ELECTRIC IMPEDANCE OF NITELLA DURING ACTIVITY*

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INTRODUCTION

There are many theories of the nature and mechanism of the processes of excitation, conduction, and recovery in irritable cells, but those which emphasize the electrical aspects of these phenomena are the most attractive and probably the most important because these effects can be measured with a high degree of precision and speed.

It is rather commonly accepted that the process of excitation of irritable cells starts as a more or less localized depolarization of the cell membrane. When the adjacent resting regions are then depolarized by the resultant current flow, the process continues as a propagated wave.

It is not surprising that both the cause and the course of the depolarization have remained vague and uncertain when most of the information has come from electrical excitation and action potential studies while the properties of the resting membrane have not been well understood. Consequently the attempts to express this picture in more quantitative form have usually encountered so many unknown factors that it has been necessary to use either poorly defined concepts or a considerable number of assumptions.

Two outstanding unknowns have been the electrical resistance and capacity of the membrane and the resistance of the cell interior. From longitudinal direct current resistance measurements on resting *Nitella*, Blinks (1930) obtained values of 10^5 Ω cm.^{2} or more which we may interpret as an ohmic membrane resistance. Since similar data by Rosenberg and Schnauder (1923) on the frog sciatic give 4.10^4 Ω cm.^{2}, we may expect that such a resistance is not peculiar to *Nitella*.

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In common with a number of other types of cells, resting membrane capacities of approximately 1μf./cm.^{2} have been found for muscle and nerve (Cole and Curtis, 1936), *Nitella* (Curtis and Cole, 1937), and the squid giant axon (Curtis and Cole, 1938). These membrane capacities have associated with them a loss, similar to that found in common dielectrics, giving them a phase angle of less than 90°, which is independent of frequency.

There is less information as to the effect of excitation on these quantities. Lullies (1930) reported a decrease in the longitudinal low frequency impedance of nerve, and Blinks (1936) showed a loss of polarizability (the change of potential difference across the membrane due to current flow from an external source) in *Nitella*. In these experiments, it was not possible to separate the membrane resistance and capacity and follow the time course of each during excitation, so that a different method of approach is necessary.

In the present work, the impedance properties of *Nitella* have been measured, transverse to the cell axis, and over a wide frequency range, during the passage of an impulse, with simultaneous records of the cell action potential under the impedance electrodes. These data give the time course of the resistance, capacity, and potential changes of the membrane during activity and so provide a more complete and quantitative description of the impulse than has been possible before.

Nitella was chosen because it has large single cells, lives in fresh water, and is quite slow in its responses. The behavior of this plant cell is analogous to that of nerve in so many respects that one might also expect that the processes of activity would be found to be similar. The structure of *Nitella* is apparently more complicated than that of nerve and has been the cause of some difficulty in the interpretation of the impedance properties of the cell at rest (Curtis and Cole, 1937) and in activity. As has been pointed out, there are two protoplasmic interfaces in *Nitella*, but since our measurements have not given any evidence which provides a means for separating the properties of the two surfaces, we shall consider the observed characteristics to be due to a single membrane.

The alternating current transverse impedance technique which has been used for the resting *Nitella* and other cells is a relatively simple and straightforward method for determining the electrical

properties of the membrane and cell interior. In order to use it for the present problem, the methods of measurement and interpretation have had to be extended considerably.

Material and Measuring Cell

Nitella flexilis, Ag., was grown in an aquarium which was balanced with guppies and radiated periodically with a neon lamp.

To obtain satisfactory action potentials, M/1000 KNO_3, M/10,000 KH_2PO_4, and M/10,000 $MgSO_4$ were added to the tap water in the aquarium. The *Nitella* grew quite readily under these conditions, and only healthy, growing cells were used for the experiments.

The cell chosen was cut away from its neighbors and immediately placed in the measuring apparatus. About half an hour was allowed for equilibration before the experiment was started. The measuring cell is essentially the same as that previously used, and is shown in Fig. 1. It is made of glass, de Khotinsky

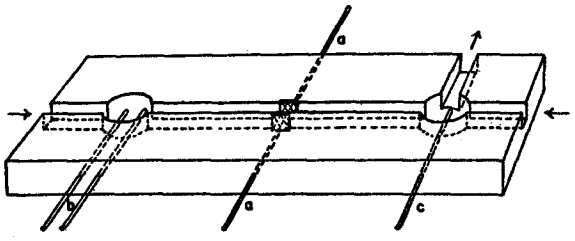

FIG. 1. Measuring cell for *Nitella*, with impedance electrodes, *aa*, stimulating electrodes, *b*, and action potential electrode, *c*. Arrows indicate the directions of flow of electrolytes.

cement, and various platinized platinum electrodes. The groove in which the *Nitella* cell was placed is 0.53 mm. by 0.55 mm. and 2 cm. long, with a depression at each end to accommodate the cut ends of neighboring cells. The impedance electrodes, *aa*, were 0.5 mm. long and were inlaid in opposite faces of the groove. This electrode length represents a compromise between the needs for measurements over as short a length of cell as possible, and a large electrode area to minimize electrode polarization corrections. The stimulating electrodes, *b*, were placed at one end and an action potential recording electrode, *c*, at the other. Capillary siphons were placed at the ends of the grooves as indicated in Fig. 1. Aquarium water or M/1000 KCl was siphoned in at the stimulating electrode end, past the stimulating and impedance electrodes, and out through the side tube. To obtain monophasic action potentials, M/10 KCl was siphoned in at the other end and out through the side tube, so that it bathed only the end of the *Nitella* under the recording electrode. This concentration of KCl was used to abolish the membrane activity because the cells seemed to last longer with KCl than with

chloroform. In this way, good monophasic action potentials were recorded, and most cells would give reproducible results for many hours and some even for days. The relatively refractory period for *Nitella* lasts from 3 to 5 minutes under the most favorable conditions, and it was necessary to allow this interval between stimuli, otherwise both the "resting" impedance and impedance change become progressively smaller.

Electrical Equipment

The impedance electrodes of the measuring cell were connected to the alternating current Wheatstone bridge (Cole and Curtis, 1937) and measurements of the parallel resistance and capacity (Cole, 1933; Cole and Curtis, 1936) were made from 0.05 to 100 kc. (kilocycles per second). The amplified output of the bridge was applied to the vertical deflection plates of a cathode ray oscillograph. A portion of the input oscillator voltage was applied to the horizontal deflection plates in such phase relation to the bridge output that a parallel resistance unbalance of the bridge caused the horizontal balance line to tip, while a parallel capacity unbalance alone gave an ellipse with a horizontal axis.

The action potentials were led off between either the midpoint of a high resistance shunting the impedance electrodes, or the grounded impedance electrode, and the recording electrode at the inactive end of the cell. These potentials were applied to the vertical deflection plates of a second cathode ray oscillograph through a differential direct current amplifier with degeneration in the common mode. Both oscillographs are photographed simultaneously with a motion picture camera at from 17 to 64 frames per second. Since motion pictures could be taken, a sweep circuit was unnecessary, and the action potential was recorded as a deflection from the base line on each picture.

The stimulating current was obtained from a condenser discharge which had a time constant of about 1 millisecond. Under these conditions it usually took about 30 volts to stimulate. The stimulating circuit was electrically isolated from the rest of the circuits to reduce the stimulus artifact and considerable care had to be taken to avoid stimulation or injury by static charges.

Procedure

The single *Nitella* cell is placed in the trough of the measuring cell, covered, and the circulation of both aquarium water and KCl started. When the impedance and resting potential have reached steady values, the bridge is balanced at one frequency and the amplifiers adjusted so that the maximum changes after stimulation remain on scale. The camera is started as the cell is stimulated, and the entire action, lasting several seconds recorded. Calibration figures are then recorded for known unbalances of both resistance and capacity. This procedure is followed at each of the nine frequencies from 0.05 to 20 kc. The cell is removed and a frequency run taken on aquarium water alone to obtain data for the capacity zero and electrode polarization (Cole and Curtis, 1937). In this way, a complete frequency run could be taken in 40 minutes.

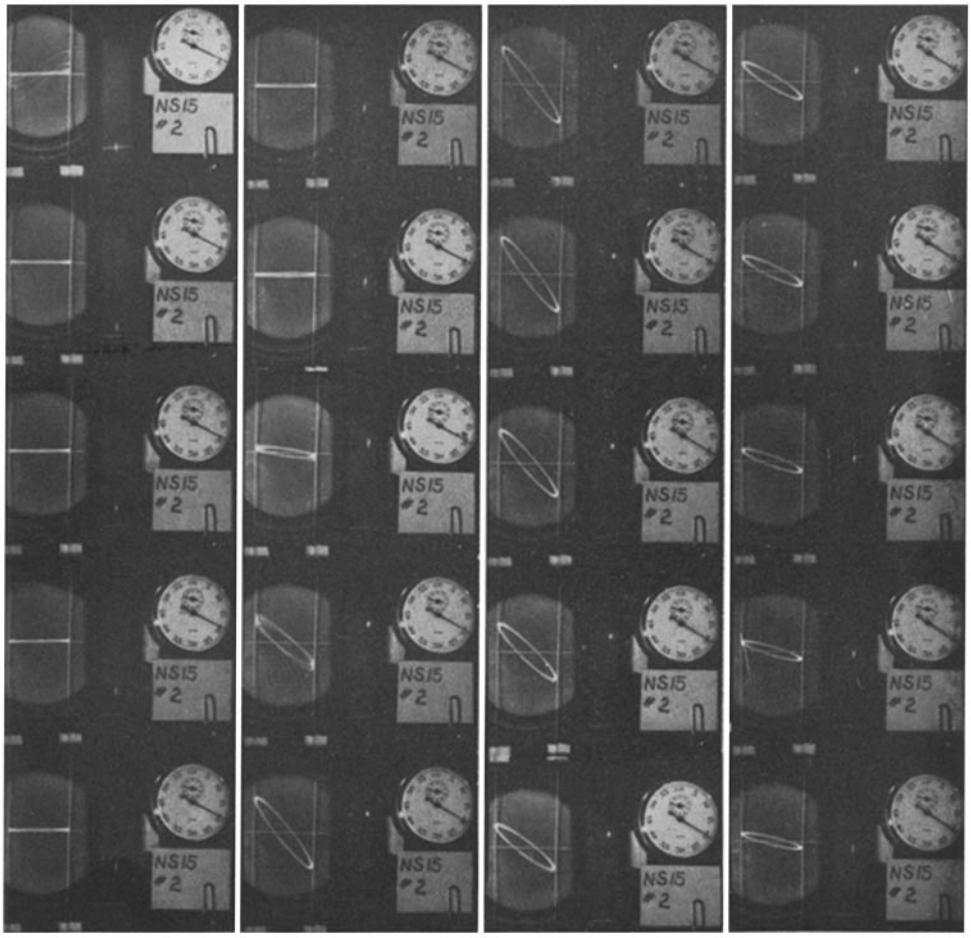

FIG. 2. Section of a motion picture record of the bridge unbalance ellipses (left), action potential spot (center), and stop watch (right) following stimulation.

A short section of a film taken at 0.2 kc. and 17 frames per second is reproduced in Fig. 2. The stimulus artifact can be seen in the first picture, and the action potential starts to rise immediately afterwards. When the action potential has nearly reached its maximum, the bridge goes off balance rather suddenly and then both subside quite gradually.

The developed film is projected and the impedance ellipses and the action potentials are measured on the screen. From the dimensions of the experimental and calibration ellipses, the changes in the parallel resistance and capacity are calculated by equations (3). The impedance at rest and at the time of each picture after stimulation

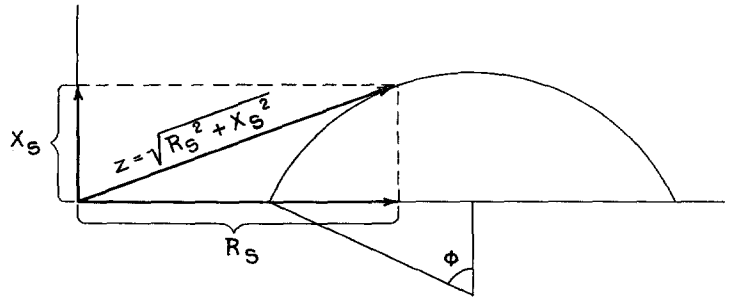

FIG. 3. Schematic impedance locus, showing an impedance vector, z, with its two components, the series resistance, R_s, and series reactance, X_s. ϕ is the membrane phase angle.

is computed as though the measurements had been made with the resistance, R_s, and the reactance, X_s, in series, by the equations

$$R_s = R_p/(1 + R_p^2 C_p^2 \omega^2) \quad \text{and} \quad X_s = R_p^2 C_p \omega/(1 + R_p^2 C_p^2 \omega^2).$$

R_s and X_s are plotted to give the impedance locus, as shown in Fig. 3. With the cell at rest, the end of the impedance vector follows a circular arc as the frequency is changed and this arc is called the frequency impedance locus. During the process of excitation, the impedance at each frequency changes so that the tip of the impedance vector follows a path, such as D in Fig. 4, which is called the time impedance locus.

RESULTS

Impedance.—It was found that there was some uncertainty as to the form of the frequency impedance locus at the time of maximum

change. This was probably due to an uncontrolled variation in the cell since the maximum change at any one frequency varied somewhat during an experiment. Since it was not possible to measure all frequencies during a single excitation, three excitations at each frequency were recorded in random order and the minimum impedances plotted in Fig. 4. It is quite apparent, in the first place, that there is no change in the extrapolated high frequency resistance. This means that the resistance of the interior of the cell, the resistance of the external fluid, and the volume concentration remain unchanged, or

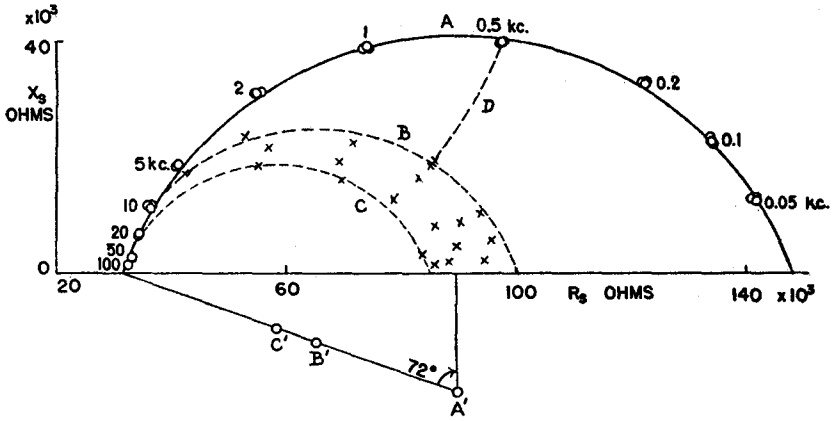

FIG. 4. Frequency impedance loci, *i.e.* series resistance, R_s, *vs.* series reactance, X_s. *A*, for *Nitella* at rest (o) and *B* and *C* for the extreme values of the maximum impedance changes during activity (×), with centers at *A'*, *B'*, and *C'*. Frequencies are given in kilocycles (kc.), and each frequency was repeated three times in random order.

else that any two, or all three, change in such a way that the infinite frequency resistance is unchanged. It is more reasonable to assume that there is no change in any of these quantities. In the second place, since the minimal excitation impedance points (×) are distributed rather well between two circular arcs representing the same phase angle as the resting cell, there is no indication of a change in the phase angle of the membrane at the height of the excitation and it will be assumed as a first approximation that there is no change of this membrane parameter during the course of the action. It will be shown that the maximum variability of the impedance changes shown in Fig. 4 rep-

resents a ± 10 per cent maximum deviation of the membrane impedance from its mean value.

During single excitations, the time course on the impedance locus has been plotted for three frequencies in Fig. 5. Equation (4) shows that if the membrane capacity alone were to change during excitation, the zero frequency extrapolation would remain unchanged and the impedance at each frequency would merely move along the resting circle. Since this is not the case, and since the membrane phase angle, the internal and external resistances, and the volume of the cell (see

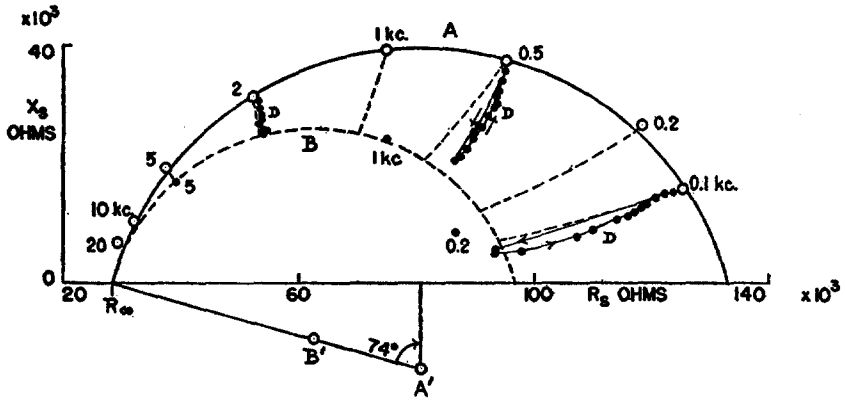

FIG. 5. Impedance loci, *i.e.* series resistance, R_s, *vs.* series reactance, X_s. The frequency loci are A, for *Nitella* at rest (○), and B, at height of activity with centers at A' and B'. The time loci, D, show how the impedance changes at 0.1, 0.5, and 2.0 kilocycles when the cell is stimulated (●). As indicated by the arrows, the time impedance locus during recovery does not exactly retrace the path of the initial change.

above) change little if at all, we must now consider the effect of a change in the membrane conductance or leakage.

The membrane conductance is thought of as due to a resistance in parallel with the membrane capacity. This conductance, which may be the representation of the ionic membrane permeability, is considered to be independent of frequency. On this assumption, it will be shown in equation (8) that if the membrane conductance alone were to change during the action, the time impedance locus would be a circular arc tangent to the resistance axis at the infinite frequency

point (R_∞), as indicated by the dotted lines in Fig. 5 and the arc C in Fig. 11. This assumption gives an approximate explanation of the observations but it is found that the experimental points quite consistently fall to one side of the arc. The deviation may be interpreted as an average maximum decrease in the membrane capacity of 15 per cent from the resting value of 0.9μf./cm.^{2} This capacity change follows a time course similar to that of the membrane conductance. The two are slightly different, however, because it can be seen on the time impedance locus that, during recovery, the impedance does not exactly retrace the path of the initial change.

In the previous *Nitella* paper (Curtis and Cole, 1937) it was shown that, within experimental error, the conductance of the resting cell membrane was so small as to be negligible in transverse impedance measurements. The membrane conductance during excitation, calculated from data at 0.05 kc. by equation (6), has been plotted in Fig. 6 as a function of time. The maximum conductance in this case corresponds to a membrane resistance of 400 ohm cm.^{2} with an average value for all experiments of 500 ohm cm.^{2} In the data of Fig. 4, the maximum (B) and minimum (C) circles correspond to membrane resistances of 350 and 287 ohm cm.^{2} or a $\pm$ 10 per cent fluctuation from the mean value for this typical *Nitella*. Furthermore, it is found that the time courses of the membrane conductances calculated from the data at frequencies shown in Fig. 5 agree closely with each other, thus supporting the assumption that the membrane resistance is *independent* of frequency.

The observed rise of conductance is considerably faster than that of the action potential. However, if a sharp, or discontinuous change of conductance were to move past the 0.5 mm. long impedance electrodes at a velocity of 1.0 cm./sec. the observed time of rise of conductance should be at least 0.05 second. Actually, this time would be still longer because of the "fringing" of current at each end of the electrodes which increases their apparent length. It is simpler to obtain the apparent length from a model experiment than by calculation. A round glass rod was placed in the measuring cell instead of the *Nitella* and resistance measurements were made for a number of positions as the rod was moved to the right and its left-hand end passed between the impedance electrodes. The end then corre-

sponded to an abrupt transition from a non-conducting to a perfectly conducting membrane, although the difference between the measured resistances with and without the rod may not be large, as is seen from equation (4). The only data taken before this glass impedance cell was broken are those of Fig. 7, but they show quite clearly that

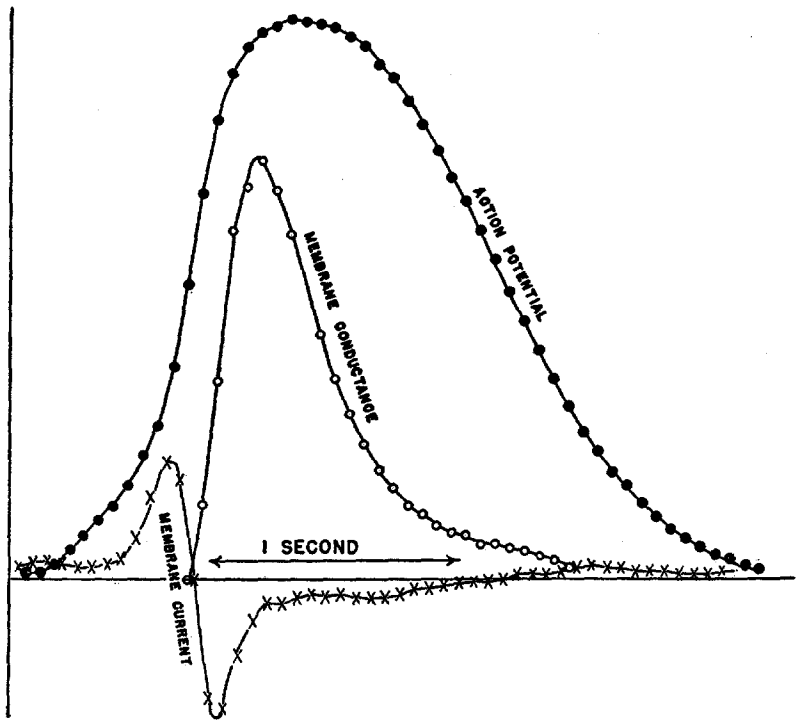

FIG. 6. Membrane conductance, monophasic action potential, and membrane current *vs.* time after stimulation. Ordinates are all in arbitrary units. Frequency 0.20 kc.

the effective length was about 1.0 mm. or twice the actual electrode length. At a velocity of 1 cm./sec. this would then correspond to a time of rise of 0.10 second and since 0.25 second or less is observed, the actual time is not more than 0.15 second. Experiments in which the cell was stimulated under the impedance electrodes indicate that the time of rise may be considerably less than 0.15 second.

If the flow of water through the cell was stopped the impedance

remained unchanged for fairly long periods of time unless the cell was stimulated. After a single stimulation, the low frequency impedance failed to return to its previous value but remained at a lower value for some time. On repeated stimulation the impedance became progressively lower, but there was an immediate return to the initial value when the water circulation was resumed. This indicates that the decrease of impedance was due to a decrease in the specific resistance of the medium which was probably due to a loss of electrolyte from the cell during excitation, that was recovered very slowly, if at all.

Action Potentials.—It is seen in Fig. 6 that the start of the membrane conductance change comes toward the maximum of the monophasic

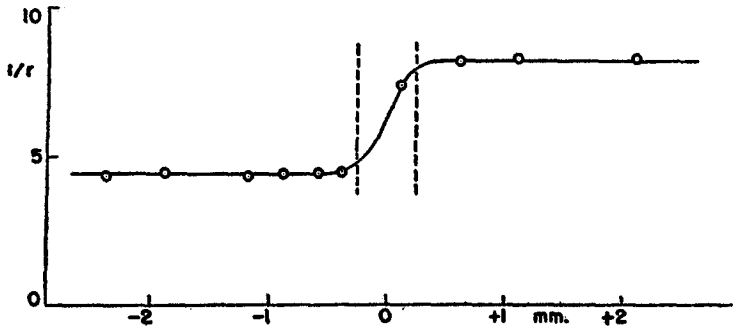

FIG. 7. A glass rod in place of *Nitella* in measuring cell is moved from left to right. Conductance, $1/r$, *vs.* distance of the end of the rod from the center of the electrodes. Negative abscissae indicate that the rod was between the electrodes. The dotted lines show the actual electrode width. Conductance is in arbitrary units.

action potential, and it is interesting to correlate it with the density of current flowing across the membrane. This is proportional to the second derivative of the action potential (equation (10)) and has been computed numerically to give the "membrane current" curve of Fig. 6. The maximum values of the outward and inward current densities are approximately equal, although the low membrane resistance should allow a considerably greater maximum current density inward than outward. The impedance electrode width should tend to equalize these maxima but the effect has not yet been calculated or investigated with a model.

Not only are experiments with cells having one end in contact with M/10 KCl not entirely satisfactory, and perhaps open to question on physiological grounds, but the cumulative errors in the numerical calculation of the second derivative led us to do a few experiments with a different technique.

The *Nitella* cell in a moist chamber was supported on 2 platinized platinum wires, 0.25 mm. in diameter, so as to make contacts on opposite sides of the cell, the wires serving as transverse impedance

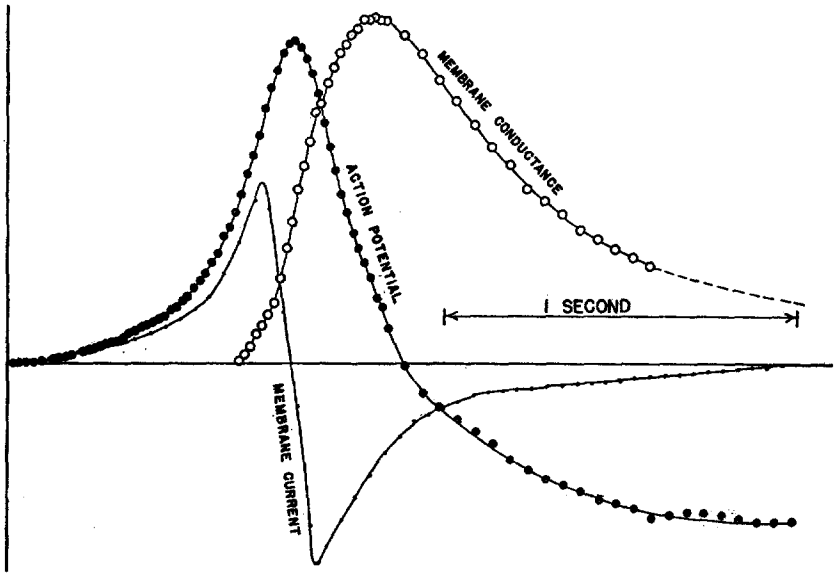

FIG. 8. Membrane conductance, diphasic action potential, and membrane current measured in a moist chamber *vs.* time after stimulation. Ordinates are all in arbitrary units. Frequency 0.20 kc.

electrodes. On each side of one impedance electrode and 1 mm. away from it a similar wire potential electrode touched the *Nitella* cell. These two were then differential electrodes giving the potential gradient over a 2 mm. stretch which included the impedance electrodes. This "diphasic" potential was then approximately the first differential of the monophasic action potential, or proportional to the external current flow parallel to the long axis of the cell, and the membrane current density its first derivative.

The impedance and potential changes were taken at 64 frames per second and the results of an action are shown in Fig. 8. Although the resting characteristics are much less steady than in the flowing medium, and the results less reproducible, it is seen that the characteristics are entirely similar. It thus seems quite certain that in general the increase in conductance and the change in direction of the membrane current from outward to inward occur at very nearly the same time.

The typical action potential of Fig. 6, which has a form similar to that of nerve and a duration of 2 or 3 seconds, is quite different from the potentials with a double peak and a 15 second duration normally found by Osterhout (1934). We have obtained the latter type of monophasic action potential by keeping the cells in a medium similar to that used by Osterhout and although the relatively refractory period was longer and the impedance changes less reproducible, the results were essentially the same as those found for the more rapid cells.

DISCUSSION

Impedance.—Although it is not yet possible to formulate our information on the electrical properties of a cell membrane in terms of a definite molecular structure, some characteristics seem fairly clear.

In Figs. 4 and 5, the extrapolated infinite frequency resistance is a common end point which is approached along one path for the resting cell as the frequency is increased and along another, at one frequency, during activity. The former path is most simply described as a decrease of the parallel reactance of the membrane and the latter as a decrease of its parallel resistance. Since also this parallel reactance corresponds to the membrane capacity found for a variety of other cells, a parallel capacity and resistance represent the membrane fairly well.

Observations on the excitability and impedance of the squid giant axon (Curtis and Cole, 1938) and the constancy of the red blood cell membrane capacity in chemical hemolysis (Fricke and Curtis, 1935) have led to the suggestion that the capacity and leakage resistance of the membrane might be relatively independent. In the present results, we have a sounder basis for this separation of the non-con-

ducting and conducting—or probably the ion impermeable, and ion permeable—aspects of the membrane.

The change in the non-conducting aspect of the membrane is important in fertilization of marine eggs and may play a considerable rôle in the action of lipoid solvents, but it is apparently a minor factor in the excitation of *Nitella*, where the phase angle is unchanged and capacity is decreased by only 15 per cent. Of course, this capacity alteration may be very significant, but we shall pass over it for the present in view of the change in the conducting aspect of the membrane.

Taking Blinks' (1930) value of 10^5 ohm cm.^{2} for the resting *Nitella* membrane, we find a 200-fold increase in the membrane conductance at the height of activity. Yet the maximum conductance, corresponding to 500 ohm cm.^{2} is far from a complete ionic permeability, which, if the resting resistance were not known, might seem to be a fairly high specific resistance when the large membrane capacity is taken as an indication of its thinness. The nearly intact capacity cannot be detected on Blinks' (1936) records because the active membrane time constant is less than 10^{-3} second and the difference between his initial and final resistances is about 0.5 per cent of the resting value. However, it seems unreasonable to picture the depolarization process during activity as a destruction or disintegration of the membrane, and one may well suppose that no more than 15 per cent of the membrane is involved.

In many of the cells and tissues which have been measured, the cell membranes have been found to have an impedance of the form $z_s = z_c(j\omega)^{-\alpha}$ (see page 56). This has been called a "polarization" impedance because this term has been applied to several types of non-living systems which have a similar but unexplained characteristic. Some of these systems are conductors and it was thought that a polarization impedance might be an expression of the ion permeability aspect of the membrane. Since a more easily acceptable measure of ion transport, *i.e.* the conductance, has been measured and its effect is probably so small as not to enter into the transverse impedance measurements of most resting cells and tissues, we are inclined to assume that the polarization impedance is essentially a dielectric impedance. The dielectric impedance is then to be

thought of as a characteristic of the ion impermeable aspect of the membrane and the constant α, and the phase angle ϕ, which is a measure of it ($\phi = \alpha\pi/2$ radians), are measures of the dielectric loss, which occurs when $\alpha < 1.0$. This loss, which might be caused by a frictional resistance to dipole rotation, is expressed as an electrical resistance, but it is not to be confused with a resistance which is an expression of ion transport across the membrane. Some time after a change, ΔV, of the potential difference across the membrane, there will be a change, ΔI, in the membrane current density and by Ohm's law, the membrane resistance for unit area is given by $\mathbf{r}_4 = \Delta V/\Delta I$. An ionic permeability of the membrane may then be defined as the number of ions transported across a unit area in unit time for a unit potential difference. The permeability on this definition is then proportional to $\Delta I/\Delta V = 1/\mathbf{r}_4$ or the membrane conductance. There will also be accumulations of electrical charge on the two sides of the membrane, $+\Delta Q$ and $-\Delta Q$ and by the definition of capacity $\Delta Q = \mathbf{c}_m \Delta V$, where $\mathbf{c}_m$ is the membrane capacity for a unit area (see Fig. 9). If now the ionic permeability be defined as the number of ions transported across the membrane in unit time for an ion pair separated by the membrane, this permeability is given by $\Delta I/\Delta Q = 1/\mathbf{r}_4\mathbf{c}_m$ which is also the reciprocal of the time constant of the membrane. Then for resting *Nitella* we find a permeability of ten ions per second for one ion pair separated by the membrane and for the maximum permeability during excitation, two thousand ions per second for one ion pair.

Action Potential.—The membrane polarization, which is made known by resting and action potentials, represents a source of electrical energy in the cell and it is preferable to speak of it and measure it, whenever possible, as an electromotive force (E.M.F.) which is a physically defined quantity. The resting and action potentials are the externally measured potential differences due to the external current (equation (14) and Fig. 12), and are not necessarily synonymous with the membrane E.M.F. Consequently if the "depolarization" be considered as a decrease in the membrane E.M.F., it is not directly measured by the external potential difference, but differs from it as indicated by equation (15) on p. 61.

It has been pointed out that the decrease in membrane resistance,

which is a "loss of polarizability," does not occur until rather late in the rising phase of the action potential, and that the reversal of the membrane current flow takes place at nearly the same point. It has also been found that the foot of the curve of the action potential up to the time of the resistance change and current reversal is nearly exponential—as it should be in front of a resting or propagated localized membrane short circuit (see equation (17)). Furthermore, preliminary experiments on the membrane resistance and potential difference at the site of sub-threshold and threshold stimuli also indicate a close parallelism between the resistance and E.M.F. For

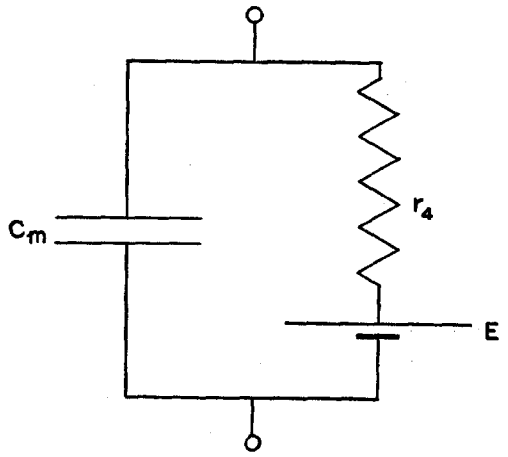

FIG. 9. Equivalent membrane circuit. c_m, r_4, and E are respectively the capacity, resistance, and E.M.F. of the membrane.

these reasons, we shall assume that the membrane resistance and E.M.F. are so intimately related that they should be considered as series elements in the hypothetical equivalent membrane circuit as shown in Fig. 9. These two elements may be just different aspects of the same membrane mechanism and the resistance may be, in part or entirely, the internal resistance of the E.M.F.

On the basis of this circuit the usual cable differential equation (equation (12)), can be set up, following Cremer (1899), Hermann (1905), and others, to describe the behavior of the fiber at any point. If the membrane E.M.F., E, is now considered as an unknown, we

should have all of the quantities necessary to calculate it. By assuming a resting membrane resistance (r_4, Fig. 9) somewhat lower than 10^5 ohm cm.^{2} the membrane E.M.F. remains nearly constant until the time of the resistance decrease, and it then follows the action potential quite closely. This is, however, merely another way of saying that the initial portion of the action potential is exponential, but to a certain extent it justifies the assumptions.

If then there is no evidence of membrane change in the initial portion of the action potential and the propagation depends upon such a change, the impulse cannot be said to have arrived. Although the conditions for the change in the E.M.F. and conductance of the membrane may have been partially achieved, the phenomenon is, up to this point, entirely passive and is to be identified with the "electrotonic spread," as shown by Bogue and Rosenberg (1934), and others. An impulse cannot be said to have passed a point or to be blocked until the membrane E.M.F. and resistance changes either have occurred or failed to take place.

It is apparent that the work to date has not shown whether excitation and propagation are possible with the assumed membrane and cell structure (*cf.* Rashevsky (1933), Rushton (1937)). This question can be answered as soon as we can set up the necessary and sufficient conditions for the E.M.F. and resistance changes to occur. One can think of many threshold conditions for this change—a current density, a transport of ions, a change of membrane potential difference, a change of membrane E.M.F., resistance, or capacity. Without a good experimental indication of which one, or combination, of these and perhaps other conditions is most important, we do not feel prepared to go into a further analysis of the processes of excitation, conduction, and recovery.

Theory

Bridge Unbalance.—When a voltage e_1 is applied to an equal ratio arm bridge having matched input and output impedances, it is found that near balance, the output voltage e_0 is given by

$$e_0 = \frac{1}{8}\frac{\dot{z} - z}{\dot{z} + z} e_1$$

where $\bar{z}$ and z are the known and unknown impedances. Owing to the failure to meet all of these conditions and the attenuation, amplification, and phase shift of the input and output systems, we have in general

$$E_0 = \mu \frac{\bar{z} - z}{\bar{z} + z} E_1$$

where E_1 and E_0 are the oscillator and amplifier output voltages respectively, and μ is the resultant complex amplification factor.

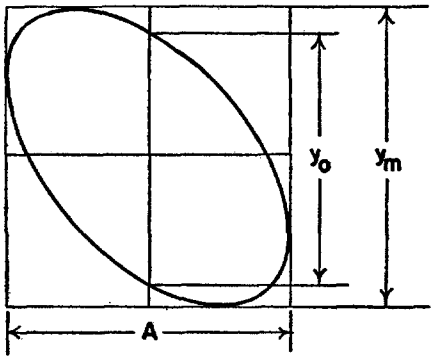

FIG. 10. Ellipse to show the measurements taken to compute the impedance changes.

Then

$$E_0 = \mu \bar{E}_1 \frac{\bar{z}z}{\bar{z} + z} \left[\frac{\bar{R}_p - R_p}{\bar{R}_p R_p} \cos \omega t + \omega(C_p - \bar{C}_p) \sin \omega t \right]$$

where $\bar{R}_p$, $\bar{C}_p$ are the parallel resistance and capacity at balance; R_p, C_p are the off-balance values, and $\bar{E}_1$ is the amplitude of the oscillator voltage. This output voltage, E_0, gives a vertical deflection on the cathode ray oscillograph which may be written, approximately,

$$y = \frac{A}{2} (l_1 \Delta R_p \cos \omega t + l_2 \Delta C_p \sin \omega t) \tag{1}$$

where ΔR_p, ΔC_p are the amounts by which the parallel resistance and capacity are off-balance, and A, l_1, and l_2 are obtained from the previous equation and the oscillograph sensitivity. In practice, the values of l_1 and l_2 are determined by direct calibration.

A portion of the oscillator output may be shifted in phase and amplitude so that it gives a horizontal deflection on the oscillograph

$$x = \frac{A}{2} \cos \omega t \tag{2}$$

We may then eliminate t between equations (1) and (2) and show that the oscillograph pattern is an ellipse which has a horizontal axis when $\Delta R_p = 0$ and degenerates into a straight line inclined to the horizontal when $\Delta C_p = 0$.

When $x = 0$, we have $y_0 = Al_2\Delta C_p$ as shown in Fig. 10. On the other hand the maximum value of y is given by

$$y_m = A\sqrt{(l_1\Delta R_p)^2 + (l_2\Delta C_p)^2}$$

Thus we have

$$\left.\begin{aligned} \Delta C_p &= \frac{y_0}{Al_2} \\ \text{and} \qquad \Delta R_p &= \frac{1}{Al_1}\sqrt{y_m^2 - y_0^2} \end{aligned}\right\} \tag{3}$$

which are the equations used for calculation.

Transverse Impedance.—The theoretical background of the impedance of suspensions of spheres and cylinders has been developed in a series of papers (Cole, 1928, 1932, 1937; Cole and Curtis, 1936; Bozler and Cole, 1935; Curtis and Cole, 1937) but will be briefly outlined here to give a basis for the extensions which are now necessary.

When the alternating current flow is perpendicular to the axis of a parallel suspension of circular cylindrical cells or to the axis of a single cylindrical cell in a nearly square cross-section of conducting medium, the specific transverse impedance, z, is given by

$$z = r_1 \frac{(1-\rho)r_1 + (1+\rho)(r_2 + z_m/a)}{(1+\rho)r_1 + (1-\rho)(r_2 + z_m/a)} \tag{4}$$

where r_1 and r_2 are the specific resistances of the external medium and cell interior, z_m is the impedance for a unit area of membrane, a is the radius of the cell, and ρ is its volume concentration in the medium.

The overall or gross impedance, z, is represented by its component series resistance and reactance, $z = r + jx$, which may be plotted as abscissa and ordinate to give the impedance locus as it varies with frequency.

Equation (4) has a well known form and many of its properties which are applicable here have been worked out in general terms (Campbell, 1911); but for simplicity a specific treatment will be followed.

When z_m is either zero or infinite, z becomes a pure resistance, r_∞ or $\bar{r}_0$. For all physically realizable values of z_m all possible values

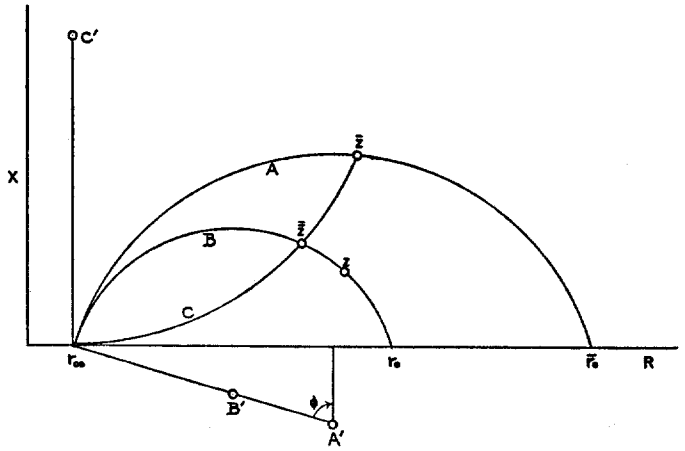

FIG. 11. Theoretical impedance loci, showing loci due to change of frequency, A and B, with centers at A' and B' and the locus C due to a change of membrane resistance, with center at C'.

of z lie on or within a circle having the segment of the r axis between r_∞ and $\bar{r}_0$ for a diameter.

It is further found by simple rearrangement that

$$z_m = a \cdot \frac{\bar{r}_0^2 - r_1^2}{\bar{r}_0 - r_\infty} \cdot \frac{z - r_\infty}{\bar{r}_0 - z} \tag{5}$$

This is a general expression which is convenient to use in special cases.

In many biological materials, z_m is a polarization or dielectric impedance, $z_m = z_3 = z_c\,(j\omega)^{-\alpha}$, where z_c is a constant, $j = \sqrt{-1}$, and ω is 2π times the frequency, and the impedance locus, as $\bar{z}$ or ω is varied, is a circular arc from r_∞ to $\bar{r}_0$ which subtends the angle

$2\phi = \alpha\pi$ at its center. When the membrane has a parallel leakage resistance, r_4, which is independent of frequency, $1/z_m = 1/z_3 + 1/r_4$, the value of r_∞ is unchanged but when z_3 is infinite, $z_m = r_4$ and $\bar{r}_0$ is decreased to another value r_0. Then as $\bar{z}$ or ω vary the impedance locus is a circular arc of angle 2ϕ from r_∞ to r_0, and the value r_4 may be calculated from equation (5) or

$$r_4 = a\frac{\bar{r}_0^2 - r_1^2}{\bar{r}_0 - r_\infty}\cdot\frac{r_0 - r_\infty}{\bar{r}_0 - r_0}. \tag{6}$$

Measurements at zero frequency where $z = r_0$ or $\bar{r}_0$ cannot verify the assumption that r_4 is independent of frequency, but when it can be shown that ϕ and r_∞ are unchanged, the value of r_0 corresponding to any value of z can be extrapolated graphically by a circular arc of angle 2ϕ through r_∞ and z. It is, however, possible to find the path which is followed by z when r_4 changes but z_3 and r_∞ remain constant, which is the membrane resistance locus at constant frequency and membrane capacity. If $\bar{z}$ is the value of z for some value of z_3 when r_4 is infinite, then equation (4) becomes

$$z = \frac{\sigma r_\infty + \bar{z}r_4/a}{\sigma + r_4/a} \tag{7}$$

where

$$\sigma = \frac{\bar{r}_0^2 - r_1^2}{(\bar{r}_0 - r_\infty)^2}(\bar{z} - r_\infty)$$

It is known that equation (7) is again a circle and that it passes through the points r_∞ and $\bar{z}$ but its center and radius must be found. Separating the real and imaginary parts, we have the two equations,

$$(\bar{r} - r)r_4/a = \bar{r}_0^2[(r - r_\infty)(\bar{r} - r_\infty) - x\bar{x}]/(\bar{r}_0 - r_\infty)^2$$

$$(\bar{x} - x)r_4/a = \bar{r}_0^2[(\bar{r} - r_\infty)x + (r - r_\infty)\bar{x}]/(\bar{r}_0 - r_\infty)^2$$

where r, x and $\bar{r}$, $\bar{x}$ are the components of z and $\bar{z}$. By eliminating r_4 we find

$$r^2 + x^2 - 2r_\infty r + [(\bar{r} - r_\infty)^2 + \bar{x}^2]x/\bar{x} + r_\infty^2 = 0 \tag{8}$$

which is the equation of a circle having its center at the point r_∞, $(\bar{r}^2 + \bar{x}^2)/2\bar{x}$ and the radius $(\bar{r}^2 + \bar{x}^2)/2\bar{x}$. This circular locus then passes through $\bar{z}$ and is tangent to the resistance axis at r_∞ as shown in Fig. 11.

When at constant frequency, z does not follow the locus required by a variation of r_4 only, z_3 must vary also, and when it can be shown that ϕ does not change, we need only the absolute value of the polarization or dielectric impedance $| z_3 |$. From equation (5) we find that when r_4 is infinite

$$| \bar{z}_3 | = a \cdot \frac{\bar{r}_0^2 - r_1^2}{\bar{r}_0 - r_\infty} \cdot \frac{\bar{u}}{\bar{v}}$$

where $\bar{u}$ and $\bar{v}$ are the lengths of the chords to $\bar{z}$ from r_∞ and $\bar{r}_0$ respectively. When r_4 is finite,

$$| z_3 | = a \frac{\bar{r}_0^2 - r_1^2}{\bar{r}_0 - r_\infty} \cdot \frac{r_0 - r_\infty}{\bar{r}_0 - r_\infty} \cdot \frac{u}{v}$$

where u and v are the chords to z from r_∞ and r_0. But if the point $\bar{z}$ is on the resistance locus as shown in Fig. 11, then we know that $| z_3 |$ has the value $| \bar{z}_3 |$ at this point so

$$| \bar{z}_3 | = a \cdot \frac{\bar{r}_0^2 - r_1^2}{\bar{r}_0 - r_\infty} \cdot \frac{r_0 - r_\infty}{\bar{r}_0 - r_\infty} \cdot \frac{\bar{\bar{u}}}{\bar{\bar{v}}}$$

where $\bar{\bar{u}}$ and $\bar{\bar{v}}$ are the chords to $\bar{\bar{z}}$ from r_∞ and r_0. We then have

$$| z_3 | / | \bar{z}_3 | = \frac{u}{v} \Big/ \frac{\bar{\bar{u}}}{\bar{\bar{v}}}.$$

The membrane capacity, $c = 1/ | z_3 | \omega \sin \phi$ and

$$c/\bar{c} = \frac{\bar{\bar{u}}}{\bar{\bar{v}}} \Big/ \frac{u}{v}. \tag{9}$$

There are other useful methods for the calculation of z_3 and r_4, as well as variations of these given and approximations which may be used judiciously.

Cable Theory.—A nerve fiber has been treated as a succession of circuit elements by Hermann (1905) and as the elements approach zero length this becomes the uniform cable which was investigated by Cremer (1899) and others, following Kelvin (1856). Although the results of the theory have been applied to whole nerves, the postulates should be much better represented by *Nitella*. There can be little question that the resting fiber has properties which

are described by such an equation and the equations are developed here for purposes of consolidation and consistency of nomenclature.

Let $\mathbf{r}_1$ and $\mathbf{r}_2$ be the resistance for a unit length of the medium, and the cell interior respectively, $\mathbf{z}_m$ the impedance between inside and outside for a unit length. Let i_1, i_2, and i_m be the external, internal, and membrane currents in the positive direction, and V_1 and V_2 be

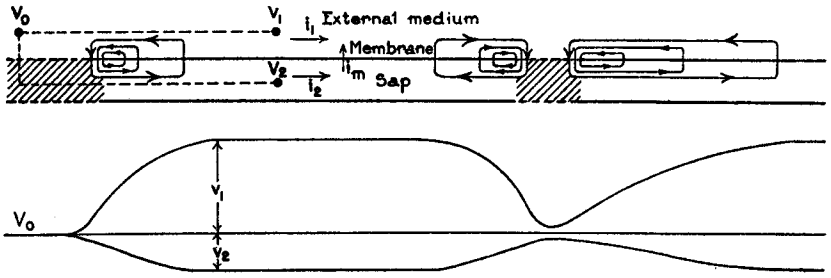

FIG. 12. Schematic drawing of *Nitella* cell to show currents and potentials in the neighborhood of a depolarized region and an inactive end at the left. i_1, i_2, and i_m are the external, internal, and membrane currents (for direction of arrows see p. 59) and the other arrows in the figure indicate the local circuit current flow. V_0 is the reference potential and V_1 and V_2 are the external and internal potentials.

the potentials outside and inside, as shown in Fig. 12. Then by Ohm's law

$$\frac{\partial V_1}{\partial x} = -\mathbf{r}_1 i_1; \quad \frac{\partial V_2}{\partial x} = -\mathbf{r}_2 i_2; \quad V_1 - V_2 = -\mathbf{z}_m i_m.$$

When there is no current flow to and from the system considered

$$i_1 + i_2 = 0 \quad \text{and} \quad \frac{\partial i_1}{\partial x} = -\frac{\partial i_2}{\partial x} = i_m.$$

Then

$$\frac{\partial^2 V}{\partial x^2} = -(\mathbf{r}_1 + \mathbf{r}_2) i_m \tag{10}$$

where $V_1 - V_2 = V$.

For the reasons given above, we shall assume an equivalent membrane circuit given in Fig. 9. The dielectric impedance of the mem-

brane has been found to have a phase angle of about 80°, but for an approximate analysis we shall replace it by the pure capacity $\mathbf{c}_m$ with a 90° phase angle.

Let

$$i_3 = -\mathbf{c}_m \frac{\partial V}{\partial t}; \qquad i_4 = \frac{E - V}{\mathbf{r}_4} \tag{11}$$

and

$$i_m = i_3 + i_4 = -\mathbf{c}_m \frac{\partial V}{\partial t} + \frac{E - V}{\mathbf{r}_4}$$

Substituting

$$\frac{\partial^2 V}{\partial x^2} = -\frac{\mathbf{r}_1 + \mathbf{r}_2}{\mathbf{r}_4}\left[E - V - \mathbf{r}_4\, \mathbf{c}_m \frac{\partial V}{\partial t}\right] \tag{12}$$

and when we let $\lambda = \sqrt{\mathbf{r}_4/(\mathbf{r}_1 + \mathbf{r}_2)}$, the characteristic length, and $\tau_m = \mathbf{r}_4\, \mathbf{c}_m$, the membrane time constant,

$$\lambda^2 \frac{\partial^2 V}{\partial x^2} - \tau_m \frac{\partial V}{\partial t} - V = -E \tag{13}$$

This is now the general equation relating all of the assumed characteristics of the cell as functions of space and time.

But V cannot be easily measured directly and the usual approach is by the inactive end technique. Choosing an electrode at $x = 0$ on the inactive end as the point of zero potential for V_1 and V_2, then the externally measured potential V_1 at an uninjured point x is given by

$$V_1(x) = \int_0^x \frac{\partial V_1}{\partial x}\, dx = -\mathbf{r}_1 \int_0^x i_1\, dx \tag{14}$$

In other words, the resting or injury potential is caused entirely by local current flow at the junction of the injured and uninjured regions. Assuming that the injury is of such a kind that the membrane potential is abolished, then there is no potential difference across the membrane at $x = 0$, and the potential inside the fiber V_2 at x is

$$V_2(x) = \int_0^x \frac{\partial V_2}{\partial x}\, dx = -\mathbf{r}_2 \int_0^x i_2\, dx = -\frac{\mathbf{r}_2}{\mathbf{r}_1} V_1(x),$$

since $i_1 + i_2 = 0$

Thus

$$V = V_1(x) - V_2(x) = \left(1 + \frac{r_2}{r_1}\right) V_1(x) = \kappa V_1(x) \tag{15}$$

We may now define a reduced E_1 by $E = \kappa E_1$ and drop the subscripts to obtain the same equations, where V is now the measured "monophasic" potential.

Once the impulse has been initiated and is traveling with a uniform velocity, v, we may refer it to a space or time coordinate system whose origin travels with the same velocity. Since the length of an impulse is of the order of a centimeter for velocities from a centimeter to a hundred meters per second, a space coordinate is to be preferred where $y = x - vt$.

Then

$$\frac{\partial V}{\partial x} = \frac{dV}{dy}; \quad \frac{\partial^2 V}{\partial x^2} = \frac{d^2 V}{dy^2} \quad \text{and} \quad \frac{\partial V}{\partial t} = -v \frac{dV}{dy}$$

and we have

$$\lambda^2 \frac{d^2 V}{dy^2} + v\tau_m \frac{dV}{dy} - V = -E$$

which may be rewritten for calculation,

$$E = V - \beta\bar{\lambda}^2 \left[\frac{d^2 V}{dy^2} + \frac{1}{\lambda_0} \frac{dV}{dy}\right] \tag{16}$$

where $\beta = r_4/\bar{r}_4$, $\bar{r}_4$ is the membrane resistance at rest, and $\lambda_0 = 1/v(r_1 + r_2)c_m$, the characteristic length for a non-conducting membrane. $\bar{\lambda} = \sqrt{\bar{r}_4/(r_1 + r_2)}$ is the characteristic length due to membrane conductance while λ_0 is the characteristic length due to the membrane capacity.

When we propagate a localized short circuit, so that E and r_4 have their resting values at all points except $y = 0$, where they are both zero, and $V = E - e$

$$\bar{\lambda}^2 \frac{d^2 e}{dy^2} + v\tau_m \frac{de}{dy} - e = 0.$$

Since $\bar{\lambda}$ and λ_0 are constant except at $y = 0$,

$$e = e_0 \exp(-y/\lambda_a). \tag{17}$$

Thus outside of the breakdown the action potential should be of exponential form with a characteristic length

$$\lambda_a = \frac{\bar{\lambda}}{\frac{v\tau_m}{2\bar{\lambda}} \pm \sqrt{1 + \frac{v^2\tau_m^2}{4\bar{\lambda}^2}}}$$

using the positive sign for $y>0$ which is ahead of the short circuit and the negative sign for $y<0$ which is behind it. If the velocity v is small as for *Nitella*, then $\lambda \gg \lambda_0$ and $\lambda_a = \lambda \pm \frac{v\tau_m}{2}$, approaching $\bar{\lambda}$, the resting characteristic length. For a high velocity $\bar{\lambda} \ll v\tau_m$, λ_a ahead approaches λ_0, which is independent of r_4, but behind becomes $v\tau_m$.

CONCLUSION

The transverse impedance measurements over a range of frequencies indicate that the impedance changes observed during activity are due entirely to changes in the membrane characteristics. By an extension of the transverse impedance theory it can be shown that, while there is no measurable change in the membrane phase angle (see Figs. 4 and 5), the membrane capacity decreases at the height of activity about 15 per cent below its resting value of 0.9 μf./cm.^{2} As compared with this, the change of the membrane resistance from a probable value of 10^5 ohm cm.^{2} to an average value of 500 ohm cm.^{2} at the height of activity is very great (see Figs. 6 and 8). This is taken as additional evidence that the capacity represents the non-conducting aspect of the membrane and that the phase angle difference from 90° is a measure of the dielectric loss of the membrane, while the parallel membrane resistance is the conducting or ion permeable aspect. In the processes of excitation, conduction, and recovery, these two aspects seem to be quite independent of each other, and the non-conducting aspect is very little affected.

It has been found that the membrane conductance increases very rapidly late in the rising phase of the action potential at nearly the same time that the direction of the current flow across the membrane changes from outward to inward (see Figs. 6 and 8). For this reason, we assume that the membrane E.M.F. and resistance are closely related, the latter being perhaps the internal resistance of the E.M.F.

This structure is then considered to be in parallel with the relatively inactive membrane capacity. On this basis the membrane E.M.F. is found to decrease sharply at nearly the time the conductance increases. It is then this sudden change of the E.M.F. and conductance which is the basis of the all-or-none nature of the propagated activity. Although the conditions for this change may be partially satisfied before it occurs, we must consider the properties to be those of a resting cell and the part of the action potential ahead of the change to be a purely passive propagation. With these definite measurements of the time, course, and nature of the membrane changes and with an extension of the present technique, it should be possible to establish the necessary and sufficient conditions for the change, and so be a step closer to the understanding of the all-or-none law.

SUMMARY

The changes in the alternating current impedance which occur during activity of cells of the fresh water plant *Nitella* have been measured with the current flow normal to the cell axis, at eight frequencies from 0.05 to 20 kilocycles per second, and with simultaneous records of the action potential under the impedance electrodes. At each frequency the resting cell was balanced in a Wheatstone bridge with a cathode ray oscillograph, and after electrical stimulation at one end of the cell, the changes in the complex impedance were determined from the bridge unbalance recorded by motion pictures of the oscillograph figure. An extension of the previous technique of interpretation of the transverse impedance shows that the normal membrane capacity of 0.9 μf./cm.^{2} decreases about 15 per cent without change of phase angle, while the membrane resistance decreases from 10^5 ohm cm.^{2} to about 500 ohm cm.^{2} during the passage of the excitation wave. This membrane change occurs during the latter part of the rising phase of the action potential, and it is shown that the membrane electromotive force remains unchanged until nearly the same time. The part of the action potential preceding these membrane changes is probably a passive fall of potential ahead of a partial short circuit.

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BIBLIOGRAPHY

Blinks, L. R., 1930, *J. Gen. Physiol.*, **13,** 495; 1936, **20,** 229.

Bogue, J. Y., and Rosenberg, H., 1934, *J. Physiol.*, **82,** 353.

Bozler, E., and Cole, K. S., 1935, *J. Cell. and Comp. Physiol.*, **6,** 229.

Campbell, G. A., 1911, *Tr. Am. Inst. Elect. Eng.*, **30,** 873.

Cole, K. S., 1928, *J. Gen. Physiol.*, **12,** 29; 1932, **15,** 641. 1933, Electric conductance of biological systems, in Cold Spring Harbor symposia on quantitative biology, Cold Spring Harbor, Long Island Biological Association, **1,** 107. 1937, *Tr. Faraday Soc.*, **33,** 966.

Cole, K. S., and Curtis, H. J., 1936, Electric impedance of nerve and muscle, in Cold Spring Harbor symposia on quantitative biology, Cold Spring Harbor, Long Island Biological Association, **4,** 73. 1937, *Rev. Scient. Instr.*, **8,** 333.

Cremer, M., 1899, *Z. Biol.*, **37,** 550.

Curtis, H. J., and Cole, K. S., 1937, *J. Gen. Physiol.*, **21,** 189; 1938, **21,** 757.

Fricke, H., and Curtis, H. J., 1935, *J. Gen. Physiol.*, **18,** 821.

Hermann, L., 1905, *Arch. ges. Physiol.*, **109,** 95.

Kelvin, Lord (William Thomson), 1856, *Phil. Mag.*, **11,** series 4, 146.

Lullies, H., 1930, *Arch. ges. Physiol.*, **225,** 87.

Osterhout, W. J. V., 1934, *J. Gen. Physiol.*, **18,** 215.

Osterhout, W. J. V., and Hill, S. E., 1935, *J. Gen. Physiol.*, **18,** 499.

Rashevsky, N., 1933, *Physics*, **4,** 341.

Rosenberg, H., and Schnauder, F., 1923, *Z. Biol.*, **78,** 175.

Rushton, W. A. H., 1937, *Proc. Roy. Soc. London, Series B*, **124,** 210.