TRANSVERSE ELECTRIC IMPEDANCE OF NITELLA*

By HOWARD J. CURTIS and KENNETH S. COLE

(From the Department of Physiology, College of Physicians and Surgeons, Columbia University, New York)

(Accepted for publication, August 2, 1937)

The large single cells of *Nitella* are very favorable material for the study of electrical characteristics. They can be found 5 cm. or more long and about 0.5 mm. in diameter. The single cell has a thin protoplasmic layer between the cellulose cell wall and the central vacuole. Presumably the bulk of the protoplasm is a relatively good electrical conductor but both the inner and outer protoplasmic surfaces are selectively permeable and each has a high electrical resistance and capacity. It has not been possible to differentiate the electrical properties of these two surfaces, so for simplicity, combined effects of both may be called the membrane resistance, capacity, or impedance.

Blinks (1930) measured the direct current resistances of living and dead cells between two electrodes separated different distances along the length of the cells, in air or a moist chamber. An average membrane resistance of 250,000 ohm cm.^{2} was found, and an approximate specific resistance of 87 ohm cm. for the vacuolar sap is obtained from his results. From the time constants of the charge and discharge curves in this and a later paper (Blinks, 1936) he estimated a membrane capacity of 1.0 μf./cm.^{2} which he suspected to be a polarization capacity.

In such longitudinal measurements, the paths of current flow are partly across the cell membrane and partly along the length of the cell, but they have been expressed with some success by the core-conductor theory (Cremer, 1899; Hermann, 1905; Labes, 1932). Although the results of the theory have been applied to nerve, the postulates should be much better represented by *Nitella*. Blinks'

* Aided by a grant from The Rockefeller Foundation.

data for the direct current resistance agree quite well with this model and lead with considerable certainty to substantially the values for membrane and sap resistance which he obtained by a simpler analysis. The membrane capacity may be calculated from the transients at the make and break of the measuring current, but this is somewhat complicated and unsatisfactory. The theory predicts that the form is given by the error integral which may differ from a simple exponential by no more than 10 per cent and this is a rather small margin in the analysis of oscillograph records.

As is well known, the transient and steady state properties are fundamentally identical (*cf.* Cole and Curtis, 1936). Since *Nitella* cells can be kept constant for hours at a time, considerably more accurate steady state alternating current impedance measurements over a range of frequencies should be possible.

The analysis of the longitudinal impedance of a single fiber (Cole and Curtis, 1936) may be extended and simplified when the electrodes are narrow and sufficiently widely separated, and the cell membrane has a finite resistance and a static capacity. In this case, from preliminary measurements at the Biological Laboratory, Cold Spring Harbor, Long Island, and Blinks' data, it was apparent that the maximum reactance would be at about 30 cycles per second. It would then be necessary to make measurements at 3 cycles per second and advantageous to go even lower. Although our apparatus is now equipped to go down to 30 cycles per second, it is not expedient at present to extend the range in this direction when an alternative type of measurement is available.

In transverse measurements, where the current flow between the two parallel electrodes is everywhere perpendicular to the axes of a suspension of parallel uniform fibers, the current distribution in and around the cells is relatively simple, the important frequencies are considerably higher and in a more convenient range than for the longitudinal measurements.

Transverse Impedance Theory

The impedance equation in this case (Bozler and Cole, 1935) is analogous to the Maxwell equation for a suspension of spheres,

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

where r_1 is the specific resistance of the suspending medium, z, the specific impedance of the suspension, z_2 the equivalent specific impedance of the fibers, and ρ is their volume concentration. The circuit which is equivalent to the suspension consists of two resistances and an element whose impedance depends upon the frequency (Cole, 1928, 1932). In the derivation of this equation, a random distribution of the fibers is assumed (Cole and Curtis, 1936) but for *Nitella* it is desirable and possible to work with a single fiber. It is easily shown that the impedance of a single cylindrical cell symmetrically placed with respect to the electrodes is the same as a suspension of such cells in a rectangular array. This case has been worked out by Rayleigh (1895) who found equation (1) as an approximate solution. When the electrode separation is equal to the electrode width, the equivalent array is square and it can be shown that for a volume concentration of 0.5 or less a maximum error of 1 per cent will be made by the use of equation (1) rather than the second approximation given by Rayleigh. The theory for a measuring cell of rectangular cross-section is more involved, but when the ratio of the dimensions is not far from unity the limiting volume concentration is only slightly reduced.

On the basis of the Rayleigh analysis, we shall use equation (1) and others which are derived either from it, or in a similar manner.

EXPERIMENTAL

Nitella flexilis was purchased from a tropical fish dealer and kept in aquaria with gravel and goldfish until used. Some time before measurements were to be made, a suitable cell about 0.45 mm. in diameter was cut loose from the adjoining cells, transferred to the measuring cell, and allowed to equilibrate. It was often found that slow, apparently spontaneous, fluctuations of the low frequency impedance would occur for long periods with an otherwise normal resting cell.

Although this effect could result from a change of membrane resistance, it seems more probable that it was due to a salt transfer since it nearly disappeared when the external medium was flowed continually. The cells could be maintained in the measuring cell in good condition with vigorous protoplasmic streaming for as long a period as desired.

To obtain satisfactory transverse impedance measurements on a single *Nitella* cell, it was necessary to use considerable care in the design and construction of the measuring cell. Cells of the type shown in Fig. 1, which were built up of glass with de Khotinsky cement were the most satisfactory.

The two electrodes of platinized platinum were 1.3 cm. long and inlaid into

opposite sides of the groove into which the center portion of a *Nitella* cell was placed. This groove was 0.5 mm. by 0.7 mm. and 3 cm. long, with depressions at each end which accommodated the cut ends of neighboring cells.

The top surface of the cell was polished flat and was covered with a flat glass plate after the *Nitella* cell was in place. The electrolytes used were flowed continually from a beaker into one end of the groove, through the measuring cell, and out at the other end by capillary glass siphons. It was possible to change the electrolyte concentration and reach the new equilibrium in about 5 minutes, and the impedance then remained stable and cell environment was known and constant.

The alternating current Wheatstone bridge used and the method of making the measurements is described in detail elsewhere (Cole and Curtis, 1937). The current through the measuring cell was so small that the impedance was independent of it. The impedance was measured as parallel resistance, R_p, and capacity, C_p, at sixteen frequencies from 30 cycles per second to 2.5 megacycles per second.

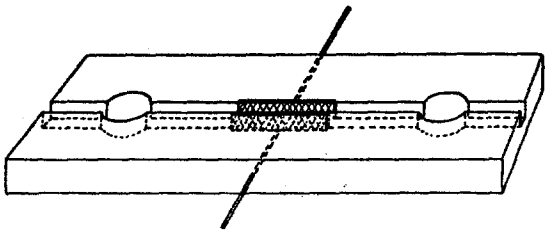

FIG. 1. Measuring cell for transverse impedance of a single *Nitella* cell.

At the lower frequencies it was necessary to make corrections, which were usually small, for the polarization impedance of electrodes.

The data were calculated[1] and plotted with series resistance, R_s, and reactance, X_s as abscissae and ordinates to give the impedance locus.

Data and Calculation

When a *Nitella* cell, suspended in an electrolyte, is equivalent to a circuit containing two resistances and a variable impedance element, the impedance locus is a circular arc, and the phase angle of the variable impedance element is half the angle subtended by lines drawn

[1] The formulae are

$$R_s = \frac{R_p}{1 + (R_p C_p \omega)^2}; \qquad X_s = \frac{R_p^2 C_p \omega}{1 + (R_p C_p \omega)^2}$$

where ω is 2π times the frequency in cycles per second.

from the two intersections of this curve with the resistance axis to the center of the circle (Cole, 1928). It is seen by the data for a *Nitella* cell which is plotted in Fig. 2, that such a circuit is a close approximation over most of the frequency range. The phase angle in this case is 77° and the average value which has been found for *Nitella* is 80° ± 4°.

If a *Nitella* cell consists simply of a non-conducting membrane surrounding an electrolyte, the volume concentration, ρ, of the cell sus-

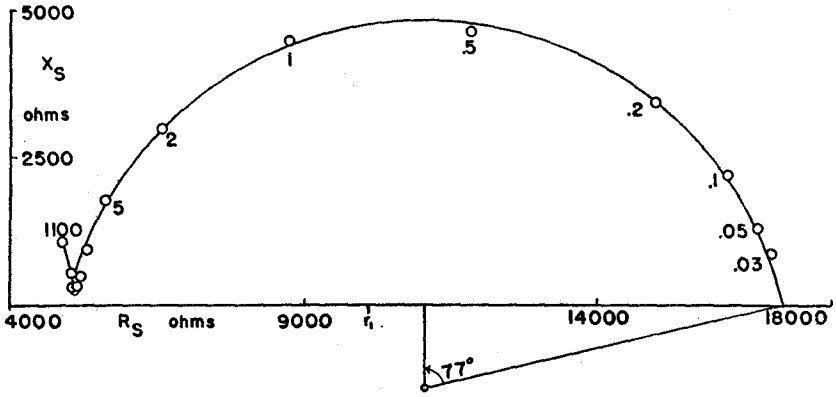

FIG. 2. Impedance locus, series resistance R_s *vs.* series reactance X_s, for a *Nitella* cell in tap water. Frequencies are given in kilocycles per second.

pended in an electrolyte of resistivity r_1 is given by (Bozler and Cole, 1935).

$$\frac{1 - r_1/r_0}{1 + r_1/r_0} = \rho \tag{2}$$

where r_0 is the low frequency resistivity of the suspension. When this equation was applied to the data of *Nitella*, however, it was found that computed values of ρ were always markedly lower than those obtained from the inner diameter of the cell wall. The most obvious explanation of this would be that the membrane is not non-conducting, but has a finite and measurable resistance. On this hypothesis the membrane resistance would have an improbable value of about 1 ohm cm.^{2} which would depend on the medium. There is, however, another explanation which is worth investigating.

Cellulose and Membrane Resistance

The *Nitella* cell is surrounded by a layer of cellulose which has been thought of as being completely permeable and therefore having a resistivity equal or at least proportional to the resistivity of the suspending medium. If, however, the cellulose wall had a resistivity independent of the suspending medium, it would be possible to explain the discrepancy in volume concentration measurements. To test this hypothesis, and at the same time obtain the other electrical constants of the cell, runs were taken in the following manner. A cell which had been carefully selected for measurement was placed in the measuring cell and tap water circulated around it. When it had become equilibrated, the parallel resistance and capacity were measured over the entire frequency range. Without disturbing the cell, 1 per cent sea water was substituted for the tap water, the procedure repeated, and again for 10 per cent sea water. The *Nitella* cell was then removed and its ends cut off. A glass rod was drawn out to approximately the internal diameter of the cellulose wall and several feet long. Such rods are always slightly tapered, and, starting at the small end, this cellulose tube was pushed as far as possible under water toward the large end. The rod was then broken at each end of the tube, placed in the measuring cell, and complete frequency runs taken for the three electrolytes. Then that portion of the tube between the electrodes was carefully cut away from the glass rod and the procedure repeated. Finally the glass rod was removed and the process repeated on the three electrolytes alone. The entire series could be completed in about 10 hours.

Table I gives specific resistances obtained from such a run. Those for the living cell are the extrapolated zero frequency values. The resistances for the suspending medium and for the uncovered glass rod were independent of the frequency and the values for the covered glass rod were nearly so. It will be noticed that for the high resistance medium the resistance with the glass rod is lower when it has the cellulose wall over it than when it is by itself, and that for the low resistance medium the opposite is the case. This in itself shows that the resistivity of the cellulose wall is neither the same as the resistivity of the suspending fluid nor even proportional to it. Comparing the resistances for the live cell and the glass-filled cell, we notice that the

former are higher in every case. This might be taken as an indication that the membrane and cell interior had a higher equivalent resistance than glass, but it seems more probable that either the cellulose fits more tightly over the living cell than over the glass rod, or better still that volume concentration, ρ, is greater in the live cell due to the turgor pressure. But from these data it is possible to assign a value to the resistivity of the cellulose.

By a method similar to that used previously for muscle (Cole and Curtis, 1936), assuming that the thickness of the cellulose, δ, is small

TABLE I

Specific Resistances in Ohm Cm. As Explained in Text

	Tap water	1 per cent sea water	10 per cent sea water
Suspending medium	9,240	1,578	180
Live cell	15,500	4,000	545
Glass rod			
Cellulose on	13,670	3,300	440
Cellulose off	20,620	3,520	397.5

in comparison to the radius, a, of the glass rod, the following formula for the cellulose resistivity, r_4, may be derived,

$$r_4 = r_1 \frac{(1 + 2\delta/a)(r_0' - r_1)(r_0 + r_1) + (r_0 - r_1)(r_0' + r_1)}{(1 + 2\delta/a)(r_0' - r_1)(r_0 + r_1) - (r_0 - r_1)(r_0' + r_1)} \cdot \frac{a}{a + \delta}$$

where r_0 and r_0' are the low frequency resistivities of the glass rod suspension with and without the cellulose wall respectively. Since we must consider both r_4 and δ as unknowns, measurements in a single medium can only define a relation between them. There is then a single value of r_4 corresponding to each possible value of δ which gives a curve when r_4 is plotted against δ. For the different media, the three curves are found as shown in Fig. 3 and if the cellulose resistance and thickness are independent of the medium, these curves should intersect at a single point. The center of the circle tangent to the three curves gives 1150 ohm cm. for the resistance and 12.8 μ for the thickness which best fit all three sets of data. Thus the cellulose resistance was so slightly affected when the conductivity of the medium was changed by a factor of more than 50, that we may

consider it to be substantially independent of these media. Direct measurements indicated that the thickness of the cellulose wall was about 10μ. Although we have not taken accurate direct thickness measurements, those that we have agree quite well with the computed values and give about 10μ for the mature cells. In one young cell the thickness obtained from the electrical measurements was 4μ while the directly measured value was 5μ. This general agreement may be

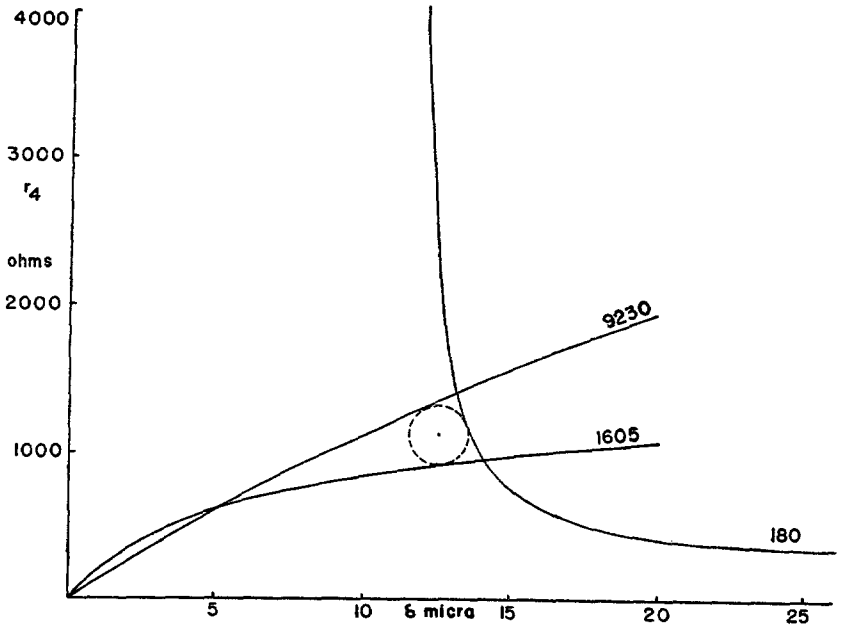

FIG. 3. Thickness δ *vs.* specific resistance of cellulose sheath r_4 from measurements in three media having specific resistances indicated.

taken as further support for the assumption of a constant cellulose wall resistance.

Turning now to the low frequency measurements of the intact cell, we prefer to delay making an assumption of the membrane resistivity and to calculate the equivalent resistivity, r'_2, which a cell representing a volume concentration, ρ, would have if it were a homogeneous conductor. In Fig. 4 the same type of graphical simultaneous solution used above gives values of $r'_2 = 27{,}000$ ohm cm. and $\rho = 50.2$ per cent.

Since a value of $\rho = 48$ per cent was estimated from the over-all cell diameter, we have some reason to consider the value of r_2' seriously. It may be noted that the intercepts of the curves of Fig. 4 on the horizontal axis are the values of volume concentration which are calculated by equation (2) if the cellulose is ignored and the protoplasmic surface assumed to be non-conducting. Also the volume

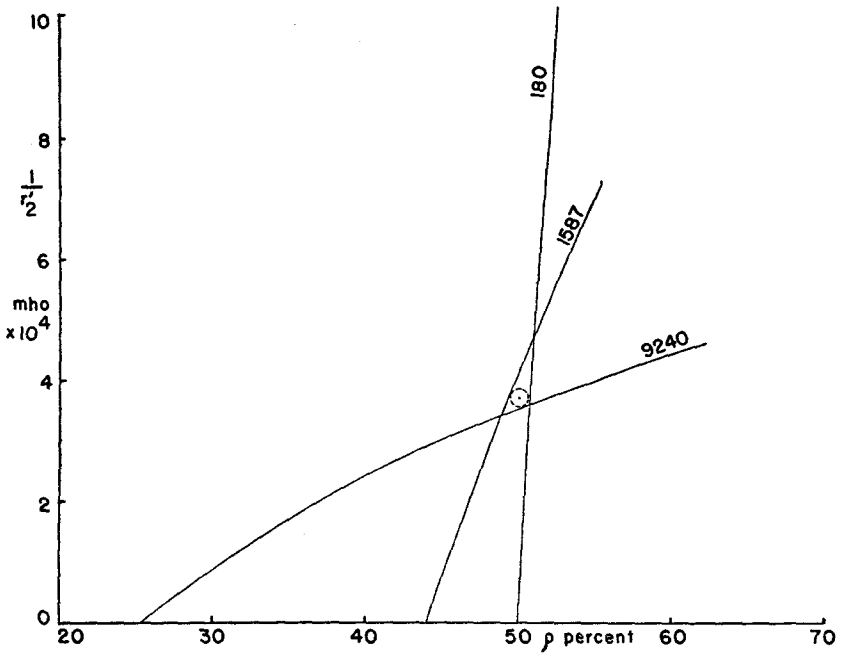

FIG. 4. Volume concentration ρ *vs.* equivalent low frequency conductance $1/r'_2$ of *Nitella* from low frequency measurements in three media having specific resistances indicated.

concentration of the glass-filled cellulose was found to be 42 per cent, which suggests that the difference between its resistance here and when it is covering the live cell is due to turgor.

If we now assume that the membrane is, within the limits of experimental accuracy, non-conducting, we have (Cole and Curtis, 1936),

$$\frac{1 - r_4/r_2'}{1 + r_4/r_2'} = \frac{a_1^2}{a_2^2}$$

where r_4 is the resistivity of the cellulose wall and a_1 and a_2 are the inner and outer radii respectively. Substituting the values of r_2 above and r_4 from the glass rod data, we find $\delta = a_2 - a_1 = 8.5\mu$, which is somewhat less than the previous value. But we may say that within our experimental limits, the membrane may be considered non-conducting.

Membrane Capacity and Internal Resistance

In previous work, the membrane capacities have been calculated at the characteristic frequency, for which the series reactance is a maximum, or better, where the series resistance takes on the mean value of the extrapolated zero and infinite frequency resistances (see Cole and Curtis, 1936). In the case of *Nitella*, the cellulose is a nuisance in that we have been unable to evolve any but elaborate, and tedious, theory and calculations for the determination of the capacity and internal resistance.

The characteristic frequencies range from 0.63 kilocycles per second for the dilute medium to about 16 kilocycles per second for the most concentrated, and in spite of the difficulties, it is rather remarkable that the capacities are constant at 0.94μf./cm.^{2} to within ± 10 per cent at the respective frequencies for the different media.

We have not been so fortunate in the determination of the internal specific resistance in the living cell. By a procedure consistent with that used in the calculation of the capacities, we obtain from 95 ohm cm. for the concentrated media to 1800 ohm cm. for the dilute media. It was to be expected that this resistivity would be relatively independent of the medium. Measurements on sap extracted from cells which had been soaking in the three media gave 58 ohm cm. $\pm$ 8 per cent, with no correlation with the medium resistance.

The internal resistances were calculated on the basis of the only infinite frequency extrapolations which could be made, as is shown in Fig. 2, but it is seen that there are indications of structures with a characteristic frequency above the present range. These probably cannot be invoked to extricate us from our present resistance difficulties, but preliminary measurements suggest that they may be the chloroplasts.

Chloroplasts

In order to investigate the effect of the chloroplasts, runs were taken on a cell with both ends cut off in the three media, and again after the chloroplasts have been washed out. The results were not particularly satisfactory because the change in resistance with frequency was very small, but it did show that the chloroplasts (or perhaps pieces of protoplasm adhering to the cell wall) have a resistance and capacity which is a function of the frequency. An effort was made to isolate the chloroplasts and measure their electrical properties independently in a measuring cell which holds 0.005 cc. of suspension. A satisfactory suspension of chloroplasts has not yet been obtained, but preliminary results agree with the dead cell data, and indicate that the impedance properties of the chloroplasts are similar to those of living cells. However, more definite conclusions will have to await further measurements.

DISCUSSION

The assumptions that *Nitella* has a non-conducting membrane, and that the cellulose wall has a resistance which is independent of the suspending medium seem to have worked out rather well. The data are consistent with them and furthermore lead to approximately correct values of the wall thickness. Blinks' longitudinal data on dead cells which have been filled with air are interesting in this regard. Assuming that the walls of his cells were 10μ thick, we compute a value of 850 ohm cm. for the specific resistance of the cellulose, which agrees quite well with our values. It seems premature to speculate about the nature of the conductance in cellulose on the basis of the present measurements, but we hope in the near future to check this behavior of cellulose in material taken from other sources.

The average membrane capacity of 0.94 μf./cm.^{2} which was obtained on these assumptions seems reasonable. It is very nearly the same as has previously been found for a wide variety of different cells, and confirms Blinks' estimate of 1 μf./cm.^{2} The average phase angle of this capacity is 80° which indicates that it is a polarization impedance only slightly removed from a static capacity.

It was suggested in the work on nerve and muscle (Cole and Curtis, 1936) that an equivalent polarization impedance element could result

from a statistical distribution of membrane static capacities and cell diameters. Further calculations on this postulate have indicated that it alone is probably not adequate, and it has not yet been possible to check it for nerve or muscle by measurements on single fibers. The present *Nitella* polarization impedance might be interpreted as a small variation of a membrane static capacity from point to point over the surface of the cell and while this is not an unreasonable picture, it is not particularly attractive.

The value of the resistivity of the sap which was measured directly on sap squeezed from the cells is probably substantially correct. In a similar manner, Hoagland and Davis (1923) obtained 89 ohm cm. Calculations made from Blinks' (1930) data for dead cells give a value of 87 ohm cm., and the same value is obtained from his transient measurements on live cells taken at very short times. Preliminary data on chloroplasts show that they undoubtedly play some part in the calculations of the resistivity of the sap from our high frequency measurements on the live cell, and on the face of it, it does not seem as though they could account for the large discrepancies observed. However, one could assume that they were packed very tightly around the inside of the living cell, being held firmly in place by the turgor pressure. Then if the turgor pressure were released by placing the cells in a strong salt solution or by killing them, the chloroplasts would not be packed nearly so tightly and would not exert nearly as much influence on the measurements. Since Blinks took his measurements with the current flow primarily parallel to the cell axis instead of perpendicular to it, he would not be troubled by this postulated phenomenon. This would explain why his results on the living cell check the direct measurements and ours do not, and explain why our measurements taken in concentrated media come quite close to the correct value whereas the ones taken in dilute media do not. However, in following out this reasoning one runs into difficulties with the capacity values, so that further speculation can be indulged in profitably only after the impedance properties of chloroplasts are better known.

It is interesting to observe that transverse measurements cannot hope to detect a membrane resistance as high as that observed by Blinks in longitudinal measurements. The problem is essentially

that of measuring the volume enclosed by the membrane with an accuracy which is impractical at present, but has been discussed in connection with marine egg measurements (Cole, 1937).

SUMMARY

Alternating current measurements have been taken on single *Nitella* cells over a frequency range from 30 to 2,500,000 cycles per second with the current flow perpendicular to the axis of the cell. The measuring cells were so constructed that electrolytes of any desired concentration could be circulated during the course of the measurements. The cellulose wall which surrounds the cell is found to play an important part in the interpretation of the results obtained. In a mature cell, this cellulose has a specific resistance of about 1000 ohm cm. which is independent of the medium in which the cell is suspended. The thickness of the wall is computed to be about 10 μ. The cell membrane is found to be virtually non-conducting, and to have a capacity of 0.94 μf./cm.^{2} $\pm$ 10 per cent and a phase angle of 80° $\pm$ 4°.

The specific resistances of the sap were difficult to compute from data on living cells and were unsatisfactory because they were very much dependent upon the medium, while measurements on extracted sap gave 58 ohm cm. $\pm$ 8 per cent which was independent of the medium. There are indications that the chloroplasts have impedance properties similar to those of living cells.

We are indebted to Mr. J. M. Spencer for his assistance.

REFERENCES

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

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

Cole, K. S., 1928, *J. Gen. Physiol.*, **12**, 29; 1932, **15**, 641. 1937, *Tr. Faraday Soc.*, **33**, 965.

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.

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

Hoagland, D. R., and Davis, A. R., 1923, *J. Gen. Physiol.*, **6**, 47.

Labes, R., 1932, *Z. Biol.*, **94**, 191.

Rayleigh, J. W. S., 1895, *Phil. Mag.*, **34**, 481.