high resistance axial electrode used to impair space clamp. Control microtip near position 7. Depolarizing pulse of 20 mv from initially hyperpolarized value of —75 mv.

nor the time course of this error is calculable since the membrane current and thus the radial current are certainly not uniform with distance. The magnitude of this error could reach maximum values of 10 to 15 mv (a few milliamperes per square centimeter of membrane times the radial axoplasm resistance of about 2 ohm cm^2), and (*b*) the potential of the external solution along the outer surface of the axon is not constant to within more than a few millivolts under conditions of extreme space clamp failure. For these reasons, and because the analysis of the distribution of voltage and current in such a complicated system has not been attempted in detail, exact quantitative considerations are not possible. However, in spite of the approximations involved, several relevant conclusions emerge from these results. These are: (*a*) the deviations are not confined to depolarizing pulses in a restricted potential range as the obvious anomalies in the current patterns are; (*b*) very large deviations from the membrane potential changes impressed at the control point occur in regions not more than a millimeter distant; (*c*) the behavior of the membrane potential on one side of the control point bears no constant relation to that on the other; and (*d*) the deviations are usually di- and sometimes triphasic.

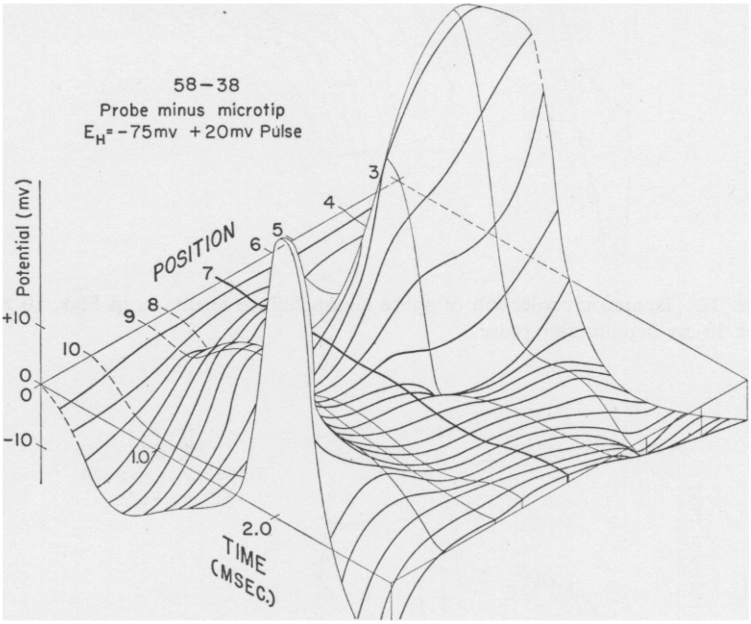

FIGURE 11 Isometric projection of data in Fig. 10.

The curves of approximate membrane potential *versus* time and distance along the axon presented in Fig. 12 have been converted into curves of the potential between the axial wire and the inner probe. This was done with the use of the curve of the potential between the axial wire and control microtip for this particular depolarizing step, which is not illustrated; *i.e.*, axial wire minus inner probe equals

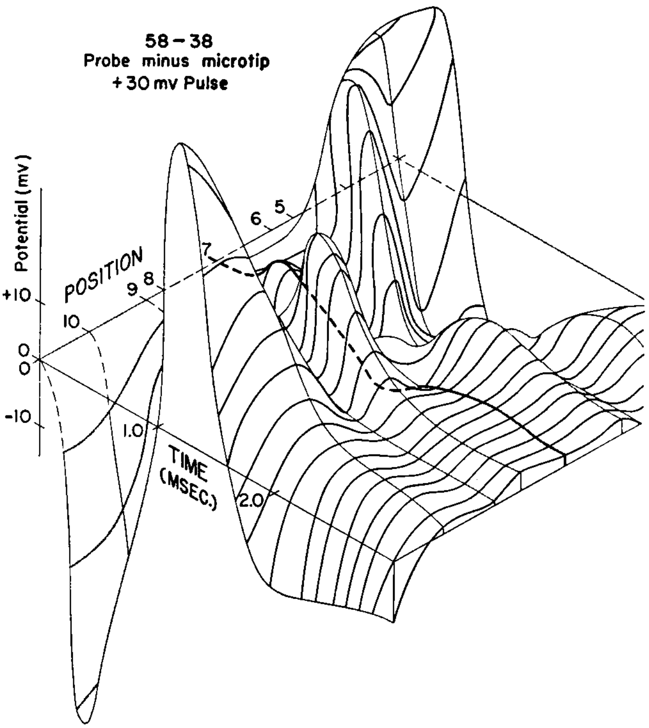

Figure 12 Isometric projection of space clamp failure results as in Figs. 10 and 11, but for 30 mv depolarizing pulse.

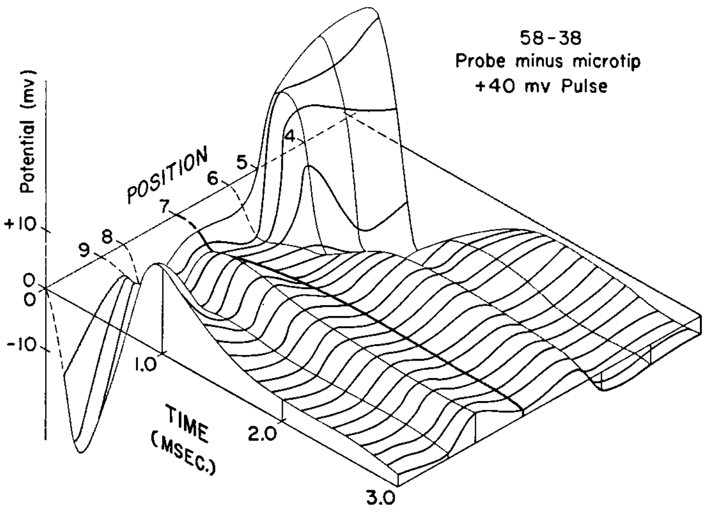

Figure 13 Isometric projection of space clamp failure results as in Figs. 10 and 11 but for 40 mv depolarizing pulse.

the difference between the axial wire minus microtip and inner probe minus microtip. The results are shown in Fig. 14. These curves have been computed in an effort to obtain an approximation to the membrane current which is flowing at any point at the same time that the potential measurements were made. Such data were not obtained with the simultaneous use of external differential electrodes because of the complexity of the experimental setup required. However, the axial wire minus inner probe potential measurements approximate the use of internal differential electrodes. If there were no longitudinal currents flowing in the axoplasm, nor any axial electrode polarization, these curves would be an exact measure of the radial current leaving the axial wire and passing through the membrane of the axon. Longitudinal currents are present, and could be estimated from the data of Fig. 13. This has not been attempted because of the errors discussed above. However, bearing in mind the presence of some inaccuracies, certain of the curves of Figs. 13 and 14 are compared in Figs. 15 and 16 and represent approximate curves of simultaneous membrane potential and current during space clamp failure. We note that

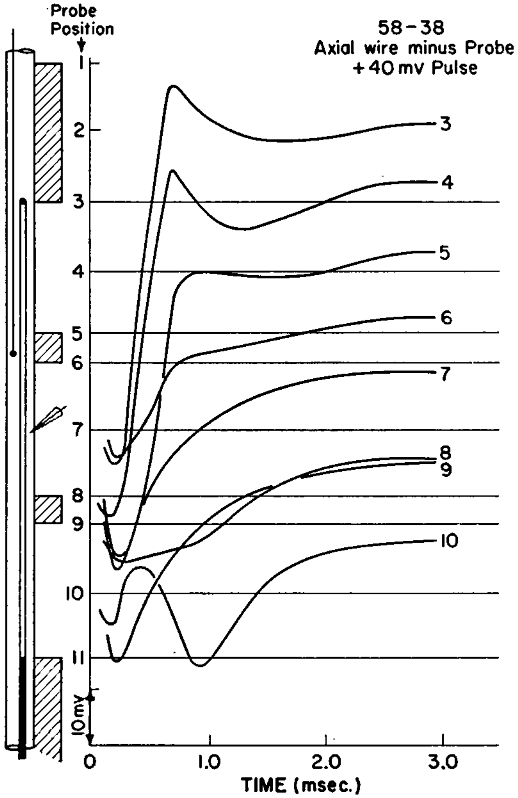

FIGURE 14 Approximate membrane current distribution during space clamp failure. These curves are the potential between the axial wire and the internal probe derived from the data shown in Fig. 13, and see text.

there is a tendency for the current curves (axial wire minus inner probe) to have the shape ordinarily seen in a proper voltage clamp experiment when the curve of membrane potential (inner probe minus microtip) is fairly flat. This is not always exactly true, and the presumption is that the discrepancies are due to longitudinal axoplasm currents. Other general tendencies occur: a well developed notch in the membrane current is associated with a membrane potential which is diphasic with the first phase negative going (Fig. 15) and not when the first phase is positive going as in Fig. 16. The dotted curves in Figs. 15 and 16 are computed potentials and currents in the "two patch" model discussed below. The relationships shown here are understandable on the basis of the two patch model but this is not meant to be in anyway exact or comprehensive. For example, it is surmised that the effect of the propagation of an action potential into the chamber from the end at just the right time might produce potential and current patterns which were indistinguishable from some of those computed on the basis of this model.

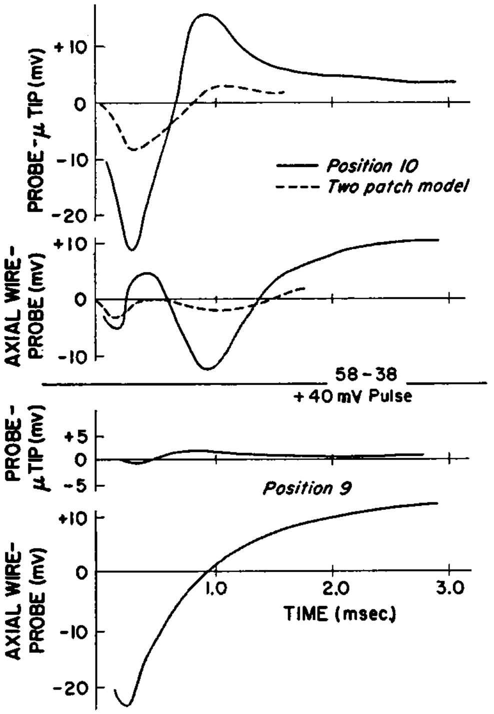

FIGURE 15 Simultaneous approximate membrane potential deviation (probe minus microtip) and membrane current (axial wire minus probe) from Figs. 13 and 14. Note that at position 9 the potential deviation is small and the current has only one inward peak. At position 10 the potential variation is initially negative going and current has pronounced notch. Dotted curves are results of computations on the two patch model which illustrate the same type of relationship. See text below.

THE SPACE CLAMP — THEORETICAL

We shall consider how well one may be expected to be able to maintain a spatially uniform and known potential over an extended region of the squid axon membrane, assuming perfect electronic control of the potential between the microtip and external reference control electrodes (see Fig. 1). We shall not analyze in any detail the results to be expected using an extended control system. Some of the results to follow apply to either system and some do not. The effects of the lateral guards will not be considered in detail.

It is important to realize at the beginning that in either the point control or extended control systems the means by which the membrane potential control is attempted is *only* through the potential which is applied between the internal and external current supplying electrodes. These electrodes represent the effector organ of the feedback apparatus.

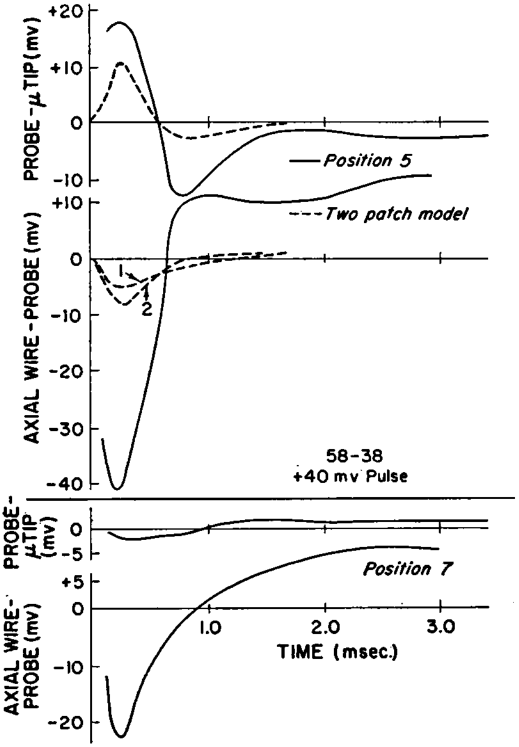

FIGURE 16 Curves as in Fig. 15. At position 7, near the microtip control the current has one inward peak. At position 10 the potential deviation is initially positive going and the membrane current has a large inward peak and no pronounced notch. Dotted curves from two patch model computations illustrate this type of relationship. Dotted current curve labeled (1) is for the controlled, (2) for the uncontrolled, patch. This is an example of a no-notch error discussed below.

Electrotonic Spread with Axial Wire. An extension of the usual simple core conductor model approximation has been devised by one of us (KSC) which includes the effect of the axial wire and the external fluid. An element of length of this model is shown in Fig. 17. Applying Ohm's and Kirchhoff's laws to the circuit of Fig. 17 and allowing the length of this element to approach zero we may write

$$\begin{aligned} d^2V_2/dx^2 &= -r_2[(V_a - V_2)/r_1 - i_3] \\ d^2V_4/dx^2 &= -r_4(i_3 - V_4/r_5) \end{aligned} \qquad (1)$$

where:

V_a	is the potential, in volts, of the axial wire relative to a ground electrode considered to be equivalent to a cylinder surrounding the axon;
V_2	is the potential, in volts, relative to ground, at a point inside the axon;
V_4	is the potential, in volts, relative to ground, of a point outside the axon;
r_1	is the equivalent radial resistance, in ohm cm, between the axial wire and the point at which V_2 is taken; it includes r_e, the axial electrode surface resistance;
r_2	is the equivalent longitudinal resistance, in ohm/cm of the axoplasm, considered to be lumped along a line;
r_3	is the equivalent radial resistance, in ohm cm, between points at which V_2 and V_4 are taken, including the membrane resistance;
r_4	is the equivalent longitudinal resistance of the external solution, in ohm/cm;
r_5	is the equivalent radial resistance of the external solution, in ohm cm, between the point at which V_4 is taken and ground;
i_j	is the current through r_j, in appropriate units for any j, positive from left to right, or top to bottom in the model as shown in Fig. 17;
x	is distance along the model from some reference point, positive to the right.

Equations (1) require no assumptions about the electrical properties of the membrane. In the steady state (in time) the membrane may be represented by an equivalent battery (V_3) in series with a resistance (r_m), in which case we may write the further relations

$$i_3 = (V_2 - V_4 - V_3)/r_3 \qquad (2)$$

$$V_m = V_3 + i_3 r_m \qquad (3)$$

The quantity ($V_2 - V_4$) will differ from the membrane potential V_m by an amount which will only be large if the membrane resistance r_m is small compared to r_3 (note $r_m < r_3$). Equation (3) may be written as

$$V_m = (V_2 - V_4)r_m/r_3 + V_3(1 - r_m/r_3). \qquad (4)$$

and it will be convenient to consider ($V_2 - V_4$) instead of V_m in most cases.

In the event (see later) that the errors introduced by neglecting the potential variations in the external solution can be tolerated, the relevant equations are obtained by setting $r_4 = 0$ in equation (1).

The solutions of the equations for electrotonic spread in the usual simple core conductor model are characterized by the fact that the variable x is always multiplied by a constant, $1/\lambda$, where λ is referred to as the "space constant" or "characteristic length" of the nerve. It is a complication of the model shown in Fig. 17 that instead of one space constant being sufficient, two are required. The solution of equations (1) under steady state conditions (in time) given by equation (2) and the condition that V_2 and V_4 are bounded, for an infinite length of nerve, x greater than zero, is

$$\begin{aligned} V_2(x) - V_2(\infty) &= Ae^{-x/\lambda_1} + Be^{-x/\lambda_2}, \\ V_4(x) - V_4(\infty) &= Ce^{-x/\lambda_1} + De^{-x/\lambda_2}, \end{aligned} \qquad (5)$$

where λ_1 and λ_2 are the positive roots of the equation

$$\left[r_2\left(\frac{1}{r_1} + \frac{1}{r_3}\right) - \frac{1}{\lambda^2}\right]\left[r_4\left(\frac{1}{r_3} + \frac{1}{r_5}\right) - \frac{1}{\lambda^2}\right] - \frac{r_2 r_4}{r_3^2} = 0. \qquad (6)$$

Values for the Model. The resistivity of axoplasm will be taken to be about twice that of sea water and assumed to be 40 ohm cm. For a 500 micron diameter axon the longitudinal internal resistance will be about 15,000 ohm/cm; the convergence resistance between a 75 micron diameter axial wire and the axon membrane will be about 12 ohm cm. The values to be used for the external resistances were arrived at by fitting the model to the results of measurements of the distribution of current outside a 500 micron diameter wire which was insulated except for one narrow ring, and positioned in the cell in place of the axon. A space constant of about 0.1 cm was found at a distance of about 100 microns from the surface of the wire and at about 0.1 cm from the surface of the wire the lines of current flow were

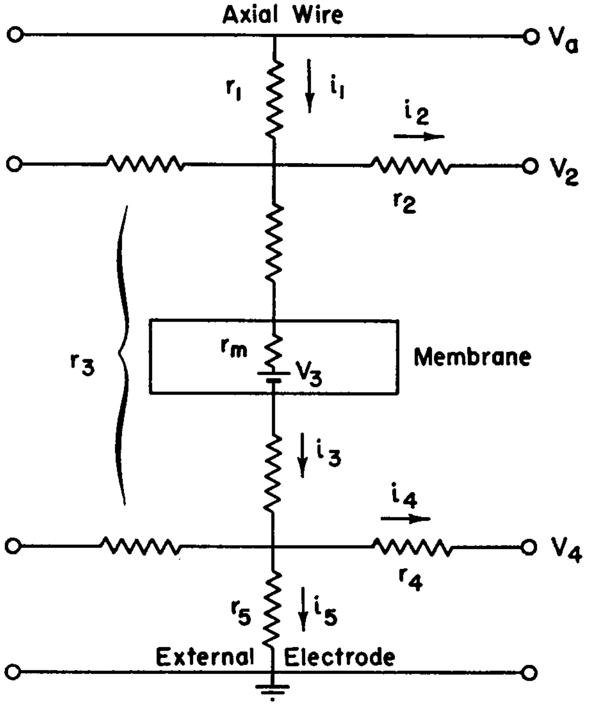

FIGURE 17 An element of length of the model taken to represent a squid axon containing axial wire. The external fluid is represented by resistances r_4, r_5, and a portion of r_3. For details see text.

virtually parallel. The values for r_4 and r_5 were chosen so that the model fits these data in the limit where the membrane resistance (hence r_3) is infinite. Thus, for r_3 infinite, equation (4) gives $1/\lambda^2$ as equal to r_4/r_5 or r_2/r_1. We take $1/\lambda^2_2$ to be (for r_3 infinite) r_4/r_5 and $1/\lambda^2_1$ to be r_2/r_1. We thus have $r_4/r_5 = 100$. r_4 is taken to be 300 ohm cm as being close to the longitudinal resistance of an annular cylinder with inner diameter 500 microns, outer diameter 2500 microns. Thus $r_5 = 3$ ohm cm. We do not lose any generality by assuming that the sea water at a distance of 0.1 cm is at ground potential. The values for the model thus become: $r_1 = (r_e + 6)$ ohm cm; $r_2 = 15{,}000$ ohm/cm; $r_3 = (r_m + 9)$ ohm cm; $r_4 = 300$ ohm/cm; $r_5 = 3$ ohm cm, where r_e is the axial electrode surface resistance, in ohm cm.

We are now in a position to calculate results from the model and to answer the question of the error introduced by simplifying the model by assuming the external fluid to be isopotential everywhere. Representative values of λ_1 and λ_2 are shown in Table I according to equation (6). Approximate values for the space constant assuming external isopotentiality for the appropriate axial electrode surface resistances are included in Fig. 3. The coefficients (A, B, C, D) in Equation 5 are determined by the parameters of the system and include two arbitrary constants, which could be, for example, $V_2(0)$ and $V_4(0)$. The relative values of these coefficients determine the relative contributions of the term involving λ_1 and λ_2 and this relationship is not constant for the various conditions listed in Table I. It is for this reason that the value of λ for the single space constant approximation in Table I varies from being almost equal to λ_1 for a low resistance electrode and the active state to being almost equal to λ_2 for a high resistance electrode and the resting state.

TABLE I

AXON MODEL SPACE CONSTANTS

λ_1 and λ_2 are the positive roots of equation (6). Parameter values used (see Fig. 17) are: $r_1 = (r_e + 6)$ ohm cm; $r_2 = 15{,}000$ ohm/cm; $r_3 = (r_m + 9)$ ohm cm; $r_4 = 300$ ohm/cm; $r_5 = 3$ ohm cm. The membrane resistance (r_m) is taken to be 5000 ohm cm for the resting state and 50 ohm cm for the active state. The surface resistance of the axial electrode (r_e) is considered for a perfect electrode ($r_e = 0$), a hopeless electrode for space clamping ($r_e = 50$ ohm cm), and for no electrode ($r_e = \infty$). The single space constant approximation is for the assumption of external isopotentiality.

r_e ohm cm	r_m ohm cm	λ_1 cm	λ_2 cm	λ (single space constant approximation) cm
0	50	0.0191	0.0978	0.0191
50	50	0.0436	0.0990	0.0438
∞	50	0.0624	0.1016	0.0627
0	5000	0.0200	0.1000	0.0200
50	5000	0.0624	0.1000	0.0608
∞	5000	0.1000	0.5778	0.5779

The error introduced by the isopotential assumption is illustrated by considering specifically the error which would be introduced for the case in which the membrane properties are the same everywhere and a short circuit is introduced across r_3 at some point. Call this point $x = 0$. We then have that $V_2(0) = V_4(0)$. By integrating the current through r_1 from minus to plus infinity and putting it equal to the integral of the current through r_5 we arrive at another relation between $V_2(0)$ and $V_4(0)$. We shall not present the algebra here, but represent this relation by

$$M_1[V_2(0) - V_2(\infty)] + M_2[V_4(0) - V_4(\infty)] = 0,$$

and we have

$$V_2(0) = V_4(0) = \frac{M_1 V_2(\infty) + M_2 V_4(\infty)}{M_1 + M_2}.$$

We can derive an expression for the isopotential assumption error as

$$\frac{V_4(0) - V_4(\infty)}{V_2(\infty) - V_4(\infty)} = \frac{1}{1 + M_2/M_1} = R. \tag{7}$$

Actually carrying out the above indicated steps we get

$$\frac{M_2}{M_1} = \sqrt{\frac{r_1 r_2}{r_4 r_5}} \left[\frac{\sqrt{r_2 r_5[1 + (r_1 + r_5)/r_3]} + \sqrt{r_1 r_4}}{\sqrt{r_2 r_5} + \sqrt{r_1 r_4[1 + (r_1 + r_5)/r_3]}} \right]. \tag{8}$$

Now, equation (7) gives the variation in the external potential divided by the potential across the membrane far from the short circuit. This is a measure of the fractional error introduced if we should take the external fluid to be isopotential. Note that R does not depend upon the values of V_a, the axial wire potential, or of V_3, the open circuit membrane potential. Using the values given above we may calculate R for various cases. Allowing the axial electrode surface resistance (r_e) and the membrane resistance (r_m) to vary, we reach a maximum error for zero r_e ($r_1 = 6$) and infinite r_m, in which case $R = 0.09$, or about 10 per cent error. The minimum r_e which is realizable, however, is about 20 ohm cm, for which, with r_m infinite the maximum R is 0.045, or about 5 per cent error. A lower bound for R, which is easily computed is with $r_3 = 0$. Under these conditions, equation (8) reduces to r_2/r_4 and thus $R = 0.0196$, independent of electrode resistance. The range of error to be expected is thus between 2 and 5 per cent. This amount is felt to be small enough so that for most cases the assumption of external fluid isopotentiality is justified. We shall refer to the model under these conditions as the single space constant model.

For the condition of external fluid isopotentiality the differential equations (1) reduce to

$$\frac{d^2 V_2}{dx^2} = r_2(1/r_1 + 1/r_3) V_2 - (r_2 V_a/r_1 + r_2 V_3/r_3), \tag{9}$$

which may be written

$$\lambda^2 \frac{d^2 V_2}{dx^2} = V_2 - V_2(\infty), \tag{10}$$

where λ is the characteristic length, or space constant and $V_2(\infty)$ is the value of V_2 which would obtain if the membrane potential were uniform with distance. We see from (9) and (10) that

$$\lambda^2 = \frac{1}{r_2(1/r_1 + 1/r_3)} \tag{11}$$

and

$$V_2(\infty) = (r_3 V_a + r_1 V_3)/(r_1 + r_3). \tag{12}$$

Thus we have a model which is equivalent to the usual core conductor model, for the steady state in time, if we replace the membrane resistance by the parallel resistance of r_1 and r_3 and the open circuit membrane potential by the expression (12). For the most part we shall consider V_2 as if it were the membrane potential, but we must keep in mind that it is actually the potential between some point in the axoplasm and ground. We may, if necessary, use the correct expression (equation (4) with $V_4 = 0$ for the external isopotential case).

THE TWO PATCH MODEL

Genesis of the Model. In any attempted voltage clamp arrangement the membrane potential and current in one region of the axon will exert an effect at some other region in two different ways; by the direct flow of current in the axon and by the influence that one region may have on the potential of the axial wire *via* the external feedback apparatus. Because the results of the above analysis indicate a rather short space constant for passive electrotonic spread (*cf.* Fig. 3 and Table I), in the neighborhood of 0.5 mm, which we have confirmed experimentally (Cole and Moore, 1960), it would seem reasonable that regions separated by more than this distance might interact mainly by the second of the two ways mentioned. This is equivalent to neglecting the coupling due to that part of the membrane between the regions considered; *i.e.*, assuming a space constant of zero. These considerations led one of us (RET) to devise the simplified models shown in Fig. 18 to represent the axon for a point control arrangement (Fig. 18*A*) and an extended control arrangement (Fig. 18*B*). Even on the assumption of a zero space constant the extended control system is not adequately represented by only two patches. No extensive computations have been made for this model and we shall not consider it in detail. For the point control system and the zero space constant approximation, however, two patches are sufficient. For the squid axon in sea water the empirical equations of Hodgkin and Huxley are considered to be appropriate for each individual patch because of the observation that the membrane current measured with external differential electrodes in a region known to be voltage-controlled appears to behave in this way (*cf.* Figs. 5 to 8 and Cole and Moore, 1960).

Computations with the Model. For computations with the model we consider patches (Fig. 18*A*) with the lumped characteristics of 1 cm^2 of membrane as given by the empirical equations of Hodgkin and Huxley at 6.3°C. In every case the applied potential step is from an initially hyperpolarized value of −20 mv (with respect to resting potential). The purpose of the computations was to determine the potential across and the current through the second patch when the first patch was subjected to a potential step. These computations were done in December, 1958, with the use of the analog computer which R. FitzHugh has been using to analyze the properties of the Hodgkin-Huxley equations (FitzHugh, 1960). A preliminary report has appeared (Taylor and FitzHugh, 1959).

As was anticipated, the current through the second patch did not necessarily follow that through the first, even when the two patches were identical and the values of the series resistances r_1 and r_2 were the same.

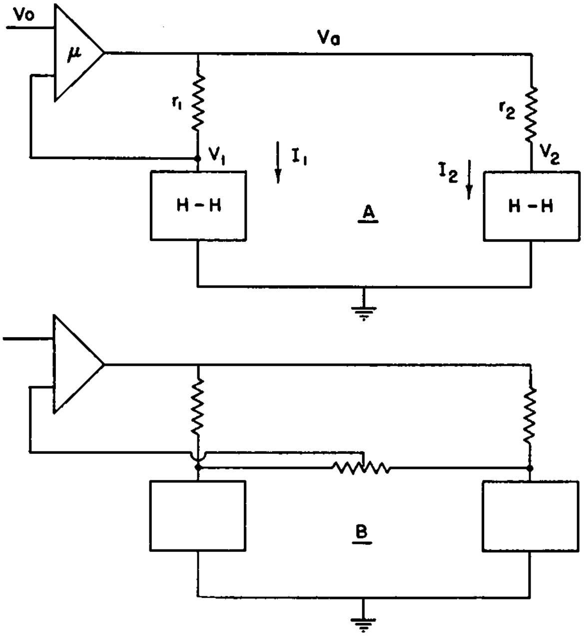

FIGURE 18 The two patch model. *A*; the zero space constant approximation for a point control voltage clamp arrangement. Boxes represent patches of membrane, one of which (subscript 1) is controlled by the electronic feedback apparatus. The other (subscript 2) is any other region of membrane. *B*; simplified model for extended control voltage clamp arrangement. This model (*i.e.*, two patches) would not be sufficient even for the zero space constant approximation, but would probably oscillate if the properties of the boxes were taken to be the Hodgkin-Huxley equations.

In addition to the equations of Hodgkin and Huxley which relate the current and potential for the equivalent membrane patches we have the following relations

$$V_1 = V_o \quad \text{(perfect clamp)}$$

$$V_a = V_1 + r_1 I_1$$

$$V_a = V_2 + r_2 I_2$$

where (see Fig. 18*A*) V_1, V_2, I_1, I_2 are the potentials across and the currents through the first and second patches respectively (currents and potentials are defined relative to resting conditions; outward current and depolarization positive), r_1 and r_2 are the total series resistances between the axial wire (at potential V_a) and the patches. Any three terminal network consisting only of pure linear resistances may be connected to the three points at which V_a, V_1, and V_2 are defined and the model as shown in Fig. 18*A* will be an exact representation (r_1 and r_2 depending on the network) so long as the potential V_1 is controlled. The computations were done by first solving the H-H equations for the first patch for a step of potential from -20 mv to V_1 at time zero. The resultant pattern of current $I_1(t)$ was then fed into the computer at a later time, multiplied by r_1, added to V_1, and the H-H equations were again solved by the computer for the condition that the pattern of potential $V_a = V_1 + I_1r_1$ was equal to $V_2 + I_2r_2$. The final output of the computer was either the potential $V_2(t)$ or the current $I_2(t)$. It was not possible to record these potential and current curves simultaneously, so that those critical cases in which the solution was not necessarily the same for successive computer runs, I_2 and V_2, may not correspond exactly to the same solution.

The current through the membrane capacity was not included in these computations. This component of the membrane current would alter the solutions in two ways: Firstly for short times compared to the time during which the membrane properties change with potential, if the feedback apparatus produced a step in V_1, the potential V_a which produces this step cannot itself be a step. It was taken to be, but auxiliary computations showed that the initial departure of V_a from a step when the capacity current was included was a transient which decayed rapidly enough not to influence the solution at times of interest here. Secondly, for longer times, the value of V_a would include a term $C_mr_2dV_2/dt$. This correction was also included in the computations in certain cases and found to be small.

A final simplification was the manner in which the non-uniformities in the actual situation were represented in the model. This was done by altering the ratio r_1/r_2. A perfectly uniform axon would be represented by a value of unity for this ratio. Other values represent either a non-uniformity in the surface resistance of the axial electrode, the axon diameter, or the condition of the membrane. For the first two the representation is accurate in the case that the membrane properties were uniform, in the third case it would only represent an alteration in membrane properties which were such that all the parameters which determine the membrane conductances in the H-H equations were multiplied by a common factor. There are many ways in which the H-H equations might be modified to represent non-uniformities. This way was chosen because of the ease with which the ratio r_1/r_2 could be changed in the computer, and while it is important to consider the effect of possible non-uniformities in membrane properties there are no data available to indicate what these might in fact be.

The Two Patch Computer Results. Figs. 19 to 23 are reproductions of the computer results which illustrate five situations in which the current through the second, uncontrolled patch was not the same as that through the first. In each case the value of V_1 and of V_2 was −20 mv before zero time. Those cases in which the currents and potentials were the same in each patch are not illustrated. (The computations were not undertaken with a view toward completely mapping the regions of the r_1, r_2, V_1 space in which various kinds of anomalous current patterns would occur, but to demonstrate that, for the model, a notch could indeed occur in the pattern of $I_2(t)$, as had been concluded from rough and intuitive arguments.)

The Uniform Model. All the r_1, r_2, V_2 combinations tried are listed in Table II. The uniform cases are those for which $r_1 = r_2$. A small notch occurred in

TABLE II

TWO PATCH MODEL COMPUTER RESULTS

r_1, series resistance of controlled patch (see Fig. 18*A*). r_2, series resistance of uncontrolled patch. V_1, potential of controlled patch during depolarizing step from an initially hyperpolarized value of −20 mv. The appearance of a notch in the current through the uncontrolled patch is indicated by "notch," a difference between the *shape* of the current pattern in the controlled and uncontrolled patches but without a notch, by "no-notch," and no difference by "same." "Unstable" indicates that in a series of computer runs both notch and no-notch patterns occurred. Underlined entries are those for which Figures appear in this paper.

r_1 ohms	r_2 ohms	V_1 mV			
		+25	+30	+40	+50
	5	Notch	Notch	Notch	—
10	10	Notch (small)	Notch (small)	Same	—
	20	No-notch	No-notch	No-notch	—
	10	—	Notch	Notch	Notch
	15	—	Notch	Notch	—
	18	—	Notch	Notch	—
	20	—	Unstable	Same	Same
20	22	—	No-notch	—	—
	24	—	No-notch	—	—
	25	—	No-notch	No-notch	—
	26	—	No-notch	—	—
	40	—	No-notch	No-notch	No-notch
	25	—	Notch	—	Notch
	30	—	Notch	—	Same
30	35	—	Unstable	—	No-notch (small)
	40	—	Unstable	—	No-notch
	60	—	Unstable	—	—
40	40	—	—	—	Notch (Not reproducible)

the current through the second patch for values of $r_1 = r_2 = 10$ ohms. Increasing this value to 20 ohms gave the results shown in Fig. 19 for potential during the step of $V_1 = +30$ mv. A more or less pronounced notch is seen in successive computer runs. The variability in the results for successive runs is related to noise in the computer. It is not known whether the sensitivity in certain cases was the result of the particular way in which the computer was programmed or is inherent in the H-H equations, but in any case the variability did not appear in those cases (large depolarizing steps) in which the current through the two patches was expected to be identical. A small bias was probably introduced because the curve follower which was used to reintroduce the curve of $I_1(t)$ into the computer slightly underestimated this function; *i.e.*, added a small increment of current in the inward direction. For this reason a strictly uniform case might be better represented by a small difference between r_1 and r_2 and the value for r_1 and r_2 at which notches just begin to appear might be slightly larger than 10 ohms. None of these considerations, however, alters the fact that for $r_1 = r_2 = 20$ ohms a very definite and pronounced notch occurred (Fig. 19). Increasing these resistances to 30 and 40 ohms (Fig. 20) accentuated the notch. The three points represented by the open squares in Fig. 3 are the (corrected) values for peak inward current and series resistance which are appropriate to the two patch computed results for series resistances in the model of 10, 20, and 30 ohms respectively, from left to right. In other words, the two patch model computations indicate that, for a uniform axon notches might be expected to begin to appear for points above and to the right of the 45° line passing through the first square on the left. The uniform model computations only resulted in anomalous current patterns of the notch type except that in the case $r_1 = r_2 = 20$ ohms, $V_1 = +30$ mv, a no-notch pattern would occasionally occur. (A no-notch pattern is illustrated in Fig. 22 and discussed in the next paragraph.) Although not illustrated in Fig. 19, this case is listed as "unstable" in Table I. A slight jar of the curve follower during the initial period of the computation was sufficient to change the course of the solution from a notch to a no-notch anomaly.

Non-uniform Model. As described above, non-uniformities are represented in the model by making r_1 differ from r_2. In every case tried, a non-uniformity of this kind introduced an anomalous current pattern in the second patch for some value of V_1 during the step. The limits of this effect were not accurately delineated (see Table II), but it is clear that for values of r_1 greater than some value which is less than 20 ohms the limits are very narrow. For example, with $r_1 = 20$ ohms, depolarizing pulse to +40 mv, a change in r_2 from 20 to 18 ohms (Table II and Fig. 21 for $r_2 = 15$ ohms) was sufficient to introduce a notch in the second patch. For larger amounts of non-uniformity the limits on the values of r_1 and r_2 are more severe as illustrated by the fact that a notch occurred for the case $r_1 = 10$ ohms, $r_2 = 5$ ohms (see Table II). A different, and potentially more dangerous, type of anomaly occurred in the computed results for the non-uniform cases when r_2 was greater than r_1. This type is illustrated in Fig. 22 and will be referred to as a "no-

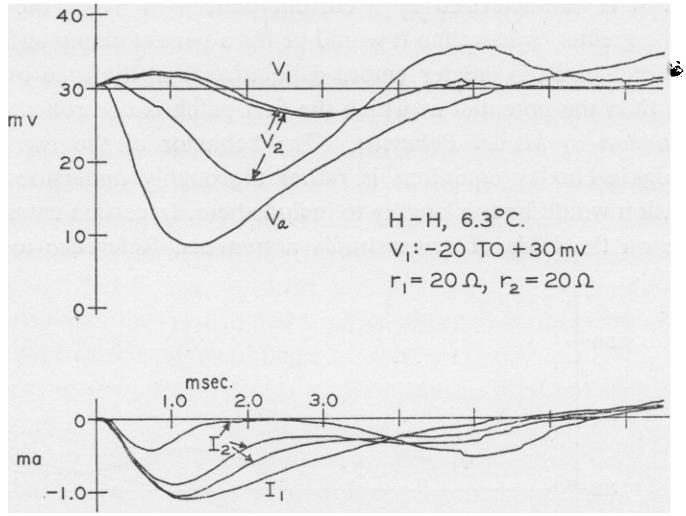

FIGURE 19 Two patch model analog computer results. Uniform case. Currents and potentials relative to resting, outward current and depolarization positive. V_1, I_1 refer to controlled patch; V_2, I_2' to uncontrolled patch; V_a is the potential of the axial wire. The three curves of I_2 and V_2 are for successive computer runs.

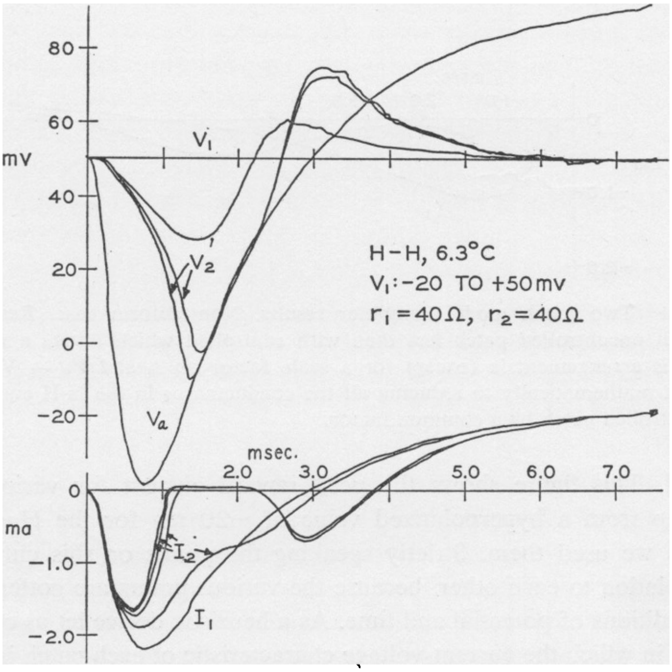

FIGURE 20 Two patch model computer results. Uniform case. Same as Fig. 19 but larger series resistances and depolarizing pulse.

notch" error. It is characterized by a current pattern in which the peak inward current may be greater or less than it would be for a perfect clamp and the potential across the second patch is greater (more depolarized) at the time of the peak inward current than the potential at which the first patch is controlled.

Discussion of Model Behavior. The behavior of the two patch model with the Hodgkin-Huxley equations is rather thoroughly understood, but an extended discussion would be too lengthy to include here. In certain cases the behavior is reasonable on the basis of some simple arguments. Reference to Fig. 24 will prove useful. This figure shows the peak inward current for various potentials during a step from a hyperpolarized value of −20 mv for the Hodgkin-Huxley equations as we used them. Strictly speaking the points on this curve bear only accidental relation to each other, because the various points are gotten under quite different conditions of potential and time. As a heuristic device let us consider a two patch model in which the current-voltage characteristic of each patch is independent of time and identical with the peak inward current curve shown in Fig. 24. Clamping the controlled patch at a potential V_0 would result in a current through this

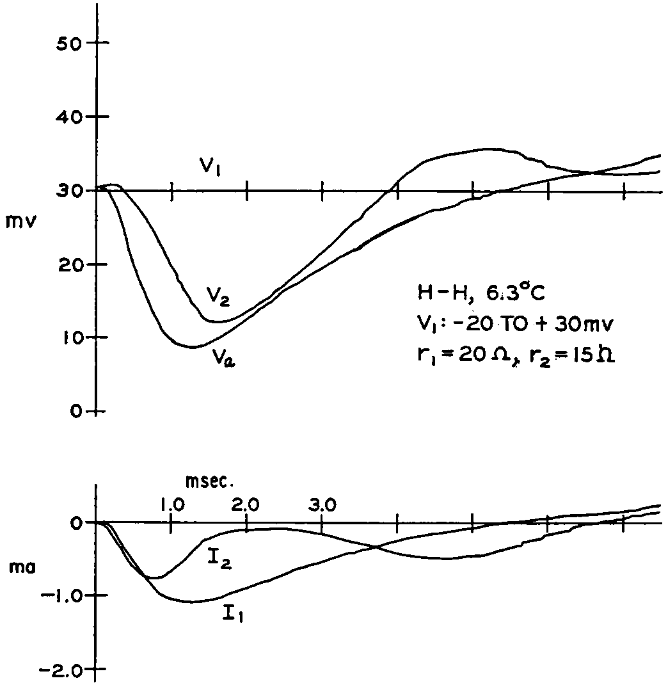

FIGURE 21 Two patch model computer results. Non-uniform case. Resistance in series with uncontrolled patch less than with controlled which favors a notch type error. This arrangement is (except for a scale factor on I_a and V_a — V_2) exactly equivalent mathematically to reducing all the conductances in the H-H equations for the uncontrolled patch by a common factor.

patch given by the intersection of the vertical line through V_0 and the curve. If the series resistance for this patch were 20 ohms the potential V_a would be given by the intersection of the line through this point of slope 1/20 with the zero current axis as indicated. Consider the second patch with identical series resistance of 20 ohms. The current and potential for the second patch will be given by the intersection of the curve and the line through V_a of slope 1/20 (labeled $r_1 = 20$ ohms in Fig. 24). However, because no feedback is involved, the intersection at the potential V_0 will be *unstable*. In the presence of noise the current through the second patch cannot be identical with that through the first, but must be the value given by one of the other two, stable, points of intersection. The final state will depend on the initial conditions and/or the exact time course of the noise. Large enough noise could drive the system from one stable condition to the other in a random fashion. If the electrical properties of the patches depend upon potential and time, as they do in the axon membrane, the entire time course of the current and potential for the uncontrolled patch may be greatly influenced by noise. This is presumably the cause of the variation which sometimes occurred in the computer

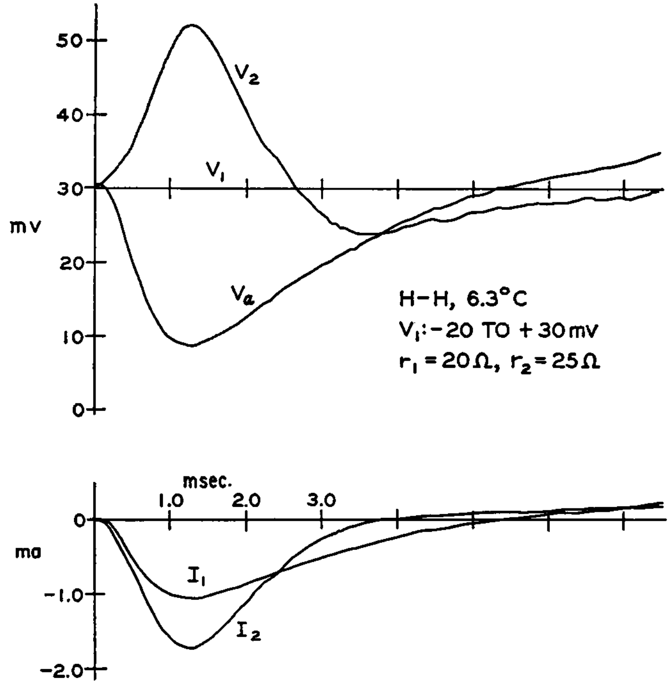

FIGURE 22 Two patch model computer results. Non-uniform case. Resistance in series with uncontrolled patch greater than controlled, favoring a no-notch type of error. This is equivalent mathematically (except for a scale factor on I_2 and $V_a - V_2$) to increasing the conductances in the H-H equations for the uncontrolled patch by a common factor. In this case the inward current through the uncontrolled patch is greater than the controlled, but it may be less in other cases.

solutions on successive runs. An extreme case of this result is illustrated in Fig. 23.

The dashed lines in Fig. 24 represent non-uniform conditions. For this example, with time independent patch characteristics and $r_2 = 15$ ohms, the current and potential of the uncontrolled patch must lie on the line of slope 1/15 passing through V_a and $I = 0$. We note that this line intersects the patch characteristic in only one point, which is near V_a. In a very rough sense this is one kind of situation which leads to the production of notches while the other case illustrated, for $r_2 = 40$ ohms, is the kind of situation which leads to the production of no-notch errors.

Let us now consider the two patch model with the patches obeying the Hodgkin-Huxley equations for the case represented by Fig. 21 where $r_1 = 20$ ohms, $r_2 = 15$ ohms with an applied step in V_1 from -20 mv to $+30$ mv. For times greater than zero the axial wire potential V_a must follow a time course given by $V_a = V_1 + r_1 I_1$ where $V_1 = +30$ mv. At short times the current through the first system, I_1, is becoming more negative, hence V_a will be less than V_1. Assume that the current through the second system, I_2, is equal to I_1. Because r_2 is less than r_1 the potential drop $r_2 I_2$ will be too small to keep V_2 equal to V_1, reducing the amount of depolarization, hence the inward current, for the second patch. This is a regenerative process leading toward the current in the second patch being very small and therefore V_2 will approach V_a. Following the time of the peak inward current through the first patch V_a will become larger, depolarizing the second patch and resulting in a transient, delayed, inward current, which we have called a notch. Because V_1 is under control and the inward current in the controlled patch is of limited duration there is no time for a second notch to occur. This requirement is not present in the extended control system and it is suggested that this is the reason that a train of oscillations may be observed under these conditions (Tasaki and Bak, 1958*a*). Computations have not been done for the system shown in Fig. 18*B*, nor for the extended, continuous model of which this is a severe simplification. Rough and intuitive arguments suggest that the system of Fig. 18*B* would oscillate under certain conditions.

In some cases the current pattern through the second patch not only varied for successive computer runs, but would sometimes show a notch and sometimes a no-notch error. These cases are represented in Table II as unstable and one of them is shown in Fig. 23.

Extensions of the Two Patch Model. Various modifications of the two patch model have been considered in an attempt to gain further insight into the space clamp problem. Some of these have been of value, but none have yielded results which are sufficiently more comprehensive than the two patch model to make them worth including here.

Conclusions from the Model. The most important conclusion from considerations of the two patch model is the very strong support that the computations provide for the assertion that the behavior of the squid axon (in sea water) in the voltage clamp arrangements which have been employed is, *without exception,* predictable from the assumption of the validity of the H-H equations. Even though the general space clamp problem is too complex to envision an early complete theoretical analysis the results to date indicate that the appearance of notches and/or oscillations during the clamping pulse provides no reason to doubt the validity of the current concepts embodied in the H-H equations and is rather to be taken as prima facie evidence of space clamp failure.

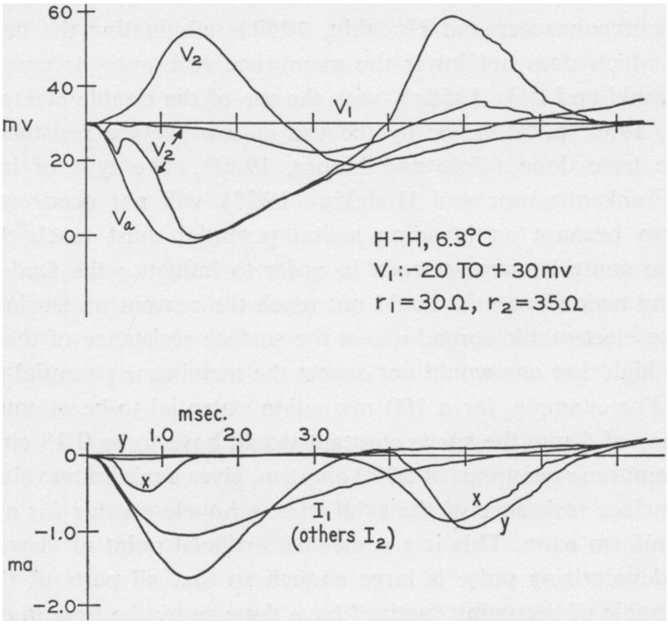

Figure 23 Two patch model computer results. Case where solution was unstable, sometimes giving a notch and sometimes a no-notch error on successive runs. Currents and potentials were recorded on different runs.

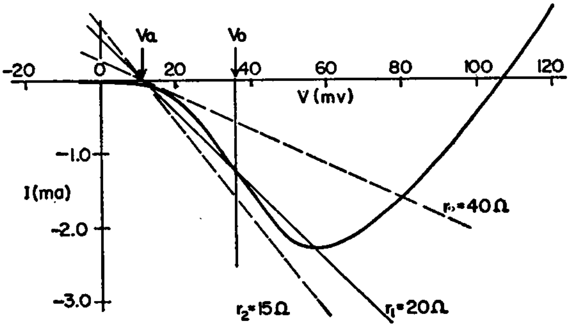

Figure 24 Curved line, value of peak inward current during a depolarizing step from a hyperpolarized value of —20 mv (relative to resting potential) to the value *V*, given by the Hodgkin-Huxley equations as used for the two patch model computations. For significance of other lines and further details see text.

EXTERNAL ACTION POTENTIAL INVASION

If a region of nerve in good condition extends beyond the end of the axial electrode, in any of the voltage clamp arrangements, an action potential will be initiated in this region for a sufficiently large depolarizing pulse and under certain conditions might invade the measuring region and produce a non-uniformity in the membrane potential, or add to that already present. Invasion of external action potentials may be avoided by eliminating the nerve in the region beyond the axial electrode (Frankenhaeuser and Hodgkin, 1957); eliminating the action potential with a drug which does not lower the membrane resistance or potential, such as urethane (Tasaki and Bak, 1958*a*), with the use of the double clamp (Tasaki and Spyropoulos, 1958, p. 312), or by the use of low surface resistance axial electrodes as we have done (Cole and Moore, 1960). The type of interaction described by Frankenhaeuser and Hodgkin (1957) will not occur with the point control system because an invading action potential must reach the immediate vicinity of the control microelectrode in order to influence the feed-back control.

An invading action potential could not reach the current measuring chamber by purely passive electrotonic spread unless the surface resistance of the axial current wire were so high that one would not expect the membrane potential to be uniform in any case. For example, for a 100 mv action potential to be attenuated to 1 mv over a distance of 6 mm the space constant would have to be 0.13 cm which, for a maximum membrane resistance of 5000 ohm cm, gives a minimum value of 254 ohm cm for the surface resistance of the axial wire, a hopeless value for a space clamp, even for a uniform axon. This is a somewhat artificial point of view, except when the applied depolarizing pulse is large enough so that all parts of the membrane which are capable of becoming "active" have done so by the time that the invading action potential appears. In this case the argument is rigorous and eliminates the possibility of notches occurring in this way for depolarizing pulses larger than those for which the peak inward current is a maximum.

For completeness we shall dispose of another possibility. If the axon were very non-uniform the inward current, during a depolarizing pulse to some value, might be small in the guard chamber, compared to its value in the center measuring chamber, but capable of becoming much larger due to the added depolarization of an invading action potential, delayed in time. Some of this current would then be drawn through the gap in the partition and appear as an extra, delayed inward current hump in the current measured in the center chamber. The magnitude of this effect depends upon the resistance of the gap, but for a value of 300 ohms (our system without grease or axon) it would have a maximum in the neighborhood of 0.1 ma/cm^2, referred to a square centimeter of membrane for a 500 micron diameter axon.

We are still left with the possibility of the interaction of an invading action potential for depolarizing pulses larger than that necessary to initiate them beyond the axial wire and smaller than that necessary to produce the maximum peak inward current. A complete understanding of this region has not been achieved. This is a complex problem, augmented by the fact that the potential range, as well as the suspected axial electrode resistance range, is the same as that for which the two patch model indicates the possibilities of notches under conditions of small amounts of non-uniformity in membrane properties. The most reasonable conclusion at the present time appears to be that there might be a range of axial electrode resistance values such that for a uniform axon the space clamp might fail and a notch might be produced only when action potentials are present beyond the ends of the axial electrode. Uniform, in this case, clearly must refer to the average properties over regions comparable in size to the axon radius. Moderate amounts of non-uniformity in membrane properties would narrow this range if it exists.

ERRORS INTRODUCED BY SMALL RESISTANCE BETWEEN VOLTAGE CONTROL POINTS

Ideally the potential which is being controlled should be the potential across the membrane capacity, but in practice it is not possible to do this without the use of compensated feed-back. A consideration of the effects of a small resistance between the control points, in series with the membrane capacity, is necessary for the evaluation of the importance of the steps taken to reduce this resistance and for a consideration of the possible effects of the presence of a suspected residual series resistance when all known series resistances are accounted for.

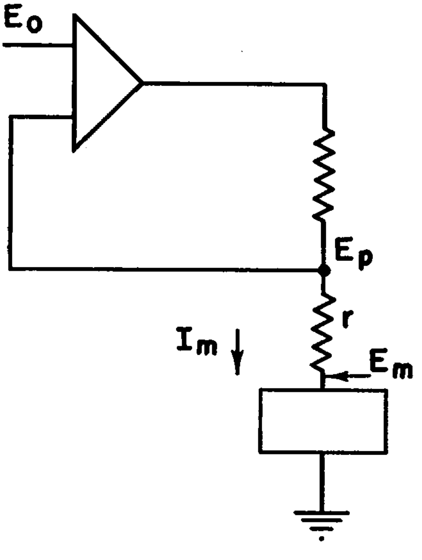

FIGURE 25 A simplified equivalent circuit relevant to the effect of a resistance in series with the membrane capacity between the potential control electrodes.

A simplified representation of the relevant equivalent circuit is shown in Fig. 25. We shall assume that $E_o = E_p$. Clearly $E_m = E_p - I_m r$. If r is very large, then during a clamping pulse to certain potentials threshold phenomena or/and repetitive activity may result. However, for solutions of the equations of Hodgkin and Huxley for the squid axon, Fitzhugh and Moore (unpublished data) have found that the magnitude of the resistance required for threshold phenomena (considerably less than that required for repetitive activity) is about twice the reciprocal of the steepest part of the peak inward current *versus* clamping potential curve. The value of this reciprocal negative slope for the Hodgkin-Huxley axon is (independent of temperature) 24 ohm cm^2 for a holding potential equal to the resting potential and 16 ohm cm^2 for a 20 mv hyperpolarized holding potential (see Fig. 24). In our experiments, for twenty-two axons for which the data are available, this value averaged 3.42 ohm cm^2, varying from 1.6 to 8.2. This difference cannot be a direct result of the greater peak inward currents in our experiments. For these twenty-two axons the maximum peak inward current averaged 3.89 ma/cm^2, as compared to 2.4 ma/cm^2 for the H-H axon prepolarized by 20 mv, or a ratio of 0.617, while the ratio of the reciprocal negative slopes is 3.42/16, or 0.214. There is no known reason, of course, why these ratios should be the same. If the axons which we used behaved in a fashion comparable to the H-H axon, and if the steepness of the peak inward current curve was not the result of a large series resistance, then serious trouble from instability would occur for an additional series resistance of 7 ohm cm^2. This value can be compared to the 50 ohm cm^2 for the H-H axon without prepolarization.

Errors are introduced for values of series resistances well below these which lead to instability. The 7 to 9 ohm cm^2 estimated by H-H and Katz (1952) to be present in the experiments with uncompensated feedback introduced errors, but probably not of a serious nature. In our experiments this resistance has been decreased (Cole and Moore, 1960) by the use of an internal microtip just under the surface of the membrane and an external reference electrode just outside the membrane, for voltage control. It is not known what the value of the final resistance is, but it should be close to the irreducible minimum without the use of compensated feedback.

Fig. 26 (axon 58-63) shows the effect of increasing this series resistance by removal of the external reference electrode on the curves of peak inward current, plotted against the clamping potential during the pulse.

Removal of the reference electrode (squares) decreases both the peak inward current and the steady state current for rather large depolarizations, while the shape of the peak inward current curve is distorted for smaller values of depolarizing pulses. The time to the peak inward current is not changed much, except for a slight increase in the region of $E_p = -40$ to -50 mv. These effects of the addition of a small series resistance are to a very good approximation completely duplicated by the results of a similar "experiment" done by FitzHugh and Moore (unpublished data) on the analog computer with the empirical equations of Hodgkin and Huxley. Similar results were found for three other nerves and for one in which an external lumped resistance was inserted between the voltage control points.

To properly evaluate the kinds of error which might result in future experiments it is worth while to consider the explanation of the effects seen in Fig. 26.

For the steady state outward current the situation is relatively simple. The line through the open circles in Fig. 26 is the steady state relation between E_p and I_m when the series resistance is zero and $E_p = E_m$. When a series resistance is introduced the current for a given E_p should be the intersection of the steady state $I_m - E_p$ curve for $r = 0$ and the load line $E = E_m = E_p - I_m r$. It follows that the reciprocal of the slope of the $I_m - E_p$ curve for $r = 0$ should differ from the same quantity for the $I_m - E_p$ curve for $r = r$ by the amount r, evaluated at the same I_m. In particular a straight line will remain a straight line and the reciprocal slopes will differ by r. This will be true no matter what kinds of non-linear elements are present so long as they give a straight line current voltage characteristic in the steady state. For this particular experiment, the slopes of the open circle line ($r = 0$) and the open squares line (series resistance in) differ by 2.7 ohm cm². This value thus

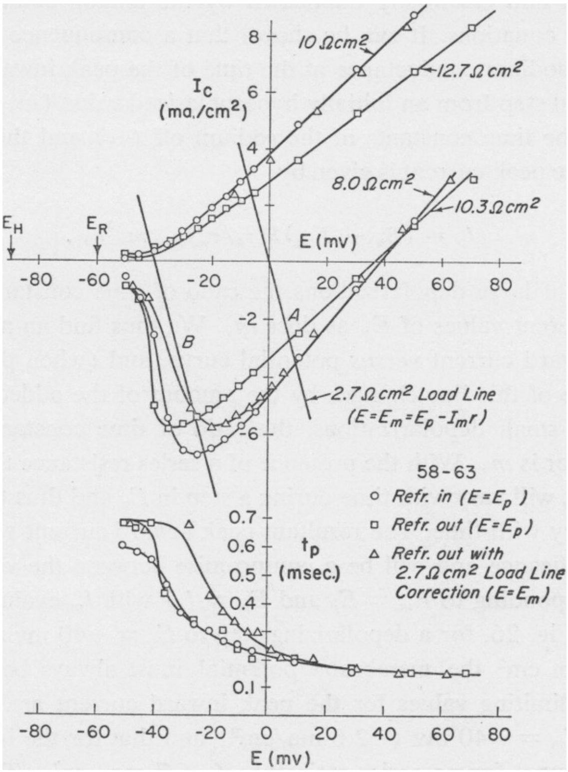

FIGURE 26 Effect of added series resistance between the potential control electrodes on the curves of peak inward and steady state outward current during depolarizing steps. Lower curve, time to peak inward current. In this case the resistance was added by removal of the external reference control electrode. Open circles, reference connected, no added series resistance. Open squares, reference removed, resulting in the addition of 2.7 ohm cm² series resistance. Open triangles, result of a simple "load line" correction (straight lines) for a series resistance of 2.7 ohm cm². Theoretically the load line correction should be possible either for the steady state or if the current voltage relation is a straight line.

represents the resistance between the point at which the reference electrode was in the solution, and ground (the nerve in the measuring chamber had an area of 0.075 cm^2 giving 36 ohms for the actual resistance).

The open square curves for the peak inward and steady state outward currents are plotted in Fig. 26 against the value of E_p. Correcting these curves for the presumed 2.7 ohm cm^2 gives the open triangles for I_m *versus* E_m. The same convention has been employed for the curves of the time to the peak inward current (t_p) in the same figure. When the currents and times are plotted against the membrane potential (E_m) the straight line portion of the peak inward current is the same with and without the series resistance and the times to the peak are the same over this range of potentials. However, for E_m between −40 and zero the peak currents and times differ considerably. The situation here is not simple, but the effects are intuitively reasonable and essentially duplicated by the analog computations with the Hodgkin-Huxley equations. It can be shown that a consequence of the H-H equations is that the sodium conductance at the time of the peak inward sodium current during a potential step from an initially hyperpolarized value ($m_o = 0$) is a function of the ratio of the time constants of the sodium off (τ_h) and the sodium on (τ_m) processes; *i.e.*, the peak current is given by

$$I_p = (E_p - E_{\mathrm{Na}})F(\tau_h/\tau_m)g_{\mathrm{Na}}m_\infty{}^3h_0 \; .$$

In the region of large depolarizations the ratio of time constants remains almost constant for different values of E_m as does m_∞. We thus find an almost straight line for the peak inward current *versus* potential curve, and (when plotted against E_p) the inverse slope of this line changes by the amount of the added series resistance. In the range of small depolarizations, the ratio of time constants is not constant with potential, nor is m_∞. With the presence of a series resistance the potential across the capacity, E_m, will vary with time during a step in E_p and thus the ratio τ_h/τ_m and M_∞ will also vary with time. The resultant peak inward current will no longer have any simple significance and will be a compromise between the values for no series resistance corresponding to $E_m = E_p$ and $E_m = I_m r$ with I_m evaluated for $r = 0$. In the example of Fig. 26, for a depolarizing step to $E_p = -40$ mv and a series resistance of 2.7 ohm cm^2 the membrane potential must always be on the load line labeled B. The limiting values for the peak inward current are that for no series resistance and $E_m = -40$ mv (−2.6 ma/cm^2) and that for the intersection of load line B and the curve for no series resistance (−6.7 ma/cm^2). The simple average of these limiting values is −4.65 ma/cm^2, compared to the measured peak inward current of −5.17 ma/cm^2. There is no simple way to arrive at *accurate* values for the effect of a small series resistance on the inward current in this range of potentials. It is probably not possible to uniquely correct for the errors introduced by a known series resistance. Distortions of the current patterns as represented in Fig. 26 might not be serious, depending upon the use to which the data are put. It is clear that in some of our experiments the addition of as little as 1 to 2 ohm cm^2 (or

7 to 14 ohm cm for a centimeter length of measuring region) would distort the peak inward current curve to such an extent that one might conclude that it was discontinuous.

Compensation. The addition of a negative resistance between the voltage control points (compensated feedback, see Hodgkin, Huxley, and Katz, 1952) will properly remove the effect of an added positive resistance. Fig. 27 represents the results of one such experiment. Removal of the reference electrode introduced a net series resistance of 3.5 ohm cm^2 (not shown in the figure). Addition of a negative resistance yielded the peak inward and steady state outward current curves (open triangles) which are virtually identical with the original (open circles) curves (neither a positive or a negative resistance added). The times to the peak inward current were also identical. Addition of still more negative resistance resulted in the curves through the open squares. This represents the addition of about 3 ohm cm^2. Since the nerve remained stable, one suspects that at least 3 ohm cm^2 was present originally between the voltage electrodes and the membrane capacity. That is to say, the open square curves are probably more accurate than the circle curves.

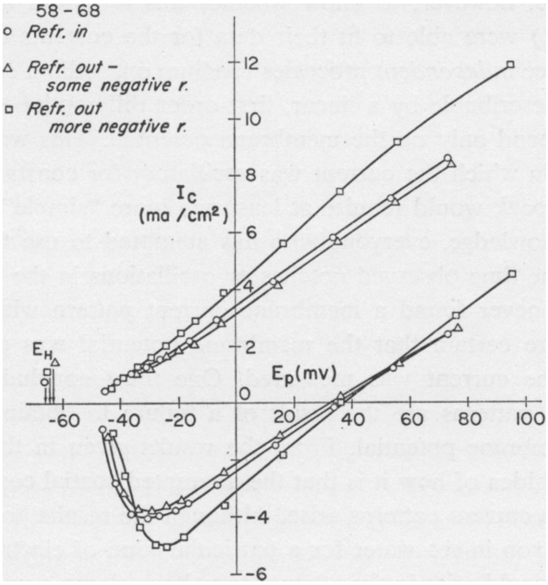

FIGURE 27 Peak inward and steady state outward currents versus potential during a depolarizing step (from a holding potential indicated by E_H). Open circles, normal conditions. Open triangles, reference removed (introducing 3.5 ohm cm^2 series resistance) and compensated by the introduction of negative resistance. Open squares, addition of a further 3 ohm cm^2 of negative resistance.

DISCUSSION

If the membrane current during an applied potential step consisted of a number of peaks, or a continued oscillation, there would be no apparent justification for the use of the membrane potential as a controlled variable in attempting to determine the electrical characteristics of the membrane. And the use of a voltage clamp apparatus would hardly seem to be an appropriate substitute for a desk calculator for eliminating the membrane capacity current. There is no reason, other than the experimental results themselves, to expect that the current through the squid axon membrane, following a step of membrane potential, would have the simple form described by Cole (1949) and Hodgkin, Huxley, and Katz (1949). It is of the greatest importance, however, to know whether this is indeed the case. Hodgkin and Huxley (1952) were able to fit their data for the currents during a potential step in terms of three *independent* processes (sodium on, sodium off, potassium on), each of which is describable by a linear, first order differential equation in which the parameters depend only on the membrane potential. This would not be practical for a system in which the current was oscillatory for constant potential; each additional current peak would require at least one more "simple" process.

While to our knowledge, everyone who has atempted to use the voltage clamp method has at some time observed notches or oscillations in the measured current patterns, we have never found a membrane current pattern with more than one peak when we were certain that the membrane potential was constant over the region in which the current was measured. One must conclude, then, that the anomalous current patterns are the result of a failure to obtain sufficient spatial control of the membrane potential. From the results given in this paper we may form a rather good idea of how it is that the attempted spatial control may fail and how the anomalous current patterns arise. Although the results presented here refer only to the squid axon in sea water for a particular kind of electrode arrangement, similar difficulties would arise in the attempt to voltage clamp any system, biological or otherwise. The empirical equations of Hodgkin and Huxley were employed in computations because they exist, and are relevant to our system, but they are incidental to the problem of the space clamp. If the oscillations were not artifacts the problems of spatial control would be essentially the same.

It is useful to distinguish between spatial control difficulties which are the result of non-uniformities in membrane properties and those which would occur in a uniform system. For our purposes a uniform membrane would be one for which the properties were the same averaged over any region comparable in size to one space constant (about 0.5 mm). Non-uniformities of significantly finer detail would not be directly measurable in terms of membrane potential. This is a fundamental limitation on the measurement of the electrical properties of any interface.

For a uniform system the spatial control would be perfect if the resistance between the axial electrode and the membrane were smaller than some given value. What this value is remains uncertain, but it is probably not greatly different from the reciprocal of the maximum negative slope of the curve of peak inward current *versus* clamping potential measured at the controlled point. In the computations on the two patch model with the Hodgkin-Huxley equations, an incipient notch occurred in the uncontrolled patch for a series resistance of 10 ohms (see Table II), while the reciprocal of the maximum negative slope of the inward current in the controlled patch was 16 ohms. Because of the presence of a known small bias in the computer (see page 186), this example may not in fact represent a perfectly uniform case, but it cannot be concluded that the limiting resistance is greater than 16 ohms (for 1 cm^2 of membrane).

In the presence of non-uniformities of membrane properties or axial electrode resistance there is no lower limit for the appearance of errors in the current through an uncontrolled region of membrane. These errors in the non-uniform case might be small and give rise to no obvious anomalies in the current-time patterns, but their complete elimination is impossible and the requirements for stability are severe. Thus a well developed notch occurred in the uncontrolled patch in the two patch model computations for a series resistance of 10 ohms in the controlled patch and 5 ohms in the uncontrolled. A rough conversion of these values to those appropriate for the axons which were used in the present investigation gives values for radial resistance which are comparable to the radial axoplasm resistance between a 75 micron diameter wire and a 500 micron diameter membrane. We must conclude that it is not possible to confidently predict the existence of an adequate space clamp for a squid axon using a 75 micron diameter axial electrode, even if it has zero surface resistance. It would appear that an independent check on the adequacy of spatial clamping must be made for each axon or the region over which the membrane current is measured must be reduced to a size comparable to an axon radius. At the present time the only feasible check on the adequacy of the space clamp and the uniformity of the axon appears to be the measurement of the membrane current distribution with external differential electrodes. If this distribution is not uniform, then almost nothing can be said about the system. A non-uniform result would certainly not indicate anything about the uniformity, or lack of uniformity of the membrane properties. Spatially uniform membrane current, however, should constitute strong evidence both that the membrane properties were uniform and that the clamp was good. An unbelievable combination of error cancellations would have to occur in order to give uniform membrane current with non-uniform membrane properties.

Extensions of Two Patch Model. From the experimental results presented here it does not appear that the stability properties of the squid axon in the point control arrangement are appreciably different from those which the two patch model would suggest. Analysis of the complete, continuous model has not been attempted and is probably impractical although some intermediate models may be useful for certain purposes. Further analysis of the behavior of the membrane in the immediate vicinity of the control point would be helpful.

A reasonable analysis of the extended control voltage clamp arrangements does not seem to be possible without the development of Hodgkin-Huxley analogs which are compact enough to be incorporated into a resistor board model for the axon.

The Node of Ranvier. It is important to know whether the membranes of other cells may show oscillations in the membrane current under conditions of constant potential for the same reasons as discussed for the squid axon. Such oscillations were not reported in nerve cell bodies by Hagiwara and Saito (1959) or by Frank *et al.* (1959) but were reported by Tasaki and Bak (1958*b*) for the frog node. However, the most careful work on the node has been done by Dodge and Frankenhaeuser (1958) and with improvements in technique, particularly with regard to the accurate measure of nodal membrane potential, the oscillations, which they had observed initially, disappeared. The situation is much the same as for the squid, the more reliable the membrane potential measurement over the current measuring region, the less the tendency to oscillate. In addition (Dodge, 1960, and personal communication), certain likely sources of error have been identified. The most important of these is probably the capacity between the outer pools in the usual gap technique methods. The existence of 10^{-14} farads of capacity in this position is sufficient to introduce differences of 10 mv between the recorded and actual membrane potential. The deviation is proportional to the first derivative with respect to time of the membrane current and appears to be adequate to explain the reported oscillations. Here, as elsewhere in this paper, we are assuming a perfect electronic system which maintains the recorded membrane potential at any predetermined value. The final conclusion from the work of Dodge and Frankenhaeuser is that it is possible to adequately voltage clamp the node of Ranvier but that special methods must be employed for the measurement of the membrane potential. Other difficulties are present in the membrane current supply and are discussed by Dodge and Frankenhaeuser (1958).

SUMMARY

An experimental and theoretical examination has been made of certain technical difficulties which may limit, and in some cases negate, the attempt to deduce the electrical characteristics of the squid axon membrane with the use of the "voltage clamp" technique. In order that data obtained with the use of this technique have any clear meaning the potential across the capacity of the membrane must be known in time (potential-time clamp) and uniform over the entire region in which the (average) membrane current is measured. Uniformity of membrane current under these conditions is not a requirement for the measuring system, but a property of the nerve membrane about which no reliable information is available at this time.

Extreme space clamp failure was induced with the use of deliberately high resistance axial current supplying electrodes and the distribution of membrane potential and current under these conditions is described. It was found that when more than one inward going membrane current peak occurred during a potential step all but the first decrease to a small value, or disappear, when the region over which the current was measured was known to be under good potential control. It is concluded that the appearance of notches or oscillations in the membrane cur-

rent during a clamping pulse is sufficient evidence for space clamp failure and that under these conditions the data cannot be used to deduce the membrane electrical characteristics. The absence of obvious anomalies in the current patterns, however, is not sufficient evidence for the conclusion that the space clamp is adequate.

Most features of the observations made during space clamp failure have been reproduced by computations using a two patch model and the empirical equations of Hodgkin and Huxley (Taylor and FitzHugh, 1959). Incomplete and largely intuitive extension of these results to the actual system provides very strong support for the conclusion that no data now available for the squid axon in sea water are inconsistent with the basic ideas embodied in the Hodgkin-Huxley equations.

Both experimentally and on the basis of incomplete but convincing theoretical arguments it is concluded that current supplying axial electrodes with surface resistance greater than about 25 ohm cm are useless for space clamping a uniform axon which can produce as much as 5 ma/cm^2 peak inward membrane current. The presence of non-uniform membrane properties would reduce the maximum allowable electrode resistance below 25 ohm cm; possibly to zero. For data to be acceptable reasonable evidence for adequate spatial clamping over the entire current measuring region must be provided. This may be done by reducing the area over which the current is measured to something less than an axon diameter, or by the measurement of membrane current distribution with external differential electrodes and rejection of any axon for which this distribution is not uniform.

The effects of a resistance between the potential reference points and in series with the membrane capacity have been described. The results are in agreement with the predictions of the empirical equations of Hodgkin and Huxley, and lead to the conclusion that a few ohm cm^2 of series resistance would result in errors which are probably uncorrectable in principle. These errors could be minor, but a series resistance of about 7 ohm cm^2 could lead to serious instability problems and might mislead one into concluding that the peak inward current *versus* potential curve was discontinuous.

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