TRANSVERSE ELECTRIC IMPEDANCE OF THE SQUID GIANT AXON*

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The alternating current impedance of a nerve may be measured in a moist chamber between electrodes placed along its length, as is usually done for excitation and action potential studies. The impedance of several nerves has been measured in this manner (Krüger, 1928; Lullies, 1930, 1934; Cole and Curtis, 1936). Labes and Lullies (1932) have interpreted the data of the latter on the basis of a core-conductor model with a double layer and a diffusion capacity at the axon membrane.

A more satisfactory analysis of this "longitudinal" impedance would involve not only the flow of current into and along a single axon, but also the electrical properties of the other axons in the nerve trunk and the current flow through and around them. Such an analysis would be difficult and probably too cumbersome to be useful, so it is expedient to look for an experimental technique where the results can be analyzed more easily.

A length of nerve may also be laid between two long parallel electrodes so that the direction of the current flow will be primarily perpendicular to the axon axes. For a homogeneous nerve, this "transverse" impedance may be readily derived theoretically and in a simple algebraic form (Bozler and Cole, 1935; Cole and Curtis, 1936). On this assumption of homogeneity, measurements show that the axon membrane has a polarization impedance. Unexplained effects analogous to polarization impedance are found at metal-electrolyte interfaces, in imperfect dielectrics, and internal viscosity of solids, but the

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equation which describes them all is purely empirical and the use of the term polarization impedance is an admission of ignorance. For an inhomogeneous nerve, the calculations are more involved (Cole and Curtis, 1936) but a distribution of static membrane capacities among the axons can be assumed which will also explain the data. However, the necessary range of capacities is so great as to seem quite unreasonable since the membrane capacities of a variety of other cells are within a factor of three of each other. Both of these interpretations of the transverse impedance data on whole nerves are rather unsatisfactory since they do not uniquely determine the characteristics of the individual axon.

It is therefore necessary to further simplify matters by making transverse measurements on a single axon, but this was not considered possible until we were introduced to the squid giant axon by Dr. John Z. Young (1936). We are also very much indebted to him for his assistance in preliminary experiments which were made during the summer of 1936 at the Biological Laboratory, Cold Spring Harbor, Long Island.

Procedure

The Woods Hole squid, *Loligo pealii*, was used and the dissections were made at a temperature of about 10°C. The hindmost stellar nerve was dissected free between the stellate ganglion and the point where it enters the mantle muscle and the small fibers teased away from the giant axon *in situ*. The axon was then tied off at the proper length with fine silk threads, cut free at both ends, and transferred to the conductivity cell. In large animals, these axons can be dissected clean for a length of over 5 cm. and they have diameters from 0.5 to 0.6 cm. or sometimes larger. With careful dissection and proper treatment they can be kept excitable for several hours after excision. Early measurements were made at about 2°C. but it was later possible to work with excitable axons at room temperature.

The conductivity cell constructed for these measurements is similar to one which was used for the transverse impedance measurements of *Nitella* (Curtis and Cole, 1937). Since the sea water in which the axons were measured has a much lower specific resistance than any of the electrolytes used for *Nitella*, larger electrodes were necessary to avoid serious electrode polarization difficulties. This cell, shown in Fig. 1, was built up of glass and de Khotinsky cement with a long rectangular groove in the top for the axon. At the center of each side, the wall of this groove was cut away for a length of 5.6 mm. and a depression ground

leading to a platinized platinum electrode 12 mm. by 5 mm. cemented to the glass. The top surface of the cell was then ground flat.

The entire cell was filled with sea water and covered with a flat glass plate after the axon had been placed on the plateau remaining between the electrodes, and in the grooves at either end of it.

There was then a considerable amount of sea water whose resistance was in series with the impedance of the axon, but the whole cell may be considered as made up of an inactive part whose resistance is unaffected by the axon and an active part which is equivalent to the cell used for *Nitella* measurements. The cell constants of these two parts were determined by equation (1) from measurements of the resistance with a series of small glass rods in place of the axon. As a check, a few axons were measured in a small electrode cell of the type used for *Nitella* measurements. The latter data are not very satisfactory, but to within the limit of error they agree with the measurements made with the large electrode cell.

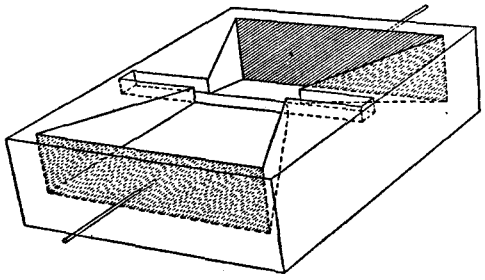

FIG. 1. Measuring cell for transverse impedance of a single axon

As soon as the axon was in place, the cell was connected to the Wheatstone bridge and the excitability tested as follows: The bridge was balanced with a small voltage, at a low frequency, 30 to 200 c. (cycles per second), a cathode ray oscillograph being used as null point detector. The amplified bridge output was connected to the vertical deflecting plates. The oscillograph figure was then an ellipse which, at balance, became a horizontal straight line. As the voltage input to the bridge, and consequently the current through the axon, was increased, a small hump appeared on the oscillograph pattern. This hump increased with the increasing input until the action potential suddenly appeared in its place.

Measurements

The general construction and operation of the alternating current Wheatstone bridge used for these measurements has been described in detail (Cole and Curtis, 1937).

The bridge input was reduced to a small fraction of that necessary to stimulate and the impedance measured as the parallel resistance, R_p, and capacity, C_p, at eleven frequencies from 1 to 2500 kc. (kilocycles per second). The excitability was tested again at the end of the frequency run and until the axon failed to respond. Then another frequency run would be taken. In many cases, the axon was left in place until, after several hours, the low frequency resistance slowly fell to a low value and the parallel capacity disappeared.

The axon was measured and removed from the cell, which was then filled with sea water. Another frequency run was taken which determined the electrode polarization corrections at the lowest frequencies and the capacity of the empty cell at higher frequencies.

The impedances were then computed as series resistance, R_s, and resistance, X_s, at the different frequencies and plotted as the impedance locus (Cole, 1928). This locus for a typical axon is shown in Fig. 2.

Transverse Impedance Theory

The equation for the specific impedance, z, between opposite faces of a nearly square cell with a cylindrical axon at the center, as given by Rayleigh (1892), and used for the *Nitella* measurements, is the same as for a uniform suspension of uniform parallel cylinders

$$\frac{1 - r_1/z}{1 + r_1/z} = \rho \frac{1 - r_1/z_2}{1 + r_1/z_2} \tag{1}$$

where r_1 is the specific resistance of the medium, ρ is the fractional part of the volume of the cell occupied by the axon, and z_2 is the equivalent specific impedance of the axon. This latter is given by

$$z_2 = r_2 + z_3/a \tag{2}$$

(Cole, 1928), where r_2 is the specific resistance of the axon interior, z_3 the membrane impedance, and a the axon radius. As has been shown when z_3 is a polarization impedance with a constant phase angle independent of frequency, the impedance locus is a circular arc with its center below the resistance axis (Cole, 1928, 1932). The phase angle, ϕ, of z_3 is then half the angle between the radii to the intercepts of the arc on the resistance axis.

It can be further shown that the membrane capacity per unit area, c_M, can be computed directly from equations (1) and (2) or more simply by the equation (Cole and Curtis, 1936),

$$c_M = \frac{k}{a\bar{\omega}r_0} \cdot \frac{1 - r_\infty/r_0}{1 - (r_1/r_0)^2} \tag{3}$$

k is the "active" cell constant, r_0 and r_∞ are the circular arc extrapolated resistances at zero and infinite frequency, and $\bar{\omega}$ is 2 π times the characteristic frequency, for which the series reactance is a maximum. Since the capacity of a polarization impedance varies with frequency and c_M has the value given by equation (3) at the characteristic frequency, the capacities in all cases have been calculated at 1 kc.

The internal resistance of the axon r_2 may be expressed in terms of the resistance of sea water, r_1, and r_∞ from equations (1) and (2), or by

$$r_2 = \frac{r_0 r_\infty - r^2}{r_0 - r_\infty} \tag{4}$$

The resistance for a unit area of membrane r_3 is computed (Cole, 1937) from the difference between the actual volume concentration ρ_n and the volume concentration ρ which is computed at low frequency by equation (1) considering the rest of z_3 to be infinite

$$r_3/a = r_1 \frac{(2 - \Delta)}{\Delta} - r_2,$$

where

$$\Delta = (\rho_n - \rho)/\rho_n.$$

Data and Interpretations

It is seen from Fig. 2 that equation (1) is an adequate representation of the data on this axon over most of the frequency range, when the membrane has a polarization impedance with a phase angle of 79°. The phase angles for other axons varied from 64° to 85° with 76° as an average value. From equation (3), it was found that the membrane capacity at 1 kc. for the axon of Fig. 2 was 0.97μf./cm.^{2} Values were obtained from 0.66 to 1.60μf./cm.^{2} and the average of twenty-two experiments was 1.07μf./cm.^{2} No significant change of these quantities with temperature was observed.

In general, the high frequency points fit the circular arc no better than in the data plotted, but from equation (4) and the values of r_∞ extrapolated by the arc, internal resistances are calculated from 1.5 to 6.9 times the resistance of sea water with an average of 4.2. Considering the difficulties involved, the volume concentrations calculated from axon diameters ρ_n and those obtained from the low frequency resistances by equation (1) assuming a non-conducting membrane, ρ, agree rather well. There is some spread in the data, and if we take the maximum deviation we obtain, at least as a lower limit, a membrane resistance of 3 ohm cm.^{2} from equation (4).

Five satisfactory experiments were obtained in which an impedance locus could be plotted on the same axon both before and after loss of

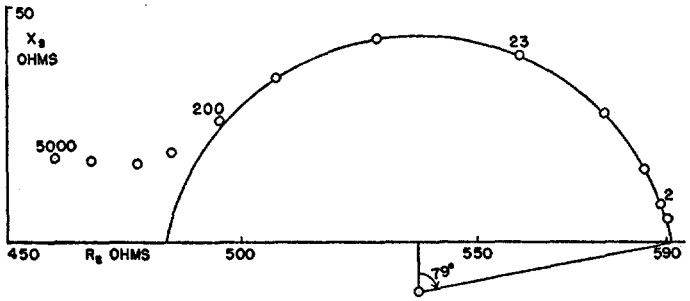

FIG. 2. Impedance locus, series resistance R_s *vs.* series reactance X_s, for a single axon at room temperature. Frequencies given are in kilocycles per second.

excitability. These axons covered a considerable range of sizes and phase angles. The constants ϕ, c_M, r_2, and r_3 were computed for each axon in the two conditions and the average change of each constant was negligible.

As has been mentioned, several hours after the axons lost excitability, the low frequency resistance and capacity would start to decrease until the membrane capacity became zero and the internal resistance was that of sea water.

DISCUSSION AND CONCLUSIONS

It is unfortunate that the data are not more consistent and reproducible from one axon to another. But the axons are delicate and are not excitable unless they have been handled quite carefully, so it may be that the results are as good as should be expected. There are

indications that the phase angle of the *Arbacia* egg membrane may be affected by rough handling (Cole and Spencer, 1938; Cole and Curtis, 1938). But since the phase angle and the other constants of the axons do not change on loss of excitability, we hardly feel justified in explaining the wide variability in the excitable axons as due to injuries which were not sufficient to prevent excitability. Transverse impedance measurements must by their very nature be made in a higher and more difficult frequency range from the longitudinal measurements and furthermore, the extra resistance incurred by the use of large electrodes alone increases the necessary precision of the measurements tenfold.

In spite of the variations, it seems reasonably certain that over a large frequency range, the squid giant axon membrane has a polarization impedance with a phase angle of about 76° and a capacity at 1 kc. of about 1.1μf./cm.^{2} Although it was suggested (Cole and Curtis, 1936) that the whole nerve data could be interpreted on the basis of a statistical distribution of diameters and static membrane capacities among the axons, further calculations made this assumption seem rather improbable, particularly in view of the fact that a polarization impedance has been found for the single cell *Nitella*. Consequently the polarization impedance is not particularly surprising, nor is the average axon membrane capacity entirely unexpected, in view of the wide variety of cell membranes which have capacities of the order of one μf./cm.^{2}

A few measurements were made on the fin nerves of the squid, with many small axons covering a wide range of diameters, which gave phase angles between 40° and 50°. This is a much lower value than has been found for any single cells or reasonably uniform material. We may assume that it is due in part to phase angles less than 90° for the individual membranes, and in part to the distribution of diameters. A similar explanation can probably be given for data on other whole nerves.

If then a polarization impedance can be an inherent characteristic of a single cell membrane, the problem of the membrane structure is somewhat clarified. Although, as has been said, there is no adequate picture of this polarization mechanism, we may now look upon it as a general problem, of which the static capacity is a special case.

Perhaps the most interesting observation is that the impedance

properties of the axon did not change when it became inexcitable. It has been observed that the resting potential does not alter markedly under the same conditions, but it seems difficult to align these two types of measurements at present. The constancy of the red blood cell membrane capacity in chemical hemolysis (Fricke and Curtis, 1935) is probably more nearly analogous. Since the phase angle and capacity are undoubtedly determined by the membrane structure and composition, we are led to assume that the changes accompanying hemolysis and loss of excitability are either slight, if the membrane is homogeneous, or else take place in only a small fractional part of the area if the membrane is not uniform. It must be remembered that suspension and transverse measurements are not suitable for the determination of the resistance, or change of resistance, of the membrane to direct current because by these methods, it is not yet possible to measure or even detect a conductivity when the resistance is above 100 ohm cm.^{2}. The *Nitella* membrane has a resistance of 100,000 ohm cm.^{2} or more (Blinks, 1930) and preliminary measurements have indicated that the normal axon membrane resistance may be of the same order of magnitude.

It might well be that excitability would be lost when this resistance fell to 1000 ohm cm.^{2}, but it does seem unreasonable to suppose that so large a change would leave the membrane phase angle and capacity unchanged if it took place over the entire membrane surface. If then, after loss of excitability, the resistance continued to decrease, more and more current would flow through the membrane and cell interior until it would become measurable at a membrane resistance of about 1 ohm cm.^{2} If it were then to go as low as 0.01 ohm cm.^{2} it would be very difficult to detect any evidence of a normal membrane polarization impedance, and as yet we have no way to determine whether the final drop in the low frequency parallel resistance and capacity is due to a disintegration of the membrane structure responsible for the polarization impedance or whether it remains essentially intact and the current flow is increasingly diverted as highly localized areas have less and less resistance.

There is not sufficient information available at present to determine the nature of the effects which were observed in the excitability tests. The preliminary humps on the cathode ray figure may be explained

as either a change of impedance or an unequalized potential. Also, it is not possible to locate and explain the origin of the action potential satisfactorily for the electrode arrangement used.

SUMMARY

The impedance of the excised giant axon from hindmost stellar nerve of *Loligo pealii* has been measured over the frequency range from 1 to 2500 kilocycles per second. The measurements have been made with the current flow perpendicular to the axis of the axon to permit a relatively simple analysis of the data. It has been found that the axon membrane has a polarization impedance with an average phase angle of 76° and an average capacity of 1.1μf./cm.^{2} at 1 kilocycle. The direct current resistance of the membrane could not be measured, but was greater than 3 ohm cm.^{2} and the average internal specific resistance was four times that of sea water. There was no detectable change in the membrane impedance when the axon lost excitability, but some time later it decreased to zero.

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