THE ELECTRIC PROPERTIES OF PLANT CELL MEMBRANES

I. THE ELECTRIC CAPACITANCE OF SUSPENSIONS OF MITOCHONDRIA, CHLOROPLASTS, AND CHLORELLA SP.

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Summary

The electric capacitance of the membranes of isolated mitochondria and chloroplasts and of *Chlorella* cells has been calculated from measurements on suspensions at frequencies from 1 kc/s to 4 Mc/s in an alternating current bridge of special design.

The capacitance per unit area is of the order of 1 μF/cm^2, similar to that of numerous animal cell membranes (nerve, red blood cell, etc.). The phase angle of the impedance representing the membrane dielectric is constant over a wide frequency range, for a given suspension, but the capacitance and dielectric loss both vary with frequency. The phase angle is less (the dielectric more "lossy") for isolated cell particles than for intact cells. Dielectric constants were calculated using measured specific capacitance and membrane thicknesses obtained from electron micrographs of chloroplasts and mitochondria. At a frequency of 1 kc/s the dielectric constant was 13 (chloroplasts) and 54 (mitochondria). The significance of the phase angle obtained in these experiments is discussed relative to Cole's (1949) suggestion that the phase angle may be related to the degree of dipole interaction in the membrane dielectric.

I. INTRODUCTION

Since the classical work on the electric resistance and capacitance of cells and cell suspensions was carried out by Fricke (1924, 1925) and later by Cole and his collaborators (see reviews by Cole 1940, 1942, 1949), little has been done to interpret membrane structure in terms of the dielectric properties. Comparatively little has been done to compare plant cell membrane properties with those of the nerve, red blood cell, marine egg, etc., which the earlier experimenters used.

Curtis and Cole (1937) examined the transverse alternating current characteristics of the *Nitella* membrane but obtained inconclusive results for the internal (sap) resistivity, suggesting that a further analysis of this cell as an electrical system is necessary. Umrath (1942) reinterpreted these results in the light of a modified theory which needs careful examination. Further, the recent results of Bennett and Rideal (1954) differ in the capacitance values, measured directly across the membrane(s), by more than an order of magnitude from those obtained by Curtis and Cole (1937) with external electrodes. These aspects are the subject of further experiments on the electric properties of *Chara*, currently proceeding.

Other work in this field of A.C. measurements on plant cells includes that of Remington (1928-9) on slices of beet tissue, which is unsatisfactory in that the current

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paths through intercellular spaces and cell walls were insufficiently defined. Iwamura (1952) showed that the electric impedance of a plasmodium of *Physarum* can be described electrically in the same terms as used with the animal experiments.

The present programme of work was designed to investigate the electrical properties of some plant cell membranes which had become of interest in other investigations (Mercer *et al.* 1955; Robertson *et al.* 1955) and eventually to try to interpret the molecular architecture of these membranes in terms of dielectric characteristics together with other known physical properties.

II. Material, Apparatus, and Experimental Method

(*a*) *Mitochondria*

Mitochondria from *Beta vulgaris* were prepared by a differential centrifugation of cell-free homogenates as described in Robertson *et al.* (1955). The final medium was 15 per cent. sucrose with KCl added to give the required conductivity. The suspension usually contained 15–30 per cent. by volume of mitochondria. A less concentrated suspension reduced the resistance and capacitance difference between suspension and medium, while more concentrated ones gave trouble with trapped air bubbles.

The time between homogenization and the electrical measurements was usually about 70 min, during which time the particles were kept at 0°C. For information about the metabolic activity of similar preparations the reader is referred to Robertson *et al.* (1955).

After measurements, the suspension was placed in narrow-bore "Pyrex" tubes and spun in the super-speed head for 10 min at 24,000 *g* to give a clear supernatant for further measurements.

(*b*) *Chloroplasts*

Chloroplasts from *Chara australis* (var. *nobilis*) were obtained by cutting and grinding clean sorted cells in glucose, straining through muslin to remove cell wall pieces, and centrifuging to bring down the chloroplasts. Starch-free cells were chosen wherever possible. The final medium was 0·5M glucose and KCl. In this tonicity the chloroplasts appeared as approximately spherical under the microscope. The time between grinding and electrical measurements was approximately 20 min.

(*c*) Chlorella

Chlorella (*pyrenoidosa*?) cells were cultured in a medium described by Emerson and Lewis (1939). After about 5 days the cells were concentrated by centrifugation, resuspended in 0·1M glucose, spun down, and finally resuspended in 0·1M glucose and KCl. The diameters of 50 or more cells were measured at a magnification of about 600× for use in calculating the specific capacitance of the cell surface.

(*d*) *Method*

The resistance and capacitance of the suspensions were measured at a number of frequencies by balancing the unknown against a substandard parallel resistance and capacitance. The former consisted of an electrolytic resistor substantially as

described by Cole and Curtis (1937) which used the separation between one fixed platinized platinum electrode and another mounted on a micrometer spindle, with KCl solution between them, as the means of varying the resistance. This resistor was calibrated occasionally for resistance and capacitance against electrode separation, using temperature-stable "Welwyn" carbon resistors and air or mica capacitors. The substandard capacitors were of two ranges, depending on the experiment. One was variable from about 11 to 1,111 $\mu\mu$F with an accuracy of $\pm 0{\cdot}1$ $\mu\mu$F*, and the other from 12 to 11,112 $\mu\mu$F with an accuracy of $\pm$ 0·2 $\mu\mu$F*.

The measuring bridge is shown in the circuit diagram (Fig. 1). By careful design of the transformer T†, the secondary outputs from 1 and 2 have been made equal in

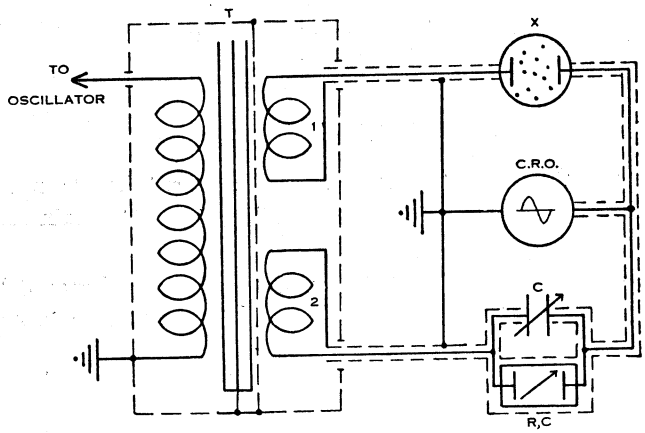

Fig. 1.—The circuit of the A.C. bridge used to measure the equivalent parallel resistance and capacitance of suspensions. The dotted lines represent electrostatic shielding. The unknown x was balanced by the resistance and capacitance of the electrolytic resistor R, C, and mica capacitors C. C.R.O. = cathode-ray oscillograph; auxiliary amplifiers not shown.

magnitude and phase to within 0·1 per cent. over the frequency range 100 c/s to 2 Mc/s., with negligible distortion as long as the secondary voltage did not exceed 0·1 V at the lowest frequency. The error in using the transformer at 4 Mc/s was small and could be allowed for in the electrolytic resistor calibration. For frequencies up to 200 kc/s, sinusoidal voltage was obtained from a "Techtron" oscillator with a 20 : 1 transformer to match into the transformer T. For frequencies above this, a small spot-frequency oscillator was used, giving several volts at 500 kc/s, and at 1, 2, and 4 Mc/s. A cathode-ray oscillograph with auxiliary amplifiers was used as the null-point indicator.

* These were standardized with the help of the Division of Electrotechnology, National Standards Laboratories, C.S.I.R.O.

† Designed by Mr. L. Medina, Division of Electrotechnology, National Standards Laboratories, C.S.I.R.O.

(e) *Measuring Cell*

The cells or cell particles were suspended for measurement in a small conductivity pipette (cell constant k = resistance/resistivity $\simeq$ 0·5) in which 0·2 ml of suspension were required to cover the electrodes. The contents were kept mixed and suspended by occasionally returning the liquid to a small glass reservoir on the end of the pipette using pressure on a rubber bulb on the other end. No effect of O_2 supply on resistance or capacitance was evident, but O_2 may never have been limiting owing to the mixing which was necessary to keep the particles in random suspension.

Mitochondria in similar suspensions at 25°C have an appreciable O_2 uptake, though less than that with added substrate (see Robertson *et al.* 1955). No evidence about the level of metabolic activity of isolated chloroplasts or of *Chlorella* cells has been obtained.

Both the electrolytic resistor and measuring pipette were kept at constant temperature (usually 25$\pm$0·02°C) in a water-bath.

III. Theory

Although the theory of this type of measurement has been developed and discussed by Fricke (1924, 1925) and Cole (1928), some of the main concepts will be repeated.

(i) The mean resistivity (r_2) in Ωcm of homogeneous spheres at low frequency in random suspension is given in terms of the resistivity of the suspension (r) and the medium (r_1) and the volume concentration ρ of the spheres, as:

$$\frac{1-r_1/r}{2+r_1/r} = \rho\left(\frac{1-r_1/r_2}{2+r_1/r_2}\right) \quad \ldots\ldots\ldots\ldots\ldots\ldots (1)$$

(Maxwell 1873).

If the spheres have a relatively non-conducting exterior surrounding a homogeneous interior of much lower resistivity, and the systems dealt with in these experiments are thought to be of this sort, then the non-conducting volume concentration ρ_0 is given approximately by

$$\rho_0 = 2\left(\frac{1-r_1/r}{2+r_1/r}\right), \quad \ldots\ldots\ldots\ldots\ldots\ldots (2)$$

where equation (**2**) is obtained from equation (**1**) by putting $r_2 = \infty$.

As Cole (1940) has pointed out, ρ in equation (**1**) would need to be determined to within 2 per cent. to reveal any difference in membrane resistivity between ∞ and 25 Ωcm^2 in his suspensions of Hipponoë eggs.

In the present series of experiments, where the volume concentration could be determined independently (using a haemocytometer), it was approximately equal to the non-conducting volume concentration ρ_0 as calculated from equation (**2**). In the absence of an accurate method of measuring ρ, we assume the suspended particles have an unknown but high resistance at low frequencies.

(ii) The capacitance per unit area is calculated from the difference in capacitance between suspension and medium, at each frequency, with correction made for the

capacitance of the electrolytic resistor. Then at low frequencies, the capacitance c_2 in F/cm² of the surface of the suspended spheres is given (Cole and Cole 1936*a*) as:

$$c_2 = \frac{2C_p k}{(2+r_1/r)(1-r_1/r)a}, \quad \ldots\ldots\ldots\ldots\ldots\ldots (3)$$

where C_p is the capacitance of the suspended particles in farads, k the cell constant, a the radius of the spheres in cm, and r_1 and r are as before.

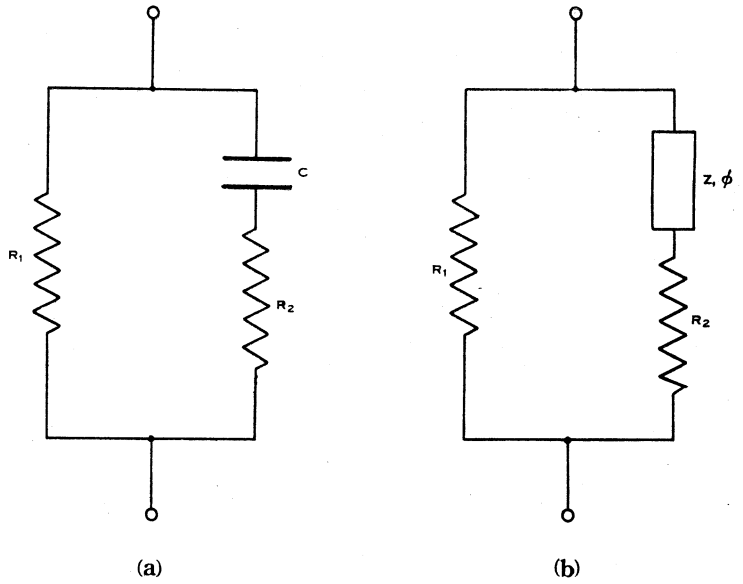

Fig. 2.—Equivalent circuits representing the electrical properties of suspensions. (*a*) The membrane is represented by a pure capacitance C, resistance to current flow through the medium by R_1, and the resistance of the interior of the suspended particle by R_2; (*b*) the membrane is represented by an impedance Z with a phase angle ϕ constant with frequency.

(iii) The resistance and capacitance data are conveniently represented by plotting for the various frequencies the series resistance R_s against series reactance X_s, both in ohms, where

$$X_s = 1/\omega C_s$$

($\omega = 2\pi \times$frequency, and C_s is the series capacitance). As the measuring bridge gives equivalent parallel values R_p and C_p, the series coordinates are calculated from the usual transformations:

$$R_s = \frac{R_p}{1+(\omega R_p C_p)^2}; \; X_s = \frac{\omega R_p^2 C_p}{1+(\omega R_p C_p)^2}. \quad \ldots\ldots\ldots (4)$$

The equivalent circuit, which is a formal representation of the electric properties of the suspension, reduced to a two-terminal network, can be shown as in Figures 2 (*a*) and 2 (*b*). C or Z represent the membrane capacitance or impedance respectively. R_1 represents the resistance to current flow in the medium between the spheres and

R_2 that due to electrolyte inside the membrane. At low frequencies when no current passes through the spheres the parallel measured resistance is R_1 while at very high frequencies the reactance of C or the impedance of Z has a low value, and current flows through R_2. The resultant resistance is R_1 and R_2 in parallel. When the membrane has a pure capacity (Fig. 2(a)) and the R_s at each frequency is plotted against the corresponding X_s, the result is a semi-circle with the centre on the R_s-axis and cutting this axis at R_1 ($\omega = 0$), and $\frac{R_1R_2}{R_1+R_2}$ ($\omega = \infty$). When, however, the membrane has a variable impedance Z with a constant phase angle (Fig. 2 (b)), Cole (1928) showed that the impedance locus is an arc of a circle with the centre below the R_s-axis. The phase angle of the impedance is then half the angle subtended at the centre of the circle by the arc. The measured capacitance and equivalent resistance representing the dielectric loss in this case are both functions of the frequency.

TABLE 1

PARALLEL RESISTANCE AND CAPACITANCE AGAINST FREQUENCY FOR A SUSPENSION OF BEET MITOCHONDRIA

f (kc/s)	1	2	5	10	20	50	100	200	500	1000	2000
R_p (Ω)	3741	3723	3705	3683	3656	3606	3542	3445	3287	3139	3012
C_p ($\mu\mu$F)	15	49	52	45	35	25·1	18·8	12·4	5·9	2·7	0·8

IV. RESULTS

(a) *Mitochondria*

The low-frequency resistance of a suspension of beet mitochondria was found to decrease continually at 25°C due to leakage of electrolyte from the interior to the medium, either because of gradual breakdown of the membranes of the whole population, or sudden complete breakdown of a certain number per unit time. For this reason the first definite results were got at 0°C with the suspension in a stirred ice-water mixture where the resistance drift was quite small.

Table 1 gives a typical set of R_p, C_p with frequency. The capacitance difference C_p between suspension and medium rises with increasing frequency and then falls, while the resistance falls continually. The decrease in C_p with increasing frequency is partly due to actual membrane capacitance decrease and partly due to the fact that the parallel capacitance depends on frequency even when C in Figure 2 (a) is constant, i.e.

$$C_p = \frac{C}{1+(\omega R_2 C)^2}. \quad \ldots\ldots\ldots\ldots\ldots\ldots (5)$$

The frequencies at which C_p become appreciably less than C depend on R_2, the element in Figure 2 representing the internal resistance.

Figure 3 shows the plot of $X_s : R_s$, these being calculated from Table 1. The impedance locus is an arc of a circle with the centre well below the R_s-axis, corresponding to the presence of a variable impedance element (cf. Fig. 2 and Section III).

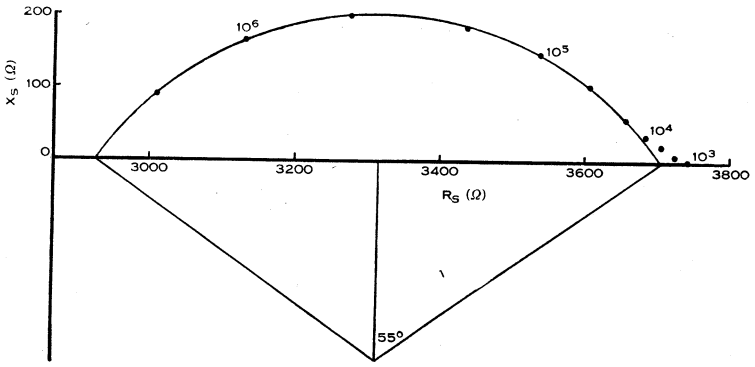

Fig. 3.—Impedance locus for a suspension of beet mitochondria. R_s = equivalent series resistance; X_s = series reactance, calculated from the measured parallel values shown in Table 1. The frequencies are in c/s.

The phase angle ϕ is constant over most of the frequencies and equals 55°. The flattening of the locus at the low-frequency end probably represents polarization of the membrane. In Figure 4 (*a*), $\log_{10}C_p$ is plotted against $\log_{10}f$, f being the frequency

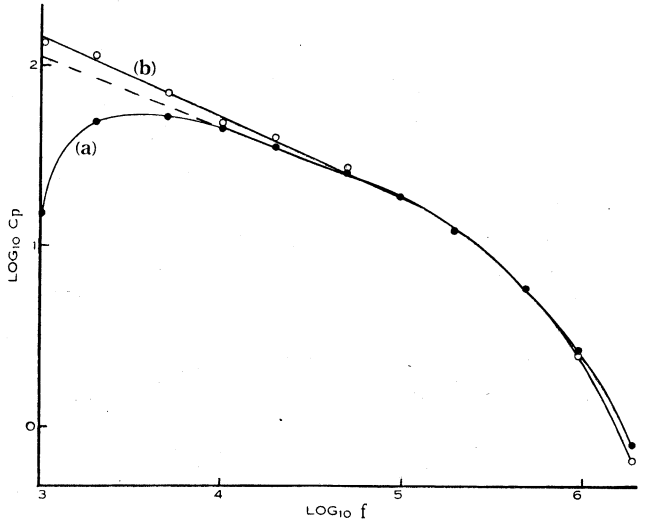

Fig. 4.—$\text{Log}_{10}C_p$ plotted against $\log_{10}f$. (*a*) For the same suspension as Figure 3 and Table 1, and (*b*) for a similar suspension.

in c/s. Over a portion of the middle-frequency range the relation is linear. At the lowest frequencies the capacitance tends to decrease with decreasing frequency and in some experiments negative capacitances were found, corresponding to an inductive reactance being present in the system. In other experiments (e.g. curve (*b*), Fig. 4)

the relation between $\log_{10}C_p$ and $\log_{10}f$ did not depart from linearity at the lowest frequency used. Differences between preparations are not yet explained.

At an arbitrary frequency of 1 kc/s the mean membrane capacitance in eight experiments was calculated, using equation (**3**), as $2{\cdot}8 \pm 1{\cdot}2\ \mu F/cm^2$. Since the effect of the membrane is negligible at infinite frequency, the extrapolation to $\omega = \infty$ enables r_2, the mean resistivity of the interiors of the mitochondria, to be calculated using equation (**1**). This was on the average three times that of the medium at 0°C (10 experiments) and one and a half times the medium resistivity at 25°C (six experiments). r_2 might be expected to be less than r_1 since, as shown by Robertson *et al.* (1955), the concentration of K^+, Na^+, and Cl^- is higher in the mitochondria than in the medium for similar external concentrations. The implications of this will be discussed in Section V.

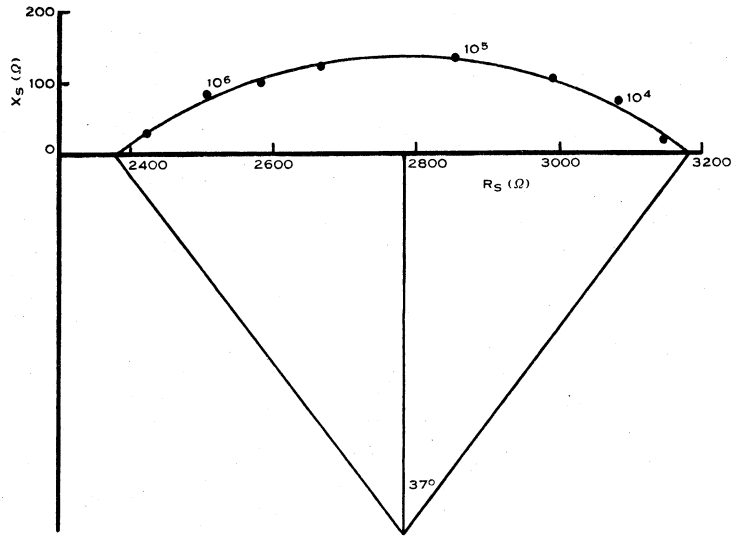

Fig. 5.—Impedance locus (X_s : R_s) for a suspension of *Chara* chloroplasts. Frequencies in c/s.

For mitochondrial suspensions measured first at 0°C and then 25°C, the impedance loci were similar to that in Figure 3, but sometimes with a smaller phase angle at 25°C. However, this difference is probably not significant as R_p was falling with time, and the readings were taken from low to high frequency which would tend to flatten out the locus.

(*b*) *Chloroplasts*

Figure 5 shows a typical impedance locus for a suspension of chloroplasts in 0·5M glucose and KCl at 25°C. The phase angle is less than that of the mitochondria, being 37°. The infinite-frequency resistance again leads to $r_2 > r_1$ in all experiments. The mean specific capacitance at 1 kc/s was $1{\cdot}6\ \mu F/cm^2$ in four experiments.

(c) Chlorella

The impedance loci at 0 and 25°C for a suspension of *Chlorella* cells in 0·1M glucose and KCl is shown in Figures 6 (*a*) and 6 (*b*). The phase angles are equal to 69° and 67° respectively. The series reactance rises again at the highest frequencies indicative of an inner membrane surrounding a more conducting region, possibly the chloroplast or nucleus. This effect is similar to that obtained by Cole and Cole (1936*a*, 1936*b*) with *Asterias* and *Arbacia* eggs and attributed to the nucleus. Because of this second impedance element in the system the extrapolation to infinite frequency carries less conviction. However, this extrapolation gave a relatively constant r_2 of 980 ± 200 Ωcm at 25°C (six experiments) independent of the medium concentration.

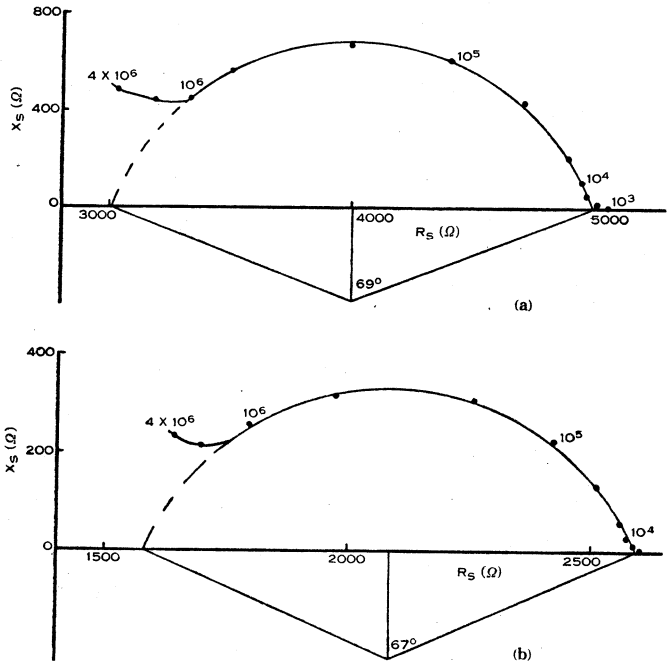

Fig. 6.—Impedance loci for a suspension of *Chlorella* cells: (*a*) at 0°C, and (*b*) at 25°C. Frequencies in c/s.

The mean capacitance/unit area of the main impedance element at 1 kc/s was $1{\cdot}0 \pm 0{\cdot}4$ μF/cm² (six experiments), while the mean phase angle was $71 \pm 8°$. These values are comparable with those quoted in Cole (1942) from his unpublished results, viz. $c_2 = 0{\cdot}33$ μF/cm², $r_2 = 460$ Ωcm, $\phi = 80°$.

V. Discussion

(a) *The Physical System*

Electron microscope and other studies (Mercer *et al.* 1955; Farrant *et al.*, unpublished data 1955) show that the chloroplast of *Nitella* and the beet mitochondrion are bounded at their surfaces by thin membranes. That of the chloroplast is 70 Å or more thick, while that of the isolated mitochondrion is *c.* 170 Å thick.

In the *Chlorella* cell, published electron micrographs (Albertson and Leyon 1954) do not show any membrane between the cell wall and cytoplasm nor around the chloroplast. However, Hodge and Hope (unpublished data 1954) by staining with phosphotungstic acid after osmium fixation, demonstrated a membrane surrounding the chloroplast of *Chlorella* but not a cytoplasmic or vacuolar membrane with certainty. The electrical measurements just described indicate that a membrane with a large capacitance surrounds a large proportion of the cell contents, since the non-conducting volume concentration was approximately equal to the total volume concentration measured by counting cells and measuring their diameters in a haemocytometer. The mean diameter of the cells was usually *c.* 5 μ.

The interior of the isolated beet mitochondrion in section does not show much organized structure of the sort found by Palade (1953) in animal mitochondria but this may be due to limitations of fixation technique. From chemical analysis the beet mitochondria contain large amounts of protein. These mitochondria, which are approximately spherical, have a mean diameter of approximately 1 μ.

The chloroplasts in 0·5M glucose, judging from the appearance of electron micrographs of sections of *Nitella* and of isolated swollen chloroplasts, probably have a slightly disorganized interior with large spaces between the groups of lamellae, due to swelling from an oblate spheriod to a sphere of diameter 8-9 μ. We can conclude that at least for the mitochondrion and chloroplast the equivalent circuit of Figure 2 is a reasonable representation of the electrical elements in the suspensions. At least one further capacitative reactance should be added for *Chlorella* but this is not important until high frequencies (>1 Mc/s) are reached.

(*b*) *The Impedance Locus*

It has been seen that in the suspensions measured, in every case when series resistance is plotted against series reactance, the points fall accurately on the arc of a circle with the centre below the R_s-axis over a considerable range of frequency. Deviations at either end of the frequency range are attributed to polarization (Figs. 3 and 6), inductive behaviour of the membrane (Fig. 4 (*a*)), or to enclosed reactances (Fig. 6). From the considerations in Section III, the circular arc locus means that the system contains a variable impedance element with the property that as the frequency changes both the capacitative and resistive (dielectric loss) components change, but the phase angle remains constant in the frequency range considered. If c' is the real or conservative part of the capacitance it can be shown that

$$c' = \frac{\bar{c}\sin(\alpha\pi/2)}{\omega^{1-\alpha}}, \qquad \ldots\ldots\ldots\ldots\ldots\ldots (6)$$

where $\bar{c}$ and α are constants. α is related to the phase angle: $\phi = \alpha\pi/2$. Thus if $\alpha = 0$, $c' = 0$; if $\alpha = 1$, $c' =$ constant; while if $\alpha = 0{\cdot}5$ (approximately the chloroplast behaviour), $c' = 0{\cdot}707\bar{c}/\sqrt{\omega}$. α is termed by Cole the "loss constant". Figure 4 shows that the variation of capacitance with frequency is consistent with equation (**6**) and the slope of the linear part enables us to calculate α and ϕ as a check on the ϕ value obtained from Figure 3. From Figure 4, curve (*a*), $\alpha = 0{\cdot}62$ and therefore $\phi = 56°$ (55° in Fig. 3). α calculated for all the experiments varied from 0·41 to 0·89 ($\phi = 37$-$80°$).

Its significance in terms of the molecular structure of the dielectric is not yet clear, although it has been found that α measured for a solution of β-lactoglobulin increased as concentration increased (Shaw, Jansen, and Lineweaver 1944). Also lowering the temperature of ice (Murphy 1934) and glycerin (Morgan 1934) increased α. Thus Cole (1949) suggests by analogy that unit loss constant means the complete replacement of viscous opposition to dipole rotation by intermolecular forces, which are negligible in dilute solutions. However, the data for ice are inadequate at the highest frequencies and other workers find $\alpha = 0$ at various temperatures (Auty and Cole 1952).

If the above suggestion is accepted tentatively, the membranes dealt with here may be thought of as lying somewhere between the completely "frozen" arrangement of molecules ($\alpha = 1$, e.g. the *Arbacia* egg) and an open aggregation with no intermolecular forces ($\alpha = 0$, a dilute "solution" of dipoles). It would be interesting to see if α (and ϕ) varied with the degree of stretching of the particle membrane during swelling (the chloroplast would be a suitable material). Interpretations of the loss

TABLE 2

SPECIFIC CAPACITANCE OF THE MEMBRANES AT 1 KC/S, MEMBRANE THICKNESS, AND CALCULATED DIELECTRIC CONSTANT

Membranes of	Specific Capacitance c_2 (μF/cm^2)	Thickness t (Å)	Dielectric Constant ϵ (at 1 kc/s)
Mitochondria	2·8	170	54
Chloroplasts	1·6	70	13
Chlorella	1·0	?	?

constant in terms of a single relaxation time ($\alpha = 0$) of the molecules following the electric field or a distribution over a large range of times ($\alpha > 0$) offers no more in the way of an explanation in physical terms (Cole and Cole 1941).

It is perhaps significant that for the cell particles in the absence of their normal environment α is less than for intact cells (*Chlorella*) and tissue (potato—Cole 1932; beet—Hope, unpublished data). Further discussion of this will be fruitful only when a sound theoretical basis is available.

(c) *The Specific Capacitance and Dielectric Constant*

The specific capacitance of the membranes studied, calculated for a frequency of 1 kc/s, together with the dielectric constant at this frequency are given in Table 2. The latter has been calculated as for a parallel-plate capacitor assuming macroscopic behaviour and

$$c_2 = \epsilon_f/(4\pi t \times 9 \times 10^{11}), \quad \ldots\ldots\ldots\ldots\ldots\ldots (7)$$

where ϵ_f is the dielectric constant at frequency f, and t the membrane thickness. c_2 is in F/cm^2 and t is in cm.

The differences in thickness and dielectric constant do not give us any confidence in supposing that plant membranes have a common structure, even allowing for uncertainties in the values of t. The zero-frequency ("static") dielectric constant is obviously larger than at 1 kc/s. Equation (**6**) suggests a value of ∞ so this equation cannot describe the membrane behaviour outside the frequency range studied. Unfortunately direct measurement of c_2 below the frequencies used here becomes very difficult and the inductive behaviour becomes prominent. Further information is needed on the change of dielectric constant with frequency as a step towards estimation of the dipole moment to clarify our ideas on the membrane structure. At low frequencies some suspensions of mitochondria and *Chlorella* were balanced by a negative capacitance, indicating an inductive reactance. This has been discussed by Cole (1947) and Teorell (1951), who attributed it to non-linearity (rectification) in the membrane.

(*d*) *The Internal Resistivity*

The constancy of the internal resistivity of the *Chlorella* cells in these investigations (980 Ωcm, independent of the medium) must mean that equilibrium with the external medium is not established in the time of the experiment. No large vacuoles are evident in the electron micrographs of sections of this cell so this resistivity is presumably that of the cytoplasm and possibly of the chloroplast as well.

On the other hand, in the mitochondrial suspensions, the evidence suggests an internal resistivity one and a half to three times that of the medium over a range of concentrations of the latter. Thus adjustment has probably been made to the new environment in which KCl and sucrose replaced sucrose. This is consistent with the results of Robertson *et al.* (1955) who found adjustment to be 50 per cent. complete in *c.* 6 min. However, in those experiments the internal concentrations of K^+, Na^+, and Cl^- were in excess of those of the medium at low concentrations. The lower conductivity of the interior in the present experiments must therefore be due to a lower mobility of ions inside the mitochondria. Thus, since the conductivity is a function of the product of concentration and ion conductance, it can be seen that the latter is less inside the mitochondria than in aqueous solution by a factor of 3 at 0°C and 1·5 at 25°C. This could be brought about by the binding of some of the ions in regions low in water and high in protein or lipo-protein concentration. The temperature coefficient of the resistivity (twice that of the medium) could also be consistent with this. Some effect similar to this has been demonstrated by Robertson and Honda (unpublished data) in their investigation of the ionic relations of beet mitochondria. The effect of a high concentration of colloid on ion conductance through increased viscosity only is probably negligible (McKenzie 1948).

Electron micrographs of sections of mitochondria (Farrant *et al.*, unpublished data 1955) generally show an interior in which OsO_4 has been strongly reduced to Os, indicating a high concentration of protein, etc., relative to the rest of the cytoplasm.

Similarly, in the chloroplast, ion mobility would probably be reduced in this way although enough data have not been assembled. For example it might be expected that r_2/r_1 would be less for more swollen chloroplasts.

VI. Conclusions

The membranes of mitochondria, chloroplasts, and *Chlorella* cells behave electrically as "lossy" capacitors in which the capacitance and equivalent resistance due to losses both change with frequency but the phase angles of which remain constant over a wide frequency range. The phase angle is possibly related to the measure of molecular interaction between dipoles in the membrane, but confirmation of this theory is still absent.

The capacitances are similar to those found in various animal cell membranes and for an artificial bimolecular film of lecithin and tanned egg albumin (Dean, Curtis, and Cole 1940), but the phase angles of the membranes of extracted cell particles are less than those of inact cells.

VII. Acknowledgments

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