

LONGITUDINAL IMPEDANCE OF THE SQUID GIANT AXON

By KENNETH S. COLE AND RICHARD F. BAKER

(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

Measurements of the transverse alternating current impedance of the squid giant axon have given a membrane capacity of about 1 microfarad/cm.² (Curtis and Cole, 1938) and from longitudinal measurements of the direct current resistance of this axon, a membrane resistance of about one thousand ohms cm.² has been found (Cole and Hodgkin, 1939). From these values the resistance and reactance components, R_s and X_s , of the membrane impedance may be calculated at various frequencies, and R_s and X_s , taken as abscissae and ordinates respectively, give the impedance locus of the membrane. This locus will be a circular arc (Cole, 1928, 1932) and, when the dielectric loss of the membrane is ignored, the locus will be the semi-circle of Fig. 1 *a*. For this simplified membrane the longitudinal impedance of the axon, as measured between two electrodes some distance apart along the length of the axon, may now be calculated by cable theory as shown below. In this manner a locus of the form shown in Fig. 1 *b* is predicted by equation (6) for the longitudinal impedance of a single axon having the membrane impedance locus of Fig. 1 *a*.

Longitudinal impedance data over a wide frequency range were first taken on the squid giant axon in connection with the direct current resistance experiments (Cole and Hodgkin, 1939) because high frequency measurements, in which the membrane impedance would be negligible, were needed to support the theory used to calculate the membrane resistance. A few sets of measurements over the complete frequency range were made primarily to determine the lowest frequency allowable for this purpose and incidentally to obtain data for comparison with the predicted theoretical impedance locus shown in Fig. 1 *b*. At frequencies above 500 cycles, the equivalent parallel capacity and resistance of the axons varied with frequency in approximately the predicted manner. At frequencies below 200 cycles, the resistance for one of the axons decreased below a maximum value

at about 200 cycles and for another axon the capacity actually went through zero. At 50 cycles a capacity of $0.01 \mu\text{f}$ had to be added to the *unknown* arm of the Wheatstone bridge to obtain a balance for this axon, as there was no provision in this equipment for measuring a net inductive reactance. All probable sources of error were found to be unimportant, and the completely unexpected presence of an inductance in the axon was apparently demonstrated. This inductance seemed to be associated with the membrane because the inductive reactance first decreased as the axon deteriorated and then was replaced by a capacitive reactance at all frequencies.

Further measurements were not possible at that time and the observations obviously needed to be confirmed and extended. All possible factors

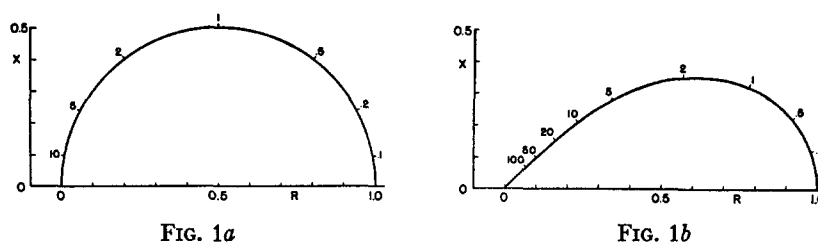


FIG. 1. Theoretical impedance loci, series resistance, R , vs. series reactance, X . (a) Locus for a membrane having a leakage resistance in parallel with a loss-free capacity. (b) Longitudinal locus, as given by equation (6), for an axon with membrane properties shown in (a). Frequencies are indicated in terms of the characteristic frequency of the membrane.

outside of the axon, from the apparatus to the oil which surrounded the interpolar stretch of the axon, should be eliminated. Then direct evidence should be obtained to localize the inductive structure in the connective tissue, the membrane, or the axoplasm. And finally, as much information as possible should be obtained about this structure and if, as suspected, the membrane was responsible, the relation of the inductance to the capacity and conductance should be determined. This program was undertaken in the summers of 1939 and 1940, and, although it is not satisfactorily completed, the results themselves are certainly indicative and when considered with those of other types of measurements seem quite conclusive.

Material and Apparatus

Young's giant axon preparation (Young, 1936) from the Atlantic squid, *Loligo pealii*, was used throughout. The dissection of the hindmost stellar nerve and the teasing of the axon from this nerve have been described (Cole and Curtis, 1939). In the effort to reduce the variability of the results extreme care was taken in the dissection and

particular attention was paid to the elimination of body fluids and to the locating and cutting of the axon branches.

The electrode system was essentially the same as previously used (Cole and Hodgkin, 1939) but with several modifications as shown in Fig. 2. One end of the axon was pulled

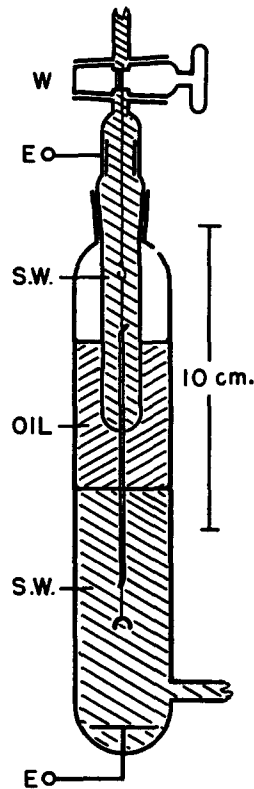


FIG. 2. Longitudinal impedance cell for the squid giant axon. The impedance is measured between the platinized platinum electrodes, E , E , in the sea water surrounding the upper and lower ends of the axon. The vertical position of the axon is varied by the stopcock windlass, W . The interpolar region is in oil and its length is varied by admitting or removing sea water at the lower end of the cell.

up through a small hole in the bottom of a tube filled with sea water and the other end hung through a layer of oil and into sea water in another vessel. Large platinized platinum electrodes made contact with the sea water at each end of the axon. The areas of the electrodes and the high resistance of the preparation made the effect of electrode polarization so small that corrections for it were unnecessary even at 30 cycles. By transferring sea water between the lower vessel and a calibrated burette the interpolar distance was easily varied and accurately measured. The entire axon could be moved up or down in the electrode system by a stopcock windlass which was hooked to the

ligature on the upper end of the axon. A slight tension was kept on the axon by a small platinum weight attached to the lower ligature.

The alternating current Wheatstone bridge and the accessory equipment have been described (Cole and Curtis, 1937), and measurements were made at frequencies from 30 cycles to 200 kc. The measuring potential applied to the axon was as small as possible and the bridge balance was independent of it except when the effect of potential was being investigated. The inductive reactances were measured as in the earlier experiments by connecting a sufficiently large known capacity (0.01 or 0.02 μf) in parallel with the unknown, or axon, arm of the bridge to give it a net capacitive reactance which could be balanced as usual. The axon reactance was then specified in terms of the "negative" parallel capacity.

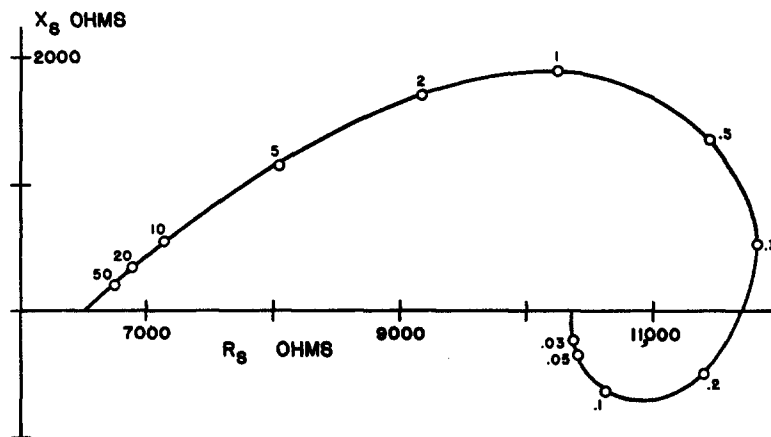


FIG. 3. Longitudinal impedance locus, series resistance, R_s , vs. series reactance, X_s , for squid giant axon. Negative, or capacitive, reactances are plotted above the resistance axis. Frequencies indicated are in kilocycles.

The series resistance and reactance, R_s and X_s , at each frequency were calculated from the observed parallel resistance and capacity (positive or "negative") and plotted as the impedance locus (Cole, 1928, 1932).

Measurements and Interpretations

The longitudinal impedance locus of Fig. 3 shows the same phenomena as were observed in the summer of 1938. The behavior at high frequencies was anticipated but the dip below the resistance axis at low frequencies is the unexpected inductive reactance. Although the first axon in the present series of experiments showed these characteristics, it was soon apparent that there was a considerable variability to be contended with. Some axons would show a negative capacity (*i.e.* positive, or inductive, reactance) at frequencies below 200 cycles over their entire length and for all interpolar distances. Other axons would have a low frequency reactance

which was positive for one interpolar distance and negative, or capacitive, for another. Occasionally the change from one to the other took place when the distance was varied as little as a millimeter. More often this reactance would change from positive to negative during the course of an experiment and there were a few cases in which a positive reactance was not found at any time, in any portion. An example of an axon without net inductive reactance is shown in Fig. 4*a*, and it is found that the low frequency portion still indicates the presence of an inductance.

The most obvious variable was that of physiological condition. There were some variations in the subthreshold phenomena but these could not be clearly correlated with the reactance measurements. These axons were

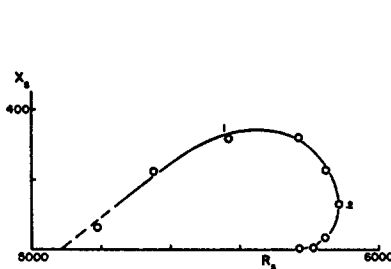
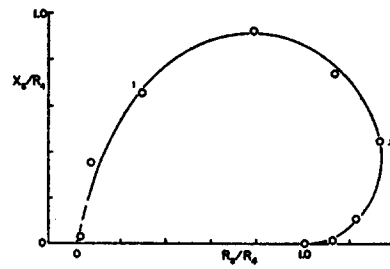
FIG. 4*a*FIG. 4*b*

FIG. 4. (*a*) Longitudinal impedance locus, series resistance, R_s , vs. series reactance, X_s , for a squid giant axon without net inductive reactance. (*b*) Membrane impedance locus, calculated from data shown in (*a*) by equation (6). Frequencies indicated are in kilocycles.

all excitable at reasonable thresholds and propagated apparently normal action potentials over their entire lengths and the survival was fair. There was a trend to indicate that the inductive reactance diminished and disappeared as the condition of the axon became poorer. This, however, failed as a generalization when several of the best axons gave capacitive reactances throughout their entire length immediately after removal from the animal. These axons, however, become inductive in the course of an hour or so.

This experiment was obviously so sensitive to some uncontrolled factor or factors that it was essential to eliminate as many uncertainties as possible. The impedance was always independent of the measuring current when this was sufficiently small, but if action potentials and harmonics generated by the non-linearity of the membrane resistance were suppressed or ignored an inductive reactance might apparently become capacitive when the

measuring current was increased. For a number of experiments, the mineral oil was not used and the interpolar stretch was in air. The inductive reactances were of about the same magnitude and as irregular as before, but the survival time of the axons was considerably shortened. Aeration of the oil had little or no effect and fresh sea water did not seem to be essential. Axons with considerable connective tissue gave results comparable to those which were carefully teased. A wide variety of wood, thread, and other "artificial" axons all failed to give an inductive reactance. At one time it was thought that the shape of the upper meniscus was a factor, but the effect of changes of the size of the orifice and the form of the meniscus was finally shown to be negligible as long as the position of the meniscus on the axon was unchanged.

As the search for constant conditions progressed, it became more and more certain that variability and the inductive reactance both lay in the axon and this was supported by the one completely reproducible observation that an inexcitable axon never gave a net inductive reactance at any frequency. Then considerable progress was made by killing one end of the axon with alcohol. With the variability of one electrode region eliminated, it was possible to show that the irregularities of impedance were probably caused by local differences in the axon which had only a slight effect on the subthreshold response, the threshold, or the action potential.

Out of the seventy-four axons investigated, the characteristics of sixty-six may be roughly classified. In nine of these, no negative capacity was found in any part of the axon at any time. Twenty-five axons showed part negative and part positive capacities at low frequencies and in many cases the latter was correlated with a visible injury, while in four cases it was apparently an initial condition. The remaining thirty-two showed negative capacities over their entire length when this was investigated. It is now felt that the initial completely capacitative reactance is the most nearly normal condition and that this is followed by the relatively stable stage in which the low frequency reactance was inductive. Subsequently this net inductive reactance disappeared and the axon became inexcitable.

When the initial capacitative phase was found, its duration was too short and the impedance was too variable for satisfactory impedance measurements to be made over a wide frequency range. As a consequence there is practically no information available about the axon in this condition. At the stage where the low frequency reactance was inductive, the axon impedance would often remain quite constant for several hours and frequency runs could be made. The behavior of the axon shown in Fig. 3 has all the typical characteristics of this stage and does not present any of the extremes encountered.

Localization of Inductive Structure

The next step was to locate the structures responsible for the inductance. As the interpolar distance is increased, the two electrode regions become practically independent and there is then no current flow across the membrane in the central portion of the interpolar region. If the inductance and capacity are both located in the membrane there should be no change in their contribution to the impedance as the interpolar distance is increased beyond five or six times the characteristic length as shown by equation (3). There is, however, current flow along the connective tissue and sea water outside, and through the axoplasm inside the membrane in this central region. If the inductance is located in either of these its contribution would depend upon the electrode separation even when this is large. This obvious and crucial experiment proved to be quite difficult to carry out because it required that the axon be uniform throughout its length and that this uniformity be maintained until the measurements were completed. It would be necessary to make a series of frequency runs with the same interpolar distance in a number of regions along the length of the axon, to prove the uniformity, before similar frequency runs could be made with varying interpolar distance. As has been indicated, sufficiently uniform fibers were not easy to obtain, and it was soon found that bridge measurements over a wide frequency range were too slow for the completion of this program. Furthermore, the capacity sensitivity of the bridge at the lowest frequencies was inadequate for the high resistances encountered at long interpolar distances, and impedance loci at four interpolar distances on each of eight axons did not demonstrate any consistent behavior at long interpolar distances. Since the resistance and reactance at 50 cycles were the most critical single measurements, attention was then centered on them. The variation of the resistance with interpolar distance was quite regular, and although the reactance was often erratic it tended to decrease with distance as often as to increase. On the other hand, for the four axons which gave smooth reactance curves the reactance was independent of distance at sufficiently large electrode separations. The data for the best of these axons are shown in Fig. 5. This may of course be fortuitous, but if the inductance was in the connective tissue or the axoplasm, the membrane capacity would have had to be larger at the longer interpolar distances. Since the membrane capacity at higher frequencies was relatively independent of physiological condition and the present measurements, taken as a whole, indicate no trend, this combination of circumstances seems rather unlikely.

It is found on page 782 that at high frequency the longitudinal current

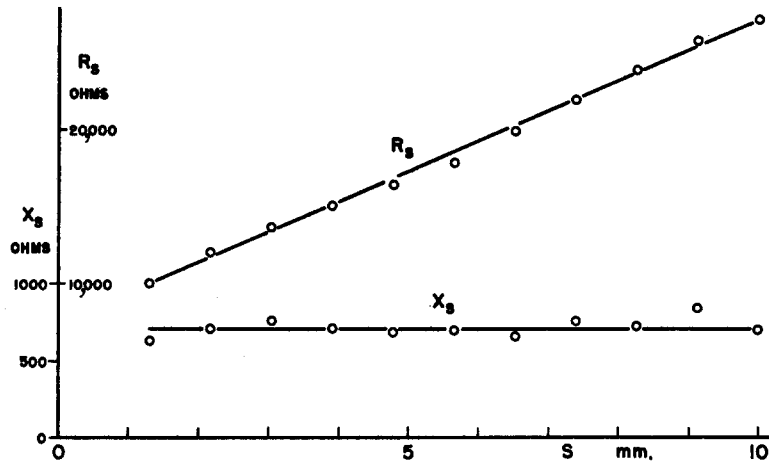


FIG. 5. Series resistance, R_s , and series reactance, X_s , at 50 cycles vs. interpolar distance, s , for squid giant axon. The reactances are inductive.

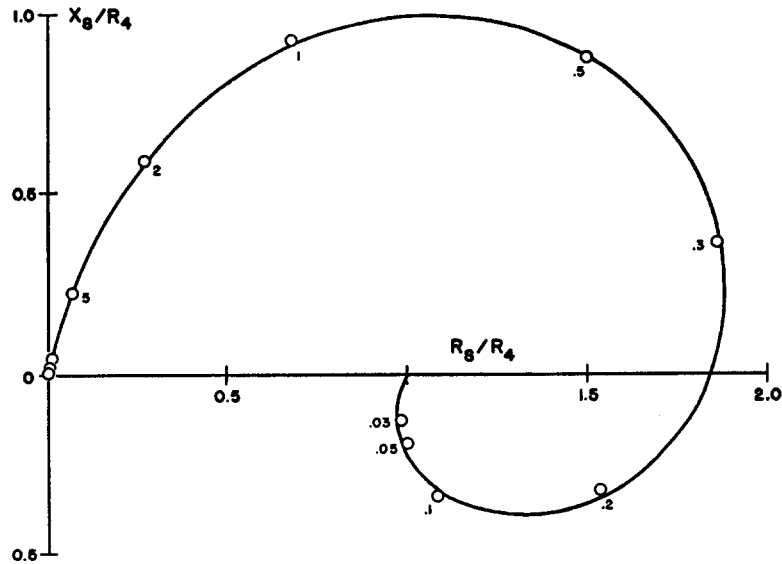


FIG. 6. Membrane impedance locus, calculated from longitudinal impedance data on axon of Fig. 3 by equation (6). Negative, or capacitive, reactances are plotted above the resistance axis. Frequencies indicated are in kilocycles.

would be carried by the axoplasm alone if the connective tissue were inductive, and by the connective tissue alone if the axoplasm were inductive. This has not been found, as is illustrated by Fig. 2*d* (Cole and Hodgkin,

1939) where the current is divided between the axoplasm and the connective tissue in the same ratio at high frequencies as it is with direct current for long interpolar distances, shown by the dotted lines of Fig. 3 in the above paper.

Consequently the experimental data lead us to assume that the axoplasm and the connective tissue are not responsible for the inductance and that it is a characteristic of the membrane.

Membrane Impedance and Equivalent Circuit

When the axoplasm and the connective tissue sheath are pure non-reactive resistances, it is found, equation (6), that the membrane impedance is easily calculated from the measured longitudinal impedance by squaring the frequency dependent part of the latter. In this way, the membrane

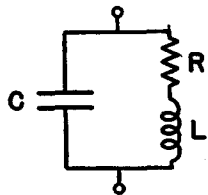


FIG. 7. An approximate equivalent membrane circuit for the squid giant axon, consisting of capacity C , resistance R , and inductance L .

impedances for the data of Figs. 3 and 4a have been determined and the loci are given in Figs. 6 and 4b respectively.

The impedance characteristics of the membrane are most simply discussed in terms of an equivalent circuit. There are, in general, many possible circuits which can represent a particular set of data (*cf.* Cole 1928, 1937) and the choice of any one should be justified by a theoretical analysis of the structure. At the present time there is not sufficient experimental evidence of the membrane structure to provide the basis for a theory which would uniquely determine a circuit. Consequently the choice will be dictated by utility, convenience, and personal prejudice, and for these reasons the circuit of Fig. 7 is proposed. This equivalent circuit can be seen to have impedances at least approximately those of the axon membranes by a comparison of the theoretical impedance loci for various values of C/L , shown in Fig. 8, with the membrane loci of Figs. 4b and 6.

Considering first the membrane impedance in Fig. 6, we see that it is not purely capacitive at high frequency because the locus does not approach the resistance axis at an angle of 90° . The approach at an angle $\phi < 90^\circ$ is, however, to be expected on the basis of an element in the membrane having a dielectric loss and an impedance $z_s = \bar{z}(j\omega)^{-\alpha}$ as has been found in a number

of biological materials. This particular value of $\phi = 75^\circ$ is close to the average of all the axons for which complete frequency data are available and is in good agreement with the values previously obtained (Curtis and Cole, 1938; Cole and Curtis, 1939). Then by considering the high frequency data alone and ignoring the inductance, a time constant of the membrane, $\tau = r_4 / |z_m| \omega = 1 / |\bar{z}|^2 \omega$, can be calculated without a knowledge

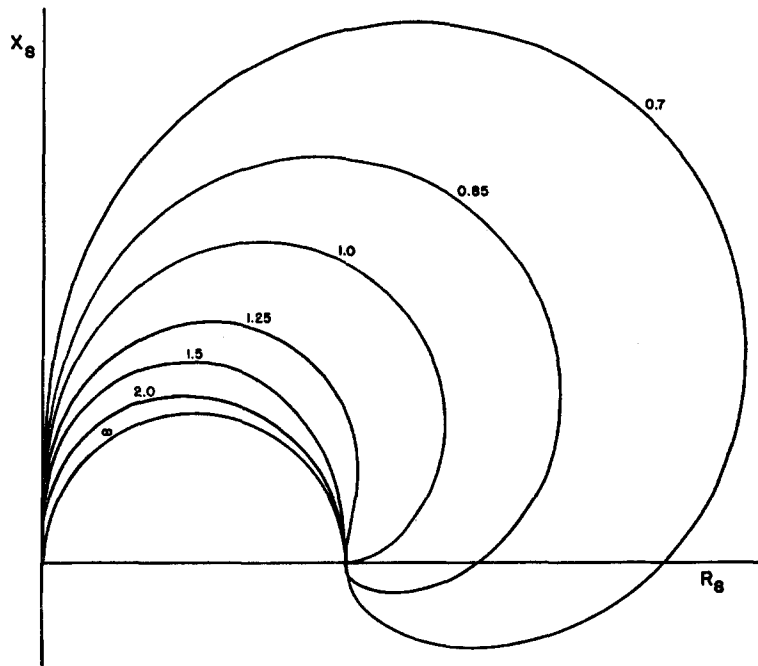


FIG. 8. Theoretical impedance loci, series resistance, R_s , vs. series reactance, X_s , for circuits of form shown in Fig. 7. The resistance R is constant throughout and the value of the damping factor, $\eta = R \sqrt{C/L}$, is given for each locus.

of the absolute value of either r_4 or z_m and we find this to be about 0.4 msec. If the membrane capacity is $1 \mu f/cm^2$, the corresponding membrane resistance is 400 ohm cm^2 , which is a reasonable value.

The resonant frequency of the membrane at which its series reactance, X_m , is zero, is about 250 cycles in the axon of Figs. 3 and 6, and other axons gave between 150 cycles to 320 cycles for this frequency. The undamped natural frequency of the capacity-inductance combination may now be calculated by equations (11 and 12). For the axon of Figs. 3 and 6, the damping factor $\eta = 0.72$ and $\nu_0 = 360$ cycles, and for the other nine axons for which the complete frequency data are satisfactory, these undamped

natural frequencies lie between 260 cycles and 380 cycles with an average value of 330 cycles. Taking a value of $1.1 \mu\text{f}/\text{cm}^2$ for the membrane capacity, this leads to a value for the membrane inductance of 0.21 henry cm^2 .

It is possible to go still further with the calculations and obtain a value for the membrane resistance, r_4 , by equation (12). For the axon of Figs. 3 and 6 the membrane resistance is 290 ohm cm^2 , while the other axons gave resistances from 260 ohm cm^2 to 420 ohm cm^2 with an average of 350 ohm cm^2 . These values agree moderately well with those obtained from the high frequencies (p. 780), are definitely lower than the resistances of 400 ohm cm^2 to 1100 ohm cm^2 obtained from direct current measurements (Cole and Hodgkin, 1939), and are higher than the 14 ohm cm^2 to 40 ohm cm^2 obtained from the membrane potential during current flow (Cole and Curtis, 1941).

Theory

Necessity of an Inductance

It seems obvious that if the reactance of any circuit in general, and the axon in particular, is inductive, there must be an inductance in that circuit. There are, however, phenomena characteristic of inductive circuits, such as "over-shoot" and oscillation, which may also be produced in circuits having only resistance and capacity, and no inductance. The interpretation and application of the present results will then be considerably simplified if it can be determined whether or not an inductance is necessary. The impedance, z , of a particular combination of lumped or distributed resistances, inductances, and capacities between two terminals can be calculated by ordinary methods, but at present we cannot assume a specific structure and must use a general analysis. We shall define first a resistance, r , as anything having a potential difference which is proportional, at every instant, to the current flowing through it, $e = ri$. Similarly for an inductance, the potential difference is proportional to the rate of change of current, $e = l \frac{di}{dt}$ and for a capacity the potential difference is proportional

to the charge, $e = \frac{1}{c} \int idt$. It then follows that energy is dissipated as heat in a resistance, but is stored in an inductance as kinetic energy and in a capacity as potential energy. These energies will vary as the current and potential difference are changed, but for alternating current, average values may be used conveniently. When an alternating current, I , flows between

the terminals of a known circuit, the average rate of energy dissipation in all the resistances, F , the average kinetic energy in all the inductances, T , and the average potential energy in all the capacities, U , can be calculated. It is not immediately obvious that there should be a connection between these quantities and the impedance, but a relation has been given by Bode (1935) which may be rewritten,

$$Z = R_s + jX_s = 2[F + j\omega(T - U)]/I^2 \quad (1)$$

where $\omega = 2\pi$ times the frequency and $j = \sqrt{-1}$. This equation is particularly useful because it applies to *any* two terminal circuits and because the quantities F , T , U are either positive or zero. If then X_s is positive, $T - U$ and, consequently, T must be greater than zero. Since this is a kinetic energy which is associated only with inductance, it follows that an inductance is necessary. On the other hand if T is zero, X_s must be negative or else zero, but it will be noticed that there may still be an inductance, giving T positive, although X_s is negative.

Location of the Inductance

Since inductance is now necessary, it must be allowed for in the calculation of the longitudinal impedance.

We shall consider the axoplasm and connective tissue to have impedances z_1 and z_2 per unit length of axon, which are perhaps of the form $z = r + j\omega l$, with a membrane impedance z_m per unit length, and obtain (Cole and Curtis, 1936, equation 1; Cole and Hodgkin, 1939, equation 1) for infinite electrodes

$$Z = \frac{z_1 z_2}{z_1 + z_2} s + \frac{2z_1^2 \lambda}{(z_1 + z_2) [\sqrt{(z_1 + z_2)/z_2} + \coth s/2\lambda]} \quad (2)$$

where s is the electrode separation and $\lambda = \sqrt{z_m/(z_1 + z_2)}$. If s is large and $\coth s/2\lambda$ approaches unity this becomes

$$Z = \frac{z_1 z_2}{z_1 + z_2} s + \frac{2z_1^2 \lambda}{(z_1 + z_2) [\sqrt{(z_1 + z_2)/z_2} + 1]} \quad (3)$$

where the second term is now independent of the electrode separation and will contribute a constant resistance and reactance. The first term, however, is inductive, if either or both of z_1 and z_2 are inductive, and this reactance will be important at large values of s . At high frequency, the overall impedance should become very high if z_1 and z_2 are both inductive, and should approach $r_1 s$ if z_2 alone is inductive or $r_2 s$ if z_1 alone is inductive. On the basis of experimental evidence (p. 777) we may assume that z_1 , z_2 are non-inductive and may be replaced by r_1 , r_2 , and that the inductance is to be found in the membrane.

Membrane Impedance

Before assuming an equivalent circuit or a location for an inductive element in the membrane, it is helpful to obtain the frequency characteristics or the impedance locus for the membrane alone from the observed longitudinal impedances for the axon as a whole. Equation (2) now becomes,

$$Z = \frac{r_1 r_2}{r_1 + r_2} s + \frac{2r_1^2 \lambda}{(r_1 + r_2) [\sqrt{(r_1 + r_2)/r_2} + \coth s/2\lambda]} \quad (4)$$

where $\lambda = \sqrt{z_m/(r_1 + r_2)}$. It still seems reasonably safe to assume that at high frequencies the current in the membrane is carried primarily by its capacity and that z_m approaches zero. Extrapolating to infinite frequency

we have $R_\infty = \frac{r_1 r_2}{r_1 + r_2} s$. For direct current, the membrane has been shown to have a resistance, r_4 , giving the overall resistance at zero frequency,

$$R_0 = \frac{r_1 r_2}{r_1 + r_2} s + \frac{2r_1^2 \lambda_0}{(r_1 + r_2) [\sqrt{(r_1 + r_2)/r_2} + \coth s/2\lambda_0]}$$

where $\lambda_0 = \sqrt{r_4/(r_1 + r_2)}$. We then find for the frequency dependent part of the longitudinal impedance

$$\bar{Z} = \frac{Z - R_\infty}{R_0 - R_\infty} = \frac{\sqrt{(r_1 + r_2)/r_2} + \coth s/2\lambda_0}{\sqrt{(r_1 + r_2)/r_2} + \coth s/2\lambda} \sqrt{\frac{z_m}{r_4}}, \quad (5)$$

and for s large,

$$\bar{Z} = \sqrt{z_m/r_4} \quad \text{or} \quad z_m = r_4 \bar{Z}^2 \quad (6)$$

Remembering now that \bar{Z} and z_m are complex quantities, we see immediately that equations (6) are conformal transformations of the simplest kind (Weaver, 1934).

If $\bar{Z} = \bar{R} + j\bar{X}$ and $z_m = r_m + jx_m$, then $r_m + jx_m = r_4(\bar{R}^2 - \bar{X}^2 + j\bar{R}\bar{X})$, from which r_m , x_m may be calculated at each frequency. Expressing both impedances in polar coordinate form

$$\bar{Z} = |\bar{Z}| e^{j\Phi} \quad \text{and} \quad z_m = |z_m| e^{j\phi_m}$$

we have

$$|z_m| e^{j\phi_m} = r_4 |\bar{Z}|^2 e^{2j\Phi} \quad (7)$$

and so

$$|z_m| = r_4 |\bar{Z}|^2, \quad \phi_m = 2\Phi$$

The absolute value of the membrane impedance, z_m , is then proportional to the square of the absolute value of \bar{Z} , the frequency dependent part of

the longitudinal impedance, and the phase angle ϕ_m of the membrane impedance is twice that of \bar{Z} . This calculation is particularly simple to carry out graphically. In the case where the membrane is assumed to be a resistance and capacity in parallel, its impedance is given by $1/z_m = j\omega c_s + 1/r_s$ and the locus of Fig. 1 *a*. The corresponding longitudinal impedance function, \bar{Z} , for such a membrane is then represented by the locus of Fig. 1 *b*. Conversely, where the longitudinal impedance data, such as Figs. 3 and 4 *a*, are available, the membrane impedance is calculated as shown in Figs. 6 and 4 *b*.

Equivalent Membrane Circuit

For many purposes it is convenient to consider the membrane characteristics in terms of an electrical circuit. The impedance of this circuit at any frequency should then be approximately that of a unit area of the membrane. The circuit shown in Fig. 7 has been chosen for reasons to be discussed later. This equivalent membrane circuit contains the capacity element C , which has the impedance $z = \bar{z}(j\omega)^{-\alpha}$ (where \bar{z} and α are constants) characteristic of many dielectrics. With an impedance of this form, relatively simple calculations appear complicated and the meaning becomes obscure to say the least. For this reason, we shall consider here, as a first approximation, the circuit in which C is a pure capacity. The impedance of the circuit is then

$$Z = \frac{(R + j\omega L)/j\omega C}{R + j\omega L + 1/j\omega C} = R \frac{1 + j\omega L/R}{1 - \omega^2 LC + j\omega RC} \quad (8)$$

If we now let $Z/R = \bar{Z} = \bar{R} + j\bar{X}$ and $\nu^2 = \omega^2 LC$, $\eta^2 = R^2 C/L$ we have

$$\bar{R} + j\bar{X} = \frac{1 + j\nu/\eta}{1 - \nu^2 + j^2\eta} = \frac{1}{(1 - \nu^2)^2 + \nu^2\eta^2} + \frac{j\nu}{\eta} \frac{1 - \nu^2 - \eta^2}{(1 - \nu^2)^2 + \nu^2\eta^2}. \quad (9)$$

η is the damping factor for the circuit, and ν is proportional to the frequency. At the undamped resonant frequency of the circuit, $\nu \equiv \nu_0 = 1$, and we have

$$\bar{R} + j\bar{X} = 1/\eta^2 + j/\eta \quad (10)$$

Also the reactance X is zero, $\bar{X}(\bar{\nu}) = 0$ at a frequency $\bar{\nu}$, other than zero or infinity, for $\bar{\nu}^2 = 1 - \eta^2$ (11) and $\bar{R}(\bar{\nu}) = 1/\eta^2$ (12) only when $\eta \leq 1$. Then $\bar{R}(\bar{\nu}) \geq 1$ and in this case with equation (10) we have a convenient method for determining ν_0 as well as η .

The loci of equation (9) have been plotted in Fig. 8 for several values of η . For $\eta = \infty$, the inductance is negligible and the locus is a semi-circle

determined by the resistance and capacity, and for $\eta = 2.0$, the circuit is critically damped. If $\eta = \sqrt{2}$, the damping is 70.7 per cent of critical or "optimum" which is often preferable to critical damping for recording instruments. The case of $\eta = 1.0$ is anomalous in that the locus approaches the zero frequency resistance along the resistance axis rather than at right angles as it does for all other values of η . As shown by van der Pol (1937) and Bode (1938) the energies stored by the inductances and capacities for direct current are equal in this case.

DISCUSSION

The concept of an inductance in a cell membrane is so foreign to our past experience and so difficult to grasp that we must inquire closely into each of the steps which has led to it before we can resign ourselves to the necessity of accepting and using it. There are observations of potentials and excitabilities which are strongly indicative of an inductive element in the membrane, but the present impedance measurements seem to be the only direct proof of the necessity of such an element. If this proof is not conclusive, evidence of all kinds may at least make an inductance seem reasonable, but if the impedance results alone are adequate proof, the conclusions may then be used as known factors in the interpretation of other phenomena. We shall therefore discuss the present results without reference to other evidence.

The only factors which have not been altered or replaced in the experimental work are the Wheatstone bridge and the squid axon. The bridge and its accessory equipment have been checked so often and in so many ways that it seems highly improbable for an error of 0.01 to 0.02 μf at frequencies of 50 to 200 cycles to appear only when a live axon was in the measuring cell. Three measuring cells with two different types of electrodes have been used and the mineral oil was changed or omitted without effect. It thus seems quite certain that the impedance characteristics are those of the axon. We are then able to prove theoretically that when an inductive reactance is measured there must be an inductance in the axon. This reactance was relatively independent of the external connective tissue on the axon which helps to eliminate it as a factor. The spatial and temporal variability encountered makes one suspect the membrane but does not eliminate the axoplasm. The experiments on the relation between impedance and interpolar distance are not completely satisfactory, but they do not consistently permit either an inductive axoplasm or connective tissue. Furthermore, none of these results eliminate an inductance from the membrane and there are several which can only be reasonably explained

by an inductive structure in the membrane alone. On the other hand, it has not yet been possible to show that an axon with inductive axoplasm or connective tissue can have an impedance locus similar to the loci found experimentally. We are then fairly well justified in assuming, until better evidence is found to the contrary, that the axon has an inductive as well as a capacitative element and that these are both located in the membrane, while the axoplasm and connective tissue are purely resistive.

With the reactive elements located in the membrane, and a sufficient distance between the electrodes, the calculation of the membrane impedance from the longitudinal impedance is found to be unexpectedly simple. Any attempt to interpret these membrane data should be regarded as speculation and accepted only tentatively, but some general features are quite obvious. The behavior at high frequencies is that of a dielectric impedance which has been referred to as the ion-impermeable aspect of the membrane (Curtis and Cole, 1938; Cole and Curtis, 1939). The phase angle and probably the capacity of this impedance agree with the values previously found from transverse measurements. The extrapolated direct current resistance of the membrane has not been carefully measured as a function of the electrode separation, but it is in better than qualitative agreement with other measurements (Cole and Hodgkin, 1939; Cole and Curtis, 1941). As before, we may consider this resistance as a measure of the ion-permeable aspect of the membrane and essentially in parallel with impermeable or capacitative portion. The equivalent circuit previously used for the membrane still seems to be satisfactory at both the very high and the very low frequencies, and we naturally wish to change these characteristics as little as possible when we introduce an inductance into the circuit. The fact that the membrane impedance has a maximum shows that the circuit is anti-resonant with the capacity and inductance in parallel. The direct current resistance must then be in series with the inductance and so we are led to the circuit of Fig. 7. As has been pointed out, many other and more complicated circuits can be found to express the data equally well or better, but we hesitate to consider a more involved situation until it is absolutely necessary for structural or phenomenological reasons. The structural implications of the elements of the present circuit and the application of the circuit as a whole to several physiological phenomena will be considered later (Cole, 1941).

There is adequate reason to believe that the membrane capacity of about $1 \mu\text{f}/\text{cm}^2$ is a structural characteristic which is relatively inert and insensitive to physiological and pathological changes. On the other hand, the accumulating evidence emphasizes the importance of the membrane

resistance and supports the growing conviction that this factor either governs or expresses the physiological condition and behavior of the cell. Unfortunately, from the available data it is not yet apparent whether or not the inductive element is a function of physiological condition. Where an undamped natural frequency can be calculated, it is surprisingly constant and is in a frequency range which is significant for a variety of other cells and phenomena. This suggests that the inductance may be as widespread and as constant as the membrane capacity.

There are some objections to the assumed circuit which are quite apparent. It seems fairly certain that as an axon deteriorates the membrane resistance decreases steadily. As this progresses the axon becomes inexcitable and finally the membrane capacity is short-circuited. If this were the only change taking place, the membrane might be initially over-damped, but would become increasingly under-damped and show a larger and larger inductive reactance as the resistance fell. This is not the case, for the inductive reactance vanished before the axon became inexcitable and never reappeared later. The circuit may be modified by the addition of a second variable resistance to include this observation, but the evaluation of an added unknown cannot be undertaken with the present data.

The characteristics shown in Figs. 4 *a* and *b* were found for a number of axons and they are particularly puzzling. From the analysis of the circuit it is found that the impedance locus should only approach the zero frequency resistance along the resistance axis in the case of optimum damping, $\eta = 1.0$, while in all other cases the locus should approach the resistance axis at right angles. It is difficult to blame the circuit for this but it seems unduly optimistic to expect the axon to adjust itself so often to this value of η . Another factor to be borne in mind is that it has been commonly assumed that the resistance in the capacitative arm, other than that representing the dielectric loss, is negligible. This assumption has not been proven and may not be justified.

An application of the present results, and in particular the inference of an inductance, to a normal squid axon and to other materials may be reasonably questioned. The net inductive reactance was not found in the axons which were judged to be the least abnormal, but there is more than a suspicion that the impedance loci of these were similar to Fig. 4 *a*, which also requires an inductance. Until it is possible to work with normal rather than surviving tissue we will not be in a position to do more. On the other hand, the stability of a good axon after it had equilibrated itself was comparable to that achieved in other experiments with this preparation, and we may assume that the process of equilibration and the steady state

were probably similar. If this is true, the longitudinal impedance measurement is at least a very sensitive index of the condition of the axon.

SUMMARY

Longitudinal alternating current impedance measurements have been made on the squid giant axon over the frequency range from 30 cycles per second to 200 kc. per second. Large sea water electrodes were used and the inter-electrode length was immersed in oil. The impedance at high frequency was approximately as predicted theoretically on the basis of the poorly conducting dielectric characteristics of the membrane previously determined. For the large majority of the axons, the impedance reached a maximum at a low frequency and the reactance then vanished at a frequency between 150 and 300 cycles per second. Below this frequency, the reactance was inductive, reaching a maximum and then approaching zero as the frequency was decreased.

The inductive reactance is a property of the axon and requires that it contain an inductive structure. The variation of the impedance with inter-polar distance indicates that the inductance is in the membrane. The impedance characteristics of the membrane as calculated from the measured longitudinal impedance of the axon may be expressed by an equivalent membrane circuit containing inductance, capacity, and resistance. For a square centimeter of membrane the capacity of 1 μ f with dielectric loss is shunted by the series combination of a resistance of 400 ohms and an inductance of one-fifth henry.

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