# MEMBRANE POTENTIAL OF THE SQUID GIANT AXON DURING CURRENT FLOW

By KENNETH S. COLE and HOWARD J. CURTIS

*(From the Department of Physiology, College of Physicians and Surgeons, Columbia University, New York, and The Marine Biological Laboratory, Woods Hole, Massachusetts)*

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## INTRODUCTION

At the time the experiments on the impedance change of the squid giant axon during current flow (Cole and Baker, 1941) were planned, it was realized that the measurement of the change of membrane potential during current flow was equally important. From the transverse impedance experiments it was possible to eliminate variations of the internal and external resistances, axon volume, and the membrane capacity and to express the change entirely as one of membrane conductance. An increase of membrane conductance could be determined satisfactorily from the measurements, but a decrease could only be approximated and the resting reference level was out of the question (Curtis and Cole, 1938). There was the further possibility that a critical change of membrane electromotive force without an appreciable change of conductance might go unnoticed. On the other hand, the change of membrane potential during current flow should give an independent measurement of the resting membrane resistance and capacity as well as changes of potential. These measurements should be particularly satisfactory for a decrease of membrane conductance, but they might not be so useful for an increase or for small changes of conductance. Although it is to be expected that the potential and impedance changes can be correlated ultimately, the membrane potential measurements are at the present time more closely associated with the extensive external potential measurements which have been made on many other nerves.

In the past, it has been possible to make potential measurements with external electrodes only, and it is an indirect and rather uncertain procedure to estimate the membrane potential from these (Cole and Curtis, 1939). With the introduction of Young's giant axon preparation from the squid (Young, 1936) and the capillary electrode technique (Hodgkin and Huxley, 1939; Curtis and Cole, 1940) it has become possible to make direct measure-

ments of the membrane potential. The present experiments were undertaken primarily to investigate the relation between the steady state change of membrane potential and the membrane current and also to determine whether or not, with high cathode polarizations, the potential was the same before and after excitation (*cf.* Cole and Baker, 1941). It was also planned to analyze the transients at the make and break of the polarizing current as completely as possible.

### *Material and Apparatus*

The giant axon from the hindmost stellar nerve of the Atlantic squid *Loligo pealii* was dissected out and teased free from small fibers. It was then placed in a transverse impedance cell consisting of a sheet of insulating material in the top of which was cut a trough about 500$\mu$ square and just large enough to accommodate the axon. Square platinized lead impedance electrodes were set flush with the sides of the trough and opposite each other. A thin glass cover slip was placed over the top of the cell after the axon was in place. As before (Cole and Baker, 1941), the polarizing current was applied by the cam contactor to the two impedance electrodes in parallel and to a remote electrode at one end of the cell. Resistances of from $1.5 \cdot 10^4$ to $5 \cdot 10^5$ ohms in series with the battery maintained approximately constant current as shown in Fig. 1.

At first the external potential was measured between one of the impedance electrodes and an electrode at the other end of the trough relative to the remote polarizing electrode. Although the alternating current impedance of a platinized electrode was only slightly affected by the current flow, the polarization potential of the electrode practically obscured the small potential change of the axon membrane. The capillary needle technique (Curtis and Cole, 1940) was then applied and the potential difference measured between an impedance electrode and the capillary of the needle whose tip was in the axoplasm midway between the two impedance electrodes. This also was unsuccessful because the electrode polarization practically obscured even this larger potential. An outside potential electrode was then constructed by imbedding a fine glass capillary in the top surface of the cell with its tip at the center of the grounded impedance electrode and flush with its surface. This capillary was filled with sea water and an electrode was fixed at the opposite end. Completely satisfactory potential measurements could now be made between the inside and outside needles with the polarizing current applied as before. When the polarizing current was applied to the cell filled with sea water, but without an axon, a potential was obtained under some conditions, having a maximum value about 10 per cent of that obtained with the axon. This effect was not investigated in detail, but corrections were made where it was measurable. The amplifier and cathode ray oscillograph have been described (Cole and Curtis, 1939).

## EXPERIMENTAL

Records were first taken of the membrane potential as a function of time for a series of polarizing currents. The membrane potentials for anode and cathode polarizing currents of 9.7, 24, and 48 $\mu$amp. are shown in Fig. 2 *a*, *b*, and *c* respectively. At the lowest value of polarizing current, which

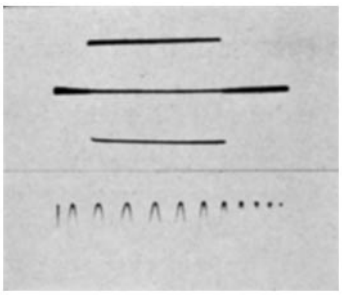

FIG. 1. Oscillograph records of cathode and anode current flow and base line made on three successive sweeps. Exponential sweep, timing, 200 cycles.

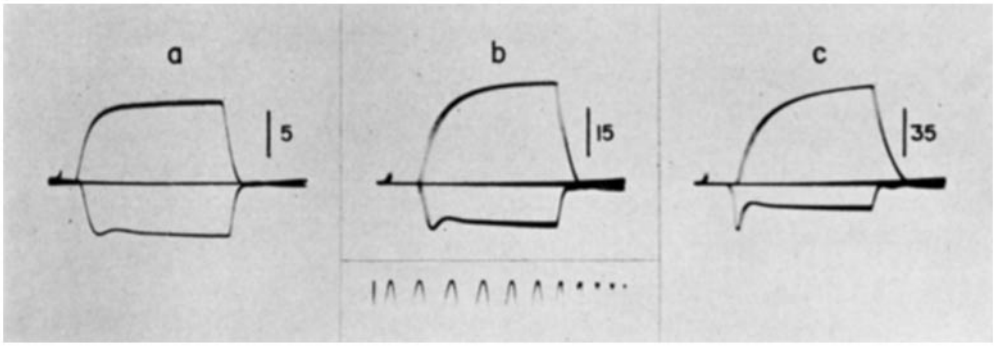


FIG. 2. Oscillograph records of change of membrane potential during current flow with the resting potential as base line, made on three successive sweeps in each case.

Anodes upward, and cathodes downward, are, respectively, increases and decreases of the membrane potential. Total current flow in $\mu$amp., (*a*) 9.7; (*b*) 23.7 (below rheobase); (*c*) 47.5 (above rheobase). Potential calibrations indicated are in millivolts. Exponential sweep, timing, 500 cycles.

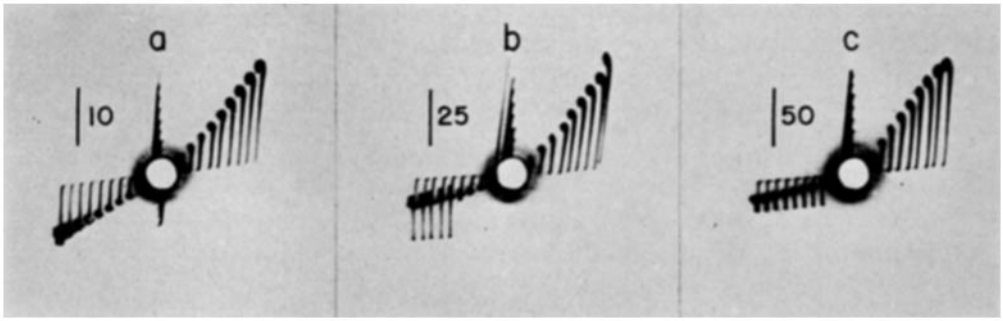


FIG. 3. Oscillograph records of change of membrane potential (ordinates) *vs.* approximate total current flow (abscissae), anode up and to right, cathode down and to left. Maximum values of current in $\mu$amp. are (*a*) 17.5; (*b*) 47.5; (*c*) 95. Current steps are each 10 per cent of the maximum. Potential calibrations indicated are in millivolts.

The dark spots corresponding to each value of current indicate the steady state change of membrane potential caused by the current. The excursions of the potential change below these steady state values on the cathodal side in *b*) and *c*) are the action potentials initiated by the make of the larger currents.

is about 0.4 rheobase, the difference between anode and cathode is seen both in the behavior of the potential at the start of the current and in the constant level of potential finally reached. Although the initial rate of rise of potential is approximately the same for both anode and cathode there is a distinct oscillation at the cathode which is not seen at the anode and the final value of potential at the cathode is somewhat lower than at the anode. When the current is increased to 24 μamp. (Fig. 2 *b*) barely sub-rheobasic, the cathode oscillation has somewhat greater amplitude, the anode rise is considerably slower, and the discrepancy between the final potentials is larger. In going to 48 μamp. (Fig. 2 *c*) nearly twice rheobase, the first maximum at the cathode has become the propagated impulse in the characteristic all-or-nothing manner. The establishment of the steady anode potential is even slower and the ratio of final potentials is still further increased.

Since there are obviously a number of factors involved in the initial or transient behavior of the potential, attention was first centered on the steady state characteristics. It then became convenient to record as much of the information as possible on a single film, and this was done by removing the sweep circuit voltage from the horizontal deflecting plates of the oscillograph and replacing it by a potential, derived from the polarizing circuit, and proportional to the polarizing current. When the current was applied (Fig. 3) the oscillograph spot gave a sudden horizontal deflection from the center point proportional to the current, and then moved vertically as the membrane potential developed as was seen in Fig. 2. At the cessation of the current, the spot returned suddenly to the vertical axis, and then descended to the center more slowly as the potential returned to its resting value. The polarizing current was applied at intervals of about 1 second and by changing the current during the off period of each interval the complete current-potential series of Fig. 3 *a*, *b*, or *c*, could be obtained in about 20 seconds. In Fig. 3 *a*, the maximum current of 17.5 μamp. was sub-rheobasic but the oscillations of potential at the cathode are apparent in the width of the spot at the higher currents. A decided departure from a linear relation between current and potential is quite evident. As we go to the maximum value of 47.5 μamp. (Fig. 3 *b*) the rheobase was exceeded and the five highest values of current gave rise to propagated impulses with the potential falling considerably below the steady value, and the curvature of the locus of the steady values is even more marked. For a maximum value of 95 μamp. (Fig. 3 *c*) there are eight points above threshold and the current-potential relations at the anode and cathode are very striking. The complete data on one axon after

correction and reduction to common potential and current scales have been plotted in Fig. 4.

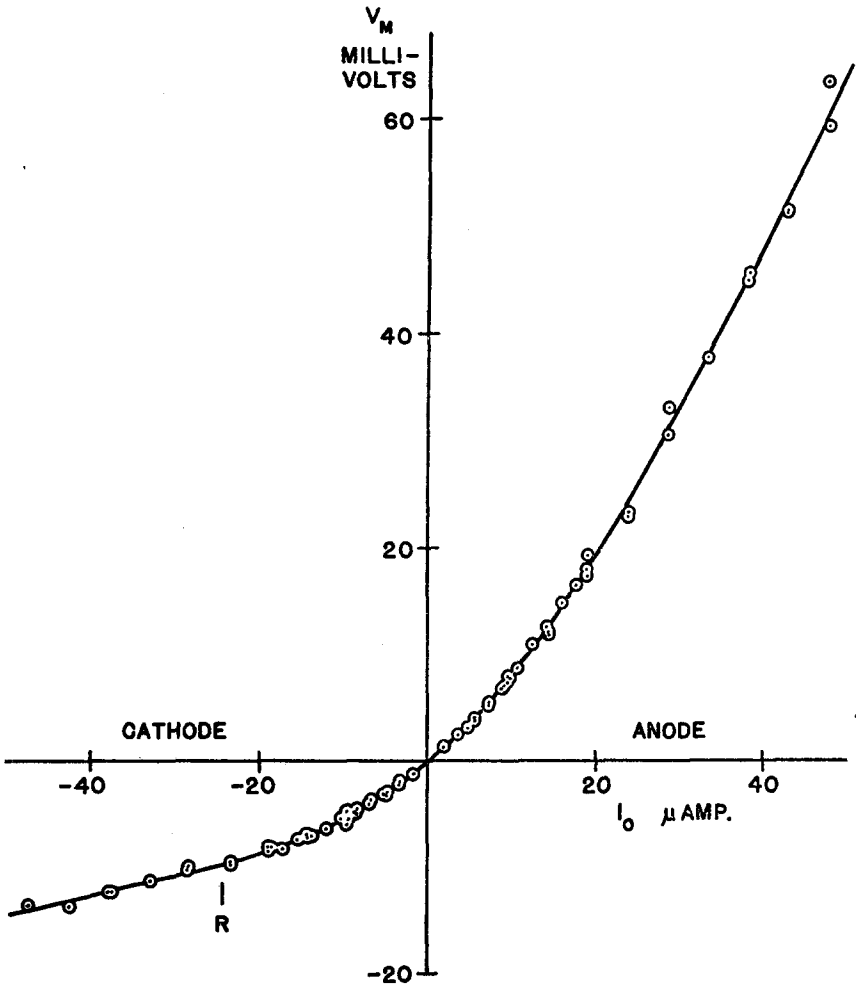


FIG. 4. Change of steady state membrane potential, $V_M$, *vs.* total current flow, $I_0$. Composite curve showing data of five experiments on the same axon. Rheobase indicated by *R*.

As the threshold for excitation rose and the fiber finally became inexcitable, the polarizing current necessary for a given change of membrane potential increased, and the potentials under the anode and cathode became more and more nearly equal to each other.

At the start of the experiments on the impedance change during current

flow it was anticipated that a large polarizing current might maintain a depolarization after an excitation. No evidence for this was found and a similar conclusion is to be drawn from the present experiments. Experiments on the potential have been performed with sub-threshold exponentially rising currents which gave the same steady state characteristic as when excitation took place at a sudden make. In Fig. 3 *b* and *c*, it is apparent that the steady state points form a continuous smooth curve up to four times rheobase and from Fig. 4 there is no certain change of form as rheobase is passed. It was also found that after a steady membrane potential had been reached in the polarized region, either anodic or cathodic, the potential returned to this same value after a distantly initiated impulse had passed through the region.

### *Calculations*

The first parameter to be computed is the resting membrane resistance This may be obtained from the curves for the change of membrane potential $V_M$, *vs.* the polarizing current, $I_0$, such as the one shown in Fig. 4. For sufficiently small polarizing currents, *i.e.* near the origin, these curves may be approximated by straight lines, and the slope, $V_M/I_0 = \overline{R}$, has the dimensions of a resistance. In this region of 1 or 2 microamperes, we are entitled to treat the membrane in the steady state as a resistance, $r_4$, for a unit length of axon, and the usual cable equation may be applied.

When $r_1$, $r_2$ are the resistances for a unit length outside and inside the axon membrane, and $i_1$, $i_2$ are the corresponding currents, it is found (Cole and Hodgkin, 1939, equation 11) that in the interpolar region (*i.e.* between the polarizing electrodes), at a distance $x$ from the midpoint between the electrodes, the membrane potential difference is $V_M = (r_1 i_1 - r_2 i_2)\lambda \tanh (x/\lambda)$, where $\lambda = \sqrt{r_4/(r_1 + r_2)}$. Then if $x$ is large, this approaches

$$V_M = (r_1 i_1 - r_2 i_2)\lambda. \tag{1}$$

By a similar procedure it is found that in an extrapolar region, *i.e.* outside the polarizing electrodes, and at a considerable distance from the origin at the end of the axon, we have again

$$V'_M = -(r_1 i'_1 - r_2 i'_2)\lambda. \tag{2}$$

For a narrow electrode—about as wide as the diameter of the axon—the membrane potential under it may be considered also as the interpolar and extrapolar membrane potential, $V_M = V'_M$. On the interpolar side,

$i_1 + i_2 = I_0$ and on the extrapolar, $i_1' + i_2' = 0$, while the interpolar and extrapolar inside currents are obviously equal, $i_2 = i_2'$. Then from (1) and (2)

$$i_2 = \frac{r_1}{2(r_1 + r_2)} I_0 \quad \text{and by (1),} \quad V_M = \frac{r_1 \lambda}{2} I_0. \tag{3}$$

So

$$\frac{V_M}{I_0} = \bar{R} = \frac{r_1 \lambda}{2} \quad \text{and} \quad r_4 = \frac{4(r_1 + r_2)}{r_1^2} \bar{R}^2 \tag{4}$$

As representative values we may take $r_1 = 1.9 \cdot 10^4$ ohm/cm., $r_2 = 1.8 \cdot 10^4$ ohm/cm. to compute $r_4$ by equation (4), then for a membrane

TABLE I

| $\bar{R}$ ohms | $r_4$ ohm cm. | $R_4$ ohm cm.$^2$ |
|---|---|---|
| 500 | 100 | 14.4 |
| 770 | 240 | 34. |
| 540 | 120 | 17. |
| 640 | 170 | 23.5 |
| 840 | 290 | 40.5 |
| (375) | (57) | (8.1) |
| 660 | 180 | 25. |
| | | 23. Average |

area per unit length of 0.14 cm.$^2$/cm. we obtain the membrane resistance, $R_4$, for a square centimeter. These values are given in Table I from the available data. The values in parentheses are for an inexcitable axon which was excluded from the average.

When the polarizing current exceeds more than a few per cent of the rheobase, it is no longer permissible to consider the membrane conductance as a resistance, $r_4$. Since as yet there is no evidence that the external and internal media may not be considered as resistances, $r_1$ and $r_2$, we may use the cable equation (Cole and Curtis, 1938, equation 10) in the form

$$\frac{d^2 V_M}{dx^2} = (r_1 + r_2) I_M,$$

where $I_M$, the membrane current density, is now to be determined as a function of $V_M$. At a sufficient distance from the electrode in the extrapolar region there is neither an appreciable polarizing current flow across

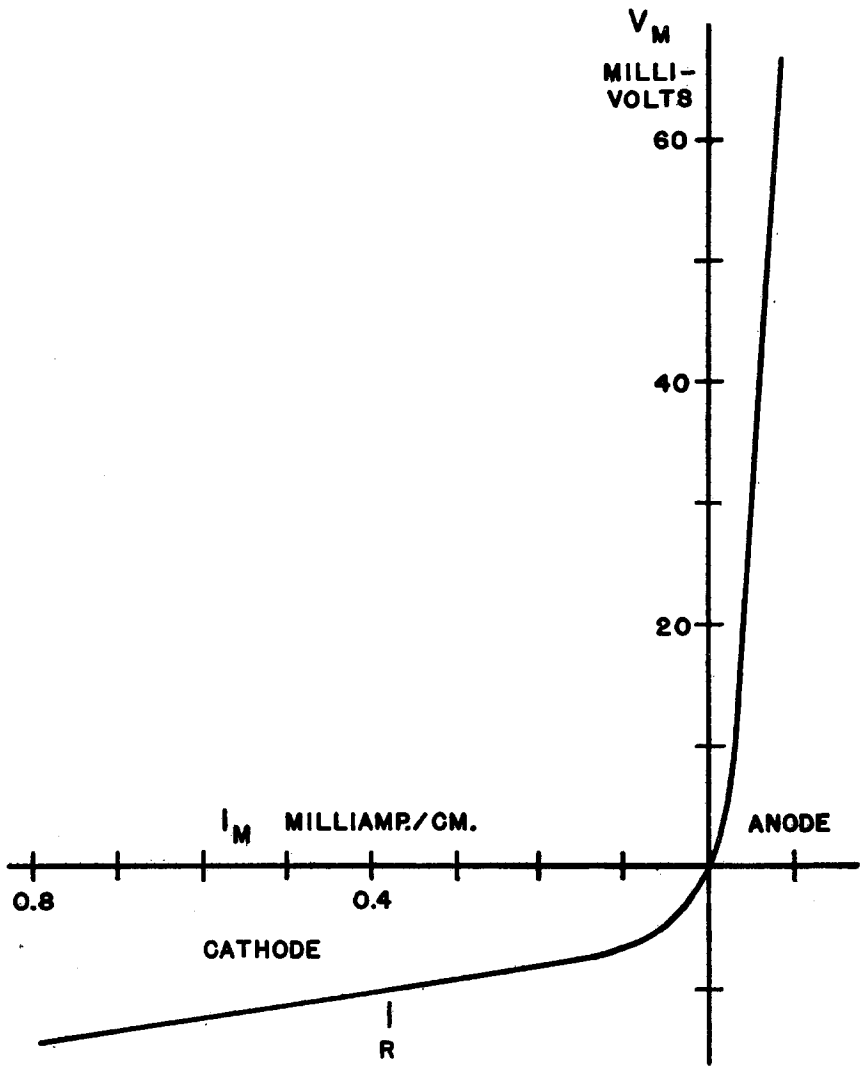


FIG. 5. Change of steady state membrane potential, $V_M$, *vs.* membrane current density, $I_M$, as calculated from data of Fig. 4 by equation (6). Rheobase indicated by $R$.

the membrane nor a potential difference caused by it. As we approach a point at a distance $x$ from the electrode, we have at each point,

$$\frac{dV_M}{dx} = (r_1 + r_2)i_1'(x)$$

and

$$\frac{d^2V_M}{dx^2} = (r_1 + r_2)\frac{di_1'}{dx} = (r_1 + r_2)^2 i_1' \cdot \frac{di_1'}{dV_M};$$

then

$$I_M = (r_1 + r_2) i_1' \cdot \frac{di_1'}{dV_M}. \tag{5}$$

Then at the electrode, by equation (3)

$$i_1' = -\frac{r_1}{2(r_1 + r_2)} I_0$$

and we find from equation (5) that

$$I_M = \frac{r_1^2}{4(r_1 + r_2)} \cdot I_0 \cdot \frac{dI_0}{dV_M} \tag{6}$$

When $dI_0/dV_M$ is independent of $I_0$ this reduces to the previous case, equation (4). We have $I_0$ and may determine $dI_0/dV_M$ from Fig. 4 for each value of $V_M$. The membrane current densities, $I_M$, then found by equation (6) and the membrane potential, $V_M$, have been plotted in Fig. 5.

The "variational" conductance, $G_4 = dI_M/dV_M$ may now be taken directly from Fig. 5. This has been plotted against the total polarizing current, $I_0$, in Fig. 6 for comparison with similar data from the impedance change during current flow (Cole and Baker, 1941, Fig. 9).

## DISCUSSION

Although the impaled axons often survived for several hours at room temperature and without circulating sea water, and the data could be taken with considerable rapidity, the reproducibility of the measurements shown in Fig. 4 was both surprising and gratifying.

The wide discrepancy between the present average value of 23 ohm cm.$^2$ for the resting membrane resistance and the average value of 700 ohm cm.$^2$ found by Cole and Hodgkin (1939) calls for some comment. There are many possible factors because the two experiments have little in common except that they are both direct current measurements on the squid axon. The first factor, which at the present time seems to be the most important, is the physiological condition of the axon. For the longitudinal resistance experiment the axon had to have a high membrane resistance,—otherwise the characteristic length would be so short that measurements could not be made with sufficient accuracy. It was also found that the high resistance correlated rather well with good physiological condition and survival. In the transverse impedance work, it was found that the impedance change during activity was also a rather sensitive index of the condition of the axon, more so than the action potential, for example. Consequently we might expect that a high membrane resistance would be

associated with a large impedance change during activity and *vice versa*. On this basis we should not be surprised at the low membrane resistance now found, because as has been pointed out (Curtis and Cole, 1940) the impedance change during activity was considerably lower immediately after impalement than it had been before. Furthermore, the most complete data, such as in Figs. 3 and 4, were obtained on axons for which the action potential had the average value of 50 mv. found by Curtis and Cole (1940). This was considerably below the maximum potentials obtained on axons in better condition. From this point of view, it seems possible that both sets of measurements may be essentially correct, and that they represent the membrane resistances of axons in different physiological conditions.

The other principal factor to be considered is the calculation of the results. In the longitudinal measurements with infinite electrodes the theory is relatively straight forward and represents the experimental conditions quite well, and furthermore, sufficient data could be taken to furnish a rather satisfactory check on the form of the theoretical expression. In the present case, however, the geometry is much more complicated and an exact solution is out of the question. The only simple approximations are those of a small axon diameter and a negligible electrode length at the point where measurements were made. An electrode length of 0.5 mm. is not negligible, but it is, after all, approximately the axon diameter. One approximation is then as good or as bad as the other and each will have to stand until both can be improved upon. This state of affairs is even more unfortunate because no reasonable experiments have been found which can either prove or disprove the validity of the equations used for calculation. Another difficulty is that direct measurements of the internal and external resistances, $r_1$ and $r_2$, could not be made, and the estimates used in the calculations may not be particularly good. As will be seen in equation (4), relatively small errors of $r_1$ have a large effect on the calculated membrane resistance, $r_4$.

The transverse impedance during current flow gave the change of membrane conductance with the total polarizing current (Cole and Baker, 1941, Fig. 8). This may be compared with the variational membrane conductance as a function of the total polarizing current obtained in the present experiments (Fig. 6). These two curves should be the same, except that a resting membrane conductance may be obtained from the present data, and an obvious similarity between them is found. Each approaches a constant value of conductance at both high anode and high cathode polarizations, and in each the change of this asymptotic value from that at rest is about ten times as large at the cathode as at the anode. The absolute

values of the conductances and of the polarizing currents are, however, considerably different. Although the two sets of measurements were not made on the same axon nor in the same measuring cell, these two measuring cells were so nearly identical that no serious difference in the flow of the polarizing current would be expected. An appraisal of the analysis used for the interpretation of the data in each experiment is again a difficult

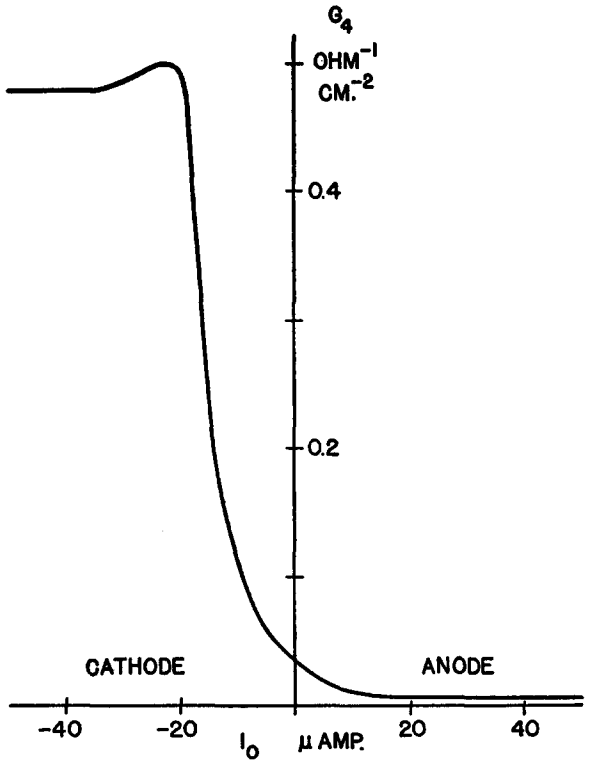


FIG. 6. Calculated membrane conductance under electrode, $G_4$, *vs.* total current flow, $I_0$.

task. As we have seen, there are a number of unsatisfactory aspects to the potential measurements, and the results of the impedance experiments also involve a number of compromises between theory and experiment which are not easily evaluated. Consequently, until the discrepancies in the magnitudes of the membrane conductance and polarizing current can be definitely ascribed to the measurements or their analysis, it is not reasonable to assume that all the differences were actually in the axons. Yet, as has been pointed out above, the most probable single factor is the puncture of the axon and its rather immediate consequences. In view of this, it may

be more appropriate at the present time to overlook the differences and consider the common characteristics of the data and analyses of the two types of experiments as good evidence in support of each.

The performance of rectifiers has been expressed in various forms depending upon the use to which the information was to be put, but the most convenient specification of the rectification characteristics of the membrane is from either the variational resistances or the conductances at rest and at the maximum and minimum values. It is found (Fig. 6) that at the cathode the conductance is about thirteen times that at rest, while at the anode it is about one-eighth the conductance at rest. Thus the ratio of the maximum conductances in the two directions is greater than one hundred to one and this is representative of the other data obtained.

It should be possible to calculate the membrane capacity from the initial rate of rise of the potential in Fig. 2 but before this could be done it was necessary to consider the amplifier characteristics. The combination of the needle electrode resistance and the input capacity of the amplifier was found subsequently to be the controlling factor which precluded the use of these data. The oscillations which appear farther along in the initial transient of the membrane potential under the cathode are particularly interesting. They agree in a general way with those found in the impedance change under similar conditions, for the frequency and amplitude are similar and the propagated all-or-none response again appears at the first maximum. Although a detailed analysis would probably be misleading because of the apparatus limitations, the oscillatory response seems quite certain, and its appearance in both types of measurement emphasizes their common basis.

## SUMMARY

The squid giant axon was placed in a shallow narrow trough and current was sent in at two electrodes in opposite sides of the trough and out at a third electrode several centimeters away. The potential difference across the membrane was measured between an inside fine capillary electrode with its tip in the axoplasm between the pair of polarizing electrodes, and an outside capillary electrode with its tip flush with the surface of one polarizing electrode.

The initial transient was roughly exponential at the anode make and damped oscillatory at the sub-threshold cathode make with the action potential arising from the first maximum when threshold was reached.

The constant change of membrane potential, after the initial transient,

was measured as a function of the total polarizing current and from these data the membrane potential is obtained as a function of the membrane current density. The absolute value of the resting membrane resistance approached at low polarizing currents is about 23 ohm cm.$^2$. This low value is considered to be a result of the puncture of the axon. The membrane was found to be an excellent rectifier with a ratio of about one hundred between the high resistance at the anode and the low resistance at the cathode for the current range investigated.

On the assumption that the membrane conductance is a measure of its ion permeability, these experiments show an increase of ion permeability under a cathode and a decrease under an anode.

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