ELECTRIC IMPEDANCE OF ASTERIAS EGGS

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Several years ago the absolute value of the alternating current impedance of suspensions of *Arbacia* eggs was measured at frequencies from 1 to 15,000 kc. (Cole, 1928*b*). It was concluded that the egg membrane capacity was not as nearly a static capacity as the capacity of the red blood cell membrane (Fricke, 1925 *a*, 1933), but was of the polarization impedance type, similar to that which could be attributed to the cell membranes of tissues (Cole, 1932, 1933; Bozler and Cole, 1935). Recent measurements (Cole, 1935) of both the resistance and reactance components of suspensions of *Hipponoë* eggs at frequencies from 1.1 to 2,300 kc. showed the membranes to have static capacities and suggested complicating phenomena at the upper end of the frequency range. It was then obvious that more complete measurements should be made not only on *Arbacia* eggs, but also on the eggs of other echinoderms.

Material

The availability and the large size of the eggs of the common starfish, *Asterias forbesi*, made them good material for this work. As soon as the animals were collected, the ovaries were removed and placed in sea water until the eggs had been shed. The eggs were washed once or twice, centrifuged lightly, and drawn into the conductivity cell. The suspension of eggs separated by jelly reached a constant resistance in 15 or 20 minutes which was maintained for an hour or two. When the suspension was removed from the cell it was found that very little cytolysis had taken place A singular scarcity of ripe males prevented tests of viability and measurements of fertilized eggs. All measurements were made between 21 and 22°C.

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Apparatus

The conductivity cell was of the burette type used for the *Hipponoë* eggs. It has a volume of 1.51 cc. and a cell constant of 14.53. The electrodes were platinized platinum.

The measurements of the suspensions were made with the conductivity equipment of the Biophysics Laboratory at Cold Spring Harbor which was placed at our disposal by Dr. Hugo Fricke. Substitution measurements of the parallel resistance and capacity were made with the Wheatstone bridge from 1 to 2048 kc. and with the resonance circuit from 4.1 to 16.4 megacycles.

An electrolytic resistor of the type employed in the work on the sartorius muscle (Bozler and Cole, 1935) and the *Hipponoë* eggs (Cole, 1935), was used
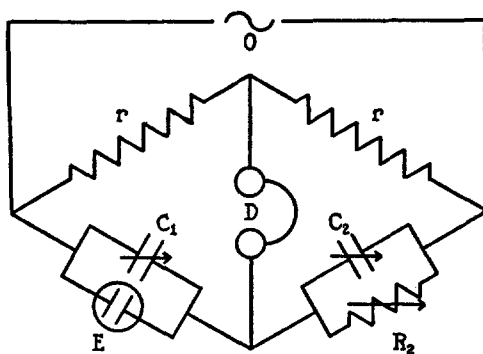

FIG. 1. Wheatstone bridge with egg cell (E), calibrated condensers C_1 and C_2 and variable resistance R_2 (r and r are fixed equal resistances), oscillator (O), and detector (D).

throughout as a variable standard of resistance. It is a modification of the micrometer electrolytic cells described by Miller, 1923; Fricke and Curtis, 1935 *b*; and Jones and Christian, 1935.

At each frequency, the bridge, Fig. 1, was balanced with the condenser C_1 set to minimum capacity. The electrolytic resistor (R_1) was then substituted for the egg conductivity cell (E) and, leaving R_2 and C_2 unchanged, the bridge was balanced by adjusting R_1 and C_1. The resistance of the suspension was then known from the calibration of the resistor at low frequency. The capacity due to the eggs was:

$$C = \Delta C + C_R - C' + \frac{\Delta L}{R^2},$$

where ΔC is the change in capacity of the parallel condenser (C_1) on substitution,

C_R is the capacity of the electrolytic resistor, C' is due to the capacity of the egg cell filled with sea water and the change in capacity of the connecting wires on substitution, and $\Delta L/R^2$ is the capacity correction due to a difference ΔL in the inductance of the leads when the circuit resistance is R. On the assumption that C_R is due to water alone, it is found that:

$$C_R = \frac{7.1}{K} \; \mu\mu f.,$$

where K is the cell constant (Fricke and Curtis, 1935 b). For the electrolytic resistor $K = K_1 M$, where K_1 is the cell constant for 1 cm. electrode separation and M is the micrometer reading in centimeters, so

$$C_R = \frac{7.1}{K_1 M} \; \mu\mu f.$$

If it is assumed that ΔL is negligible and that the conductivity cell is filled with electrolyte, then:

$$\Delta C = \frac{7.1}{K_1 M} - C'.$$

When the conductivity cell is filled with electrolytes of different conductivities so that M varies, ΔC is a linear function of $1/M$. These data for both the bridge and the resonant circuit are plotted in Fig. 2. For the former, $C' = 2.87 \mu\mu f.$, and for the latter, $C' = 1.05 \; \mu\mu f.$

The cell constant $K_1 = 0.907$, so the slopes of the lines should both be 7.83. Actually they are found to be 8.36, so that probably the glass wall of the cell and the surrounding air contribute to the capacity to this extent. The latter value is used to compute C_R. The graph further shows that ΔL is negligible.

The electrodes of the conductivity cell and the electrolytic resistor were platinized so that, with sea water in the former, the equivalent parallel capacity of each and their difference was small but it was still necessary to correct for the electrode polarization capacity at low frequencies. If C_W is the change in parallel capacity when the micrometer cell is substituted for the conductivity cell filled with sea water, and C is the difference in capacity when the cell is filled with suspension, then the capacity due to the eggs:

$$C_E = C - \left(\frac{R_W}{R}\right)^2 C_W,$$

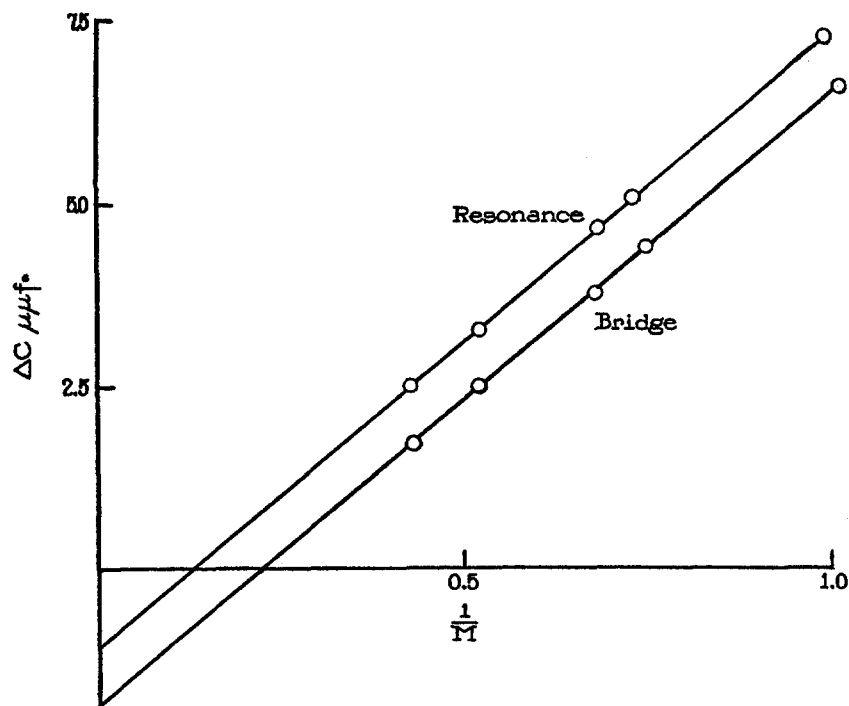where R_W and R are the parallel resistances of the sea water and the suspension respectively.

FIG. 2. Reciprocal of electrolytic resistor setting, $1/M$, vs. capacity difference, ΔC, between resistor and conductivity cell.

Data and Calculations

When a suspension of eggs is equivalent to a circuit containing two resistances and a single static capacity, the complex locus (Cole, 1928a, 1933) will be a semicircle with its center on the resistance axis. The complex plane impedance locus is obtained by plotting the series resistance R_S and the series reactance X_S, as abscissae and ordinates. These should be calculated from the parallel resistance R and capacity C_E by the formulae:

$$R_S = \frac{R}{1 + R^2 C_E^2 \omega^2}, \qquad X_S = \frac{R^2 C_E \omega}{1 + R^2 C_E^2 \omega^2},$$

where $\omega = 2\pi n$ and n is the frequency. In almost every case, however, the term $R^2 C_E^2 \omega^2$ was so small[1] that it was neglected. Then

[1] In Table I, $R^2 C^{E^2}\omega^2$ has its maximum value in No. 8 at 128 kc. and here $R^2 C_E^2 \omega^2$ = [930.5 × 117.2 × 10^{-12} × 2π × 128 × 10^3]^2 = 7.7 × 10^{-3}.

TABLE I

Suspension of Unfertilized Asterias Eggs of Diameter 124μ

Resistance of sea water, R_W = 450.0 ohms. Volume concentration ρ = 47.1 per cent. Extrapolated capacity, C_0 = 335 μμf. Cell constant, K_2 = 14.53. Temperature, 21.8°C.

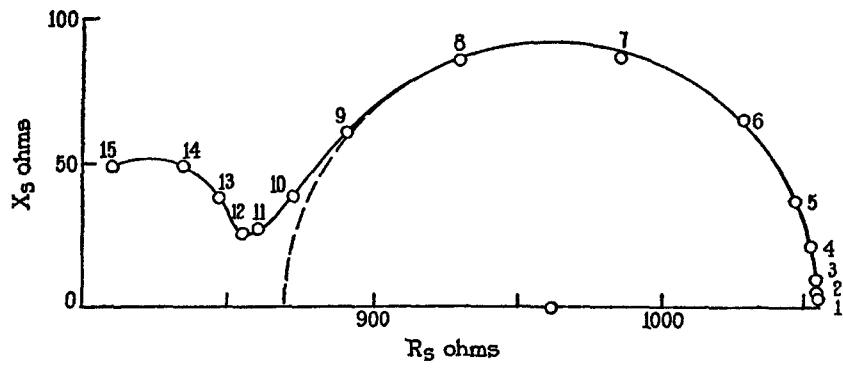| Frequency		R and R_S	C_E	X_S
No.	n			
		ohms	*μμf*	*ohms*
1	$1 \cdot 10^3$	1054.9	380.2	2.78
2	2	1054.7	360.6	5.13
3	4	1054.0	349.4	9.84
4	8	1052.5	337.7	21.39
5	$1.6 \cdot 10^4$	1047.0	323.6	37.65
6	3.2	1029.3	290.5	65.15
7	6.4	986.0	222.3	87.05
8	$1.28 \cdot 10^5$	930.5	117.2	86.05
9	2.56	890.5	47.98	61.10
10	5.12	872.6	15.98	39.05
11	$1.024 \cdot 10^6$	860.0	5.86	27.86
12	2.048	854.5	2.88	27.00
13	4.1	848.5	2.06	38.12
14	8.2	834.0	1.38	49.45
15	$1.64 \cdot 10^7$	810.0	0.73	48.95

FIG. 3. Complex plane locus, series resistance, R_S, vs. series reactance, X_S, for a suspension of unfertilized *Asterias* eggs. Frequencies given in Table I.

$R_S = R$ and $X_S = R^2 C_E \omega$. It is seen from the data for an *Asterias* suspension given in Table I and plotted in Fig. 3 that such a circuit is a close approximation over the low frequency range. It should then

also be true that at sufficiently low frequencies the parallel capacity should be constant and independent of frequency. This is, however, not the case, for in Table I and Fig. 4 the parallel capacity continues to increase as the frequency decreases without the corresponding in-
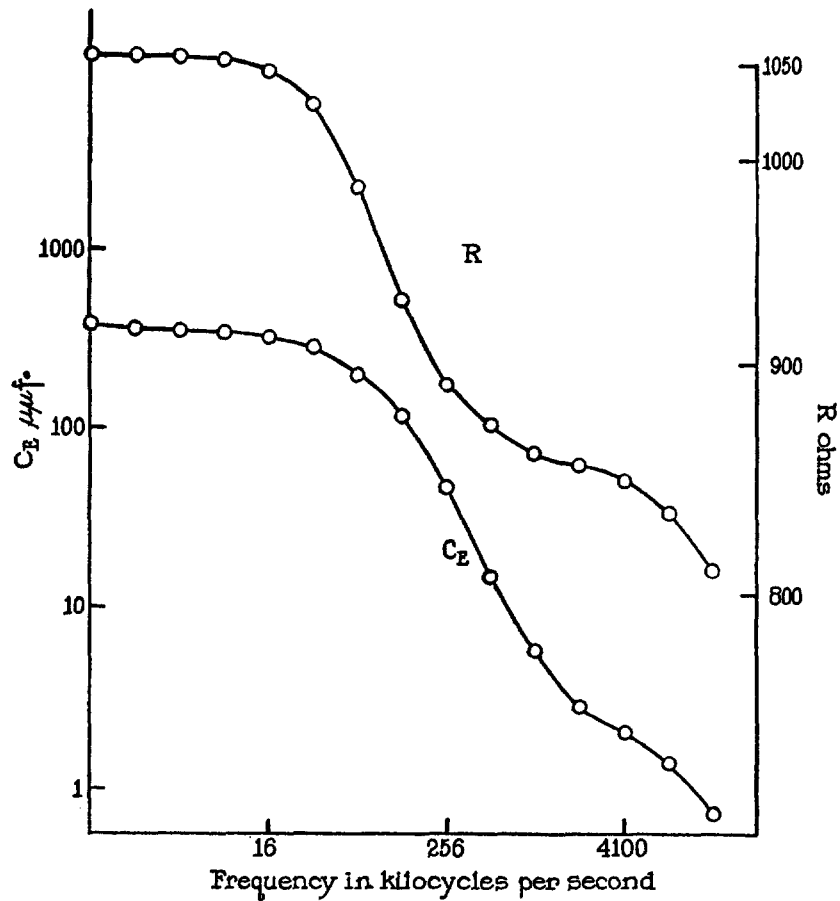

FIG. 4. Log frequency, n, vs. log parallel capacity, C_E, and log parallel resistance, R, for a suspension of unfertilized $Asterias$ eggs.

crease in resistance that would be expected if the current was still penetrating the egg interior to any appreciable extent. It has been shown that a random distribution of spheres having a uniform conducting interior and a poorly conducting membrane of capacity C_M

per unit area in a suspending medium is equivalent to the network A
of Fig. 5 (Fricke and Morse, 1925; Fricke, 1933). It is then found
that (Cole, 1928 a):

$$C_M = \frac{2\,C_0\,K_2}{\left(2 + \dfrac{R_W}{R_0}\right)\left(1 - \dfrac{R_W}{R_0}\right)a}$$

where a is the egg radius, K_2 the conductivity cell constant, R_W
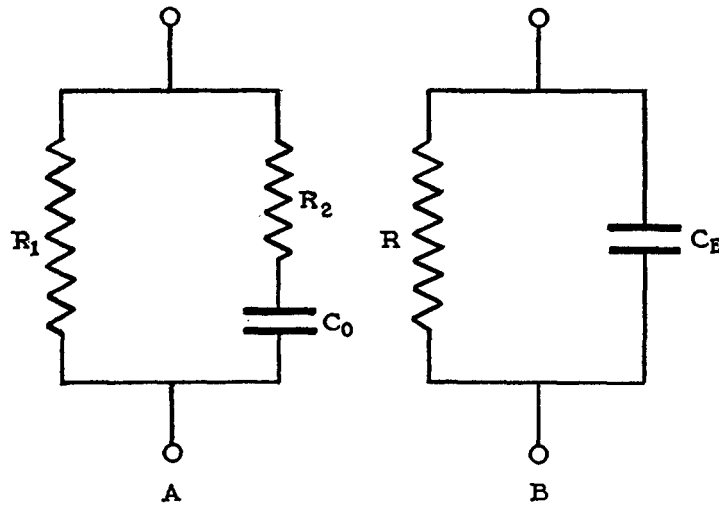and R_0 the resistances of sea water and the suspension, while C_0 is

FIG. 5. Equivalent circuits

the low frequency capacity. The problem now is to determine the C_0
which is the dominant constant over the intermediate frequency range
by an extrapolation to low frequency and thus eliminate the confusing
factors at the low frequencies which prevent the parallel capacity (C_E)
from becoming constant. When the suspension is represented by
Circuit A in Fig. 5, the equivalent parallel resistance (R) and capac-
ity (C_E) at any frequency are given by:

$$\frac{1}{R} = \frac{1}{R_1} + \frac{\omega^2\,R_2\,C_0}{1 + \omega^2\,R_2^2\,C_0^2}, \qquad C_E = \frac{C_0}{1 + \omega^2\,R_2^2\,C_0^2},$$

from which:

$$\frac{1}{R} = \frac{1}{R_L} - \left(\frac{1}{R_L} - \frac{1}{R_0}\right)\frac{C_E}{C_0}.$$ (1)

When C_E is plotted against $1/R$ a straight line should result for the range in which the above assumptions are valid. The intercept on the $1/R$ axis should correspond to the extrapolated value at the high frequency end of the circle, and this may be called $1/R_L$. At the other
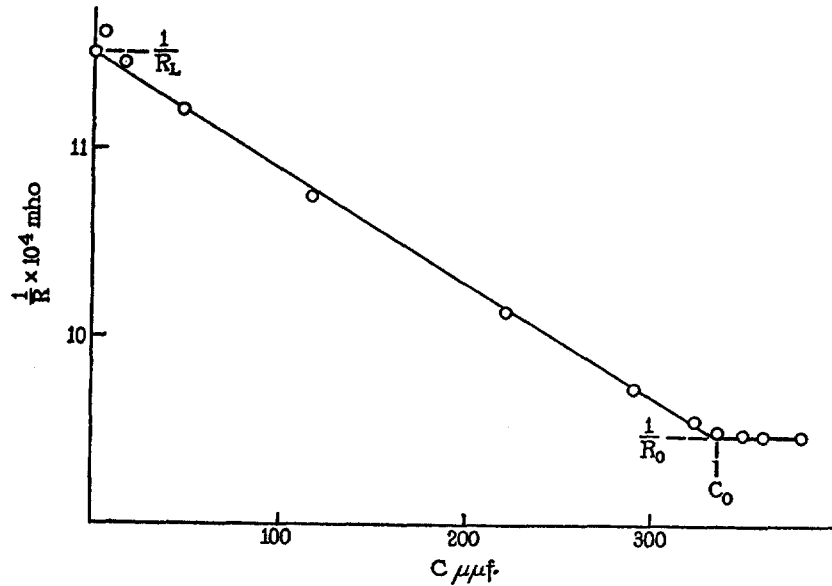

Fig. 6. Parallel capacity, C_E, vs. reciprocal resistance, $1/R$, for *Asterias* suspension. $C_0 = 335\mu\mu f$.

end where $1/R = 1/R_0$ we have $C_0 = C_E$, as shown in Fig. 6. Here $C_E = 335\ \mu\mu f.$, $R_W/R_0 = 0.421$, $a = 62\mu$, so $C_M = 1.03\mu f./cm.^2$ The average value for unfertilized *Asterias* eggs is 1.10 $\mu f./cm.^2$ Two runs taken on a single batch of eggs at different volume concentrations gave values of C_M which differed by less than 2 per cent. It is obvious from the failure of the low frequency data to follow equation (1) that the capacity which determines the penetration of current into the egg is only part of the capacity which is observed at low frequencies. Any resistance component accompanying this additional capacity was not observable.

Ignoring for the moment the complicating factors at high frequencies, we may assume that the extrapolated value of resistance R_L at the high frequency end of the semicircle represents the equivalent low frequency internal resistance which the egg would have if it were uniform. In the data represented by Fig. 3, we find $R_L/R_W = 6.93$, while the average value of all runs gives $R_L/R_W = 7.24$. For the unfertilized *Hipponoë* egg $R_L/R_W = 11$. Even before the abrupt entrance of the high frequency element there is a general tendency for the data to depart from the circle slightly. This was found to about the same extent for *Arbacia*, but was not so apparent in the *Hipponoë* data. Without data at much higher frequencies, it is hazardous to make any analysis of the high frequency effect or assign its cause to any particular part of the egg structure. Making a most unjustifiable extrapolation to infinite frequency we find that if no further undiscovered elements are present, the average high frequency internal resistance of the egg is about 4.5 times the resistance of sea water.

DISCUSSION

It should be pointed out that essentially we have used an electrolyte as our standard resistance and that any frequency dependent characteristics which electrolytes may have must be considered as possible sources of error. There are two questions which may well be mentioned here, not because they are of particular importance in this specific problem, but because they are so often asked when conductance phenomena at high frequency are discussed.

It is well known that at high frequencies the current density in the interior of a conductor is less than at the surface and that the effective resistance is greater than at low frequencies. An approximate analysis of this "skin effect" shows that for a conductor having a resistance R ohms per centimeter, the increase in resistance ΔR is given by:

$$\frac{\Delta R}{R} = \frac{\omega^2}{12 \cdot 10^{12} \, R^2}.$$

For sea water in a cell having a square centimeter cross-section, R is about 25 ohms per centimeter and the resistance error will be less than 1 per cent at a frequency of 10^9 cycles per second.

On the other hand, the resistance of an electrolyte is less at high frequencies because the effect of the electrostatic interaction between ions is reduced. For N/100 KCl, approximately the concentration used in the electrolytic resistor, this effect is less than 1 per cent at $2 \cdot 10^8$ cycles per second.

The measurements and analysis indicate that a membrane having a static capacity independent of frequency, of about $1.10\mu f./cm.^2$, controls the passage of the current through the interior of the *Asterias* egg. This is the same type of capacity and of the same order of magnitude as that found for other suspended cells, as Table II shows.

As is shown in the following paper, the apparent paradox between *Hipponoë* eggs on the one hand and *Arbacia* eggs and tissues on the other has now resolved itself into the reasons for differences between tissues and suspended single cells given by the same analysis and the explanation of the earlier results on *Arbacia* eggs.

It is extraordinarily interesting that cells differing so widely in other characteristics should have membranes so similar in both the type and the magnitude of their electric capacity. All these data naturally lead to the hypothesis of a monomolecular membrane if a dielectric constant of 3 is assumed (Fricke, 1925a). It is, however, apparent from the work which has been done on monomolecular films that such a conclusion should be taken as a warning that it is probably necessary to consider the structure and properties of the material in a thin layer rather than in bulk (Danielli, 1935; Danielli and Davson, 1935).

The volume concentration ρ was computed from the low frequency resistance R_0 on the assumption of a non-conducting membrane by the Maxwell formula for a suspension of non-conducting spheres:

$$\frac{\rho}{2} = \frac{1 - \dfrac{R_W}{R_0}}{2 + \dfrac{R_W}{R_0}}.$$

No independent measurements of the volume concentration were made. On the assumption of a membrane resistance of 25 ohms/cm.^{2} (40 ohms/cm.^{2} was the order of magnitude for frog sartorius) the resistance of a 50 per cent suspension would be 1 per cent less than if the membrane were non-conducting, whereas the accuracy of volume

concentration determinations is about ±2 per cent. It should be possible with large amounts of suitable material, adequate temperature control, and sufficient time, to increase the accuracy of the measurements of volume concentrations considerably over those made with *Hipponoë* eggs. On the other hand, several methods are available for measurement of the cell volume concentration in tissues. By such methods it may be possible to determine whether the tissues showing a polarization impedance have a markedly lower low frequency membrane resistance than have the single cells which have been found to have a static capacity. This seems to be the case from the results on frog sartorius muscle and *Hipponoë* eggs, but the data are not sufficiently accurate or numerous to justify a general and definite conclusion. From simple qualitative considerations, it can be seen that when there is selective ion permeability giving rise to a large out-of-phase back electromotive force with a phase angle of less than 90°, there must be a finite membrane conductance due to the passage of all ions to which the membrane is permeable. On this basis it might be said that probably both the total and the selective permeability of the suspended cells is low compared to tissues in general. In this connection it should be noted that the value in radians of the phase angle ϕ (as given by α, where $\alpha = \phi/\frac{\pi}{2}$) for the red cell $\alpha = 0.95$ and for the white cell $\alpha = 0.90$ is considerably less than for the three echinoderm eggs (for which α is very close to unity). This would seem to be in line with the relative permeabilities of the red cell and *Arbacia* egg to water.

At present there are not sufficiently accurate data available to warrant an extended discussion of the low frequency capacity effect but we are inclined to the obvious conclusion that it is the same type of phenomenon as that observed for the red cell by Fricke and Curtis (1935 a). It was at first felt that this effect might well be due to the selective permeability of the membrane which represented a high impedance as compared with the reactance of the static capacity of the non-permeable portions of the membrane.

The experiments of Errera (1923, 1932), Fricke and Curtis (1935 c), and others on colloids and the suggestion of Bikerman (1934) lend weight to the view that this is associated with surface conductance.

A further support is found in the data of Briggs on cellulose at 1000 cycles where the von Smoluchowski conductance was unexpectedly low (Cole, 1932). Urban, White, and Strassner (1935) also state that the von Smoluchowski conductance of pyrex glass is very slight at 1000 cycles. It is not possible, however, to choose between a normal and a tangential conductance explanation of the data on suspended cells.

At the higher bridge frequencies, 256 and 512 kc., there is a tendency for the points to lie above the circles which best fit the lower frequency points. This is found in the data for unfertilized *Arbacia* eggs, but was present to a lesser degree in the data for unfertilized *Hipponoë* eggs. It is not yet certain whether this can be due to the presence of the high frequency element.

The intercept of the high frequency end of the circle on the resistance axis was interpreted quite hesitantly for the *Hipponoë* data because it gave so high a value for the internal resistance as compared with the early data for the *Arbacia* egg and those for the red and white blood cell. The finding of the high frequency element, which the *Hipponoë* data hinted at, involves the necessity of considering its cause along with the equivalent internal specific resistance. It was decided in the work on *Hipponoë* that the cause of the high value for the low frequency internal resistance was not the nucleus. If the cytoplasm had the resistance of 3.6 times that of sea water as found for *Arbacia*, the nuclear volume would have to be 60 per cent of the egg volume. Since the *Arbacia* figure is now found to be considerably higher and *Hipponoë*, *Asterias*, and *Arbacia* all give about the same value, it is advisable to reconsider the possibility of a nuclear effect, since another element is now known to be present. It is interesting to note that from the data on non-conducting lecithinated red blood cells (Fricke, 1933) there is no indication of the entrance of a similar high frequency element, although, if present, it might be expected to have at least a slight effect at the highest frequency.

If we consider the nucleus to have a very low internal resistance, a volume of 2 per cent of the egg volume is estimated. The effect on the value of the specific resistance of the cytoplasm is then relatively slight and we arrive at an average value of 217 ohm cm., or about 7 times that of sea water.

On the other hand, if the nucleus is assumed to have the same resistance as the cytoplasm, the figure is 5 per cent and the internal specific resistance becomes 136 ohm cm. or 4.5 times sea water. This is in marked contrast to the red and white blood cells and sartorius muscle when considered in relation to their normal environment, but it is interesting to notice that the absolute values of the internal resistance are not so widely divergent, as is shown in Table II.

The acceptance of so high a value of the cytoplasmic resistance would necessitate the presence of a large amount of undissociated material in order to maintain osmotic equilibrium with sea water and would indicate that in different cells the variations in the osmotic

TABLE II

Suspended cell	c_M	Internal resistance		Reference
	µf/cm.²		*ohm cm.*	
Red blood cell....	0.95	2 × plasma	140	Fricke and Curtis, 1934a
White blood cell...	1.0	2 × plasma	140	Fricke and Curtis, 1935a
Yeast...........	0.60	—	—	Fricke and Curtis, 1934b
Hipponoë				Cole, 1935
Unfertilized.....	0.87	11 × sea water	203	
Fertilized.......	2.18	(18 × sea water)	(349)	
Asterias				This paper
Unfertilized.....	1.10	4–7 × sea water	136–225	
Arbacia				Cole and Cole, 1936
Unfertilized.....	0.72	4–6 × sea water	120–186	
Fertilized.......	3.10	(11 × sea water)	(336)	

pressure of the media are met to a large extent by changes in concentration of non-ionized substances.

The volume concentration of membrane-covered material in the cytoplasm is apparently still too great to be due solely to the nucleus, but it will not be possible to discuss the effect in detail until measurements are made at frequencies considerably higher than 16 million cycles. It has been interesting, however, to calculate by a series of approximations and extrapolations the order of magnitude of the "nuclear" membrane capacity to be $0.1\mu f./cm.^2$ at 8 million cycles.

In view of the interpretations given above, it might be quite instructive to observe the effect of high intensities of alternating current at

various frequencies. It is easy to show that when the egg interior has the heat conductivity of water, it would be very difficult to maintain appreciable temperature gradients in the egg interior. On the other hand, it seems that below 10 kc. very little current penetrates the egg and any effects are due to heat from the medium. At 100 kc. there is a high potential difference and a large current flow across the plasma membrane. At 1,000 kc. the "nuclear" membrane is the controlling factor, while it will probably be necessary to go higher than 100 million cycles to be certain of a good nuclear penetration.

It is not possible to estimate whether similar factors enter into the differences observed between diathermy and short-wave therapy without complete measurements on some of the tissues involved (*cf.* Schereschewsky, 1926; Schereschewsky and Andervolt, 1928). Hemingway (1932) has shown that to within 2 per cent the body acts like a pure resistance but it should be pointed out that for *Asterias* suspensions the reactance at 8 million cycles is less than 10 per cent of the resistance and that it contributes less than 1 per cent of the impedance.

SUMMARY

The alternating current resistance and capacity of suspensions of unfertilized eggs of *Asterias forbesi* have been measured at frequencies from one thousand to sixteen million cycles per second.

The plasma membrane of the egg has a static capacity of $1.10\mu f/cm.^2$ which is practically independent of frequency. The suspensions show a capacity dependent on frequency at low frequencies which may be attributable to surface conductance.

The specific resistance of the cytoplasm is between 136 and 225 ohm cm. (4 to 7 times sea water), indicating a relatively high concentration of non-electrolytes.

At frequencies above one million cycles there is definite evidence of another element of which the nucleus is presumably a part.

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