ELECTRIC IMPEDANCE OF HIPPONOË EGGS

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(Accepted for publication, December 20, 1934)

The results on the alternating current impedance of *Arbacia* eggs (Cole, 1928 *b*) indicated that a polarization type of impedance predominated at the surface of the egg. This is also the case for many tissue membranes (Fricke, 1931; Cole, 1932; Bozler and Cole, 1935) whereas the impedance of the membranes of red blood cells, (Fricke, 1925) and yeast (Fricke and Curtis, 1934) is predominantly that of a static capacity. Because of the similarity between the eggs and tissues and since the analysis of data can be much more complete for suspensions of spherical cells than for tissues, it was desirable to extend the previous work. Measurements of the resistance and capacity should completely determine the polarization characteristics of the membrane as was not possible from the measurements of scalar impedance made on *Arbacia* eggs. The work on muscle (Bozler and Cole, 1935) and *Laminaria* (with B. M. Hogg, unpublished) emphasized the importance of the zero frequency conductance of the membrane, but the measurements of the volume concentration of the *Arbacia* suspensions were not accurate enough to be used for this purpose.

Preparation of Material

Some difficulty was experienced in obtaining a sufficient quantity of *Arbacia* eggs from a single female, so it was decided to work with *Hipponoë esculenta* one female of which yields as much as 100 cc. of settled eggs between October and January in Bermuda.

The urchins were either injured and allowed to shed the eggs into sea water or the ovaries were removed and placed in sea water. The suspensions were strained through coarse bolting cloth. It was found that with jelly present, the volume concentration of settled or lightly centrifuged eggs was often less than 20 per cent. This jelly was difficult to remove entirely by shaking without injury to the eggs, but a mild shaking removed part of it and gave 30 per cent to 50 per cent

concentrations of eggs in good condition. The rather heavy suspension was then placed in a large crystallizing dish with 2 or 3 liters of sea water and allowed to settle. The supernatant sea water and detached jelly were siphoned off and the eggs given another such washing. Before measurement, the suspension was cleared of jelly-free eggs and excess sea water by light centrifuging.

Apparatus

Conductivity Cell.—It was planned to use a bubbler cell of the type developed for the *Arbacia* eggs, but the *Hipponoë* eggs were in better condition after standing in jelly than after stripping and bubbling. The conductivity cell was made in the form of a burette, 0.64 cm. inside diameter and 24 cm. long, including the burette stop-cock at the lower end. Cylindrical electrodes, 0.5 cm. long, were sealed into the wall of the cell, 9.3 cm. apart. This cell could be conveniently filled by suction and delivered 5.08 cc. from the fiducial mark above the upper electrode. The electrodes were platinized platinum and the cell constant was 29.5. When the suspensions were diluted with sea water and inseminated after an hour in the cell, normal fertilization and cleavage took place in practically all of the eggs. Within 10 or 15 minutes after the eggs were drawn into the cell, the resistance and capacity reached steady values which only changed slowly over a period of hours, if there were no appreciable temperature changes. Most of the measurements were made at temperatures between 21 and 24°C.

Wheatstone Bridge.—The parallel resistance and capacity measurements were made with the same alternating current Wheatstone bridge used for the muscle impedance (Bozler and Cole, 1935) at eleven frequencies from 1.08 kc. (kilocycles per second) to 2.32 10^3 kc. The substitution method was employed at all frequencies. The egg cell and precision condenser in parallel were first balanced directly against another air condenser and electrolytic resistor (Bozler and Cole, 1935) in parallel on the other arm of the bridge. The egg cell was then replaced by a second electrolytic resistor and the bridge again balanced by adjustment of this resistor and the precision condenser.

The resistance of the unknown was then found from calibrations of the electrolytic resistor made before and after each run. The capacity of the unknown was given by the sum of (1) the change in capacity of the precision condenser, (2) the capacity of the electrolytic resistor, and (3) the difference in capacities of the leads to the cell and to the resistor. The error due to changes of inductance was negligible. An entire frequency run could be made in less than 15 minutes.

Measurements and Results

Volume Concentration and Membrane Resistance

The specific resistance, r, of a suspension of homogeneous spheres is given by the Maxwell equation

$$\frac{1 - r_1/r}{2 + r_1/r} = \rho \frac{1 - r_1/\hat{r}_2}{2 + r_1/\hat{r}_2} \tag{1}$$

where ρ is the volume concentration and r_1 and $\hat{r}_2$ are the specific resistances of the medium and the spheres respectively. Since the suspended cells are not homogeneous they may more reasonably be assumed to consist of an electrically homogeneous interior of specific resistance, r_2, surrounded by a thin membrane having a parallel capacity C_0 and resistance r_3 per unit area. When the radius of the sphere is a, it can be shown (Cole, 1928 *a*) that the specific resistance $\hat{r}_2$ of the equivalent homogeneous sphere is

$$\hat{r}_2 = r_2 + r_3/a .$$

At sufficiently low frequencies, the reactance of the membrane, $1/C_0\omega$, will be large compared with its resistance r_3 and the specific resistance of the suspension, r, will approach its limiting value r_0. If the membrane is non-conducting, r_3, and consequently $\hat{r}_2$, is infinite so

$$\frac{1 - r_1/r_0}{2 + r_1/r_0} = \frac{\rho_0}{2},$$

where ρ_0 is the "non-conducting volume concentration," (Fricke and Curtis, 1935). It cannot in general be assumed that the membrane is a non-conductor at low frequencies, so that an independent measure of ρ is necessary to determine $\hat{r}_2$. If it is then found that there is no significant difference between ρ and ρ_0 it may be concluded that, within the experimental error, the membrane is non-conducting and the cells are equivalent to non-conducting spheres.

The volume concentrations of the *Arbacia* suspensions were calculated from the centrifuged volume of eggs. The method seemed quite unreliable, as Gerard and Rubenstein (1934) have pointed out, and could not be used for *Hipponoë* eggs.

The hemoglobin colorimetric method for the measurement of volume concentration has been very successful for blood (Ponder and Saslow, 1930) and preliminary experiments by Ponder at Cold Spring Harbor on *Asterias* eggs indicated that it might be generally applicable to marine eggs. Unfortunately the hemoglobin seemed to be absorbed by the jelly in such large amounts that it could not be used, and the dye indigo carmine was then tried. It does not penetrate *Arbacia*

eggs and is not appreciably absorbed by the *Hipponoë* jelly. A series of measurements all gave volume concentrations ρ considerably higher than the non-conducting concentration ρ_0. The calculated values of membrane resistances were quite low and not at all consistent with each other. From the capacity measurements, there was reason to suspect a high membrane resistance which gave additional cause to suspect the determinations. It seems probable that sufficient colloidal material, from the jelly, remained in the color sample from the suspension, to falsify the color match against the standard made up from fresh sea water.

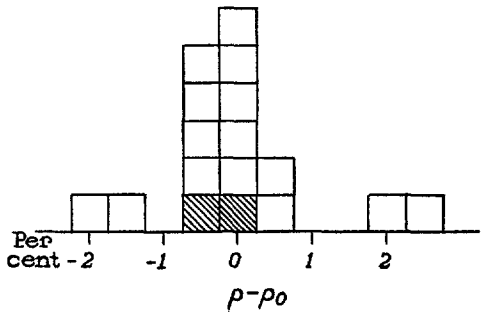

FIG. 1. Frequency distribution of the differences of the titration percentage volume concentration, ρ, and the low frequency non-conducting percentage volume concentration, ρ_0, for *Hipponoë* eggs. Open blocks for unfertilized suspensions, and shaded blocks for fertilized suspensions.

The next attempt was essentially a conductance titration. The egg suspension in the cell, 5.08 cc. was run into 10 cc. of iso-osmotic dextrose solution, the eggs removed by centrifuging, and the resistances of the supernatant solution and sea water measured. From their ratio, and a calibration curve, the volume of sea water added to the dextrose, and consequently the egg volume concentration of the original suspension, ρ, could be determined. At the same time, a standard containing the amount of sea water corresponding to the non-conducting volume concentration, ρ_0, was measured as a check.

The frequency distribution of the differences, $\rho - \rho_0$ is shown in Fig. 1. Two suspensions of fertilized eggs are included and two determinations have been omitted. In one of the latter some cytolysis

took place and in the other the eggs settled slightly in the conductivity cell and left clear sea water above, which was not removed.

There is no evidence to indicate any injury or salt leakage of the eggs in the dextrose solution. The surface capacity and internal resistance of the eggs were not affected and there are no systematic differences between ρ and ρ_0. The eggs with their attached jelly probably assumed a somewhat regular arrangement in the cell, but this did not seem to invalidate the application of the Maxwell equation. It may be concluded that within the limits of experimental error, the cell membranes of both the unfertilized and fertilized *Hipponoë* eggs are non-conducting.

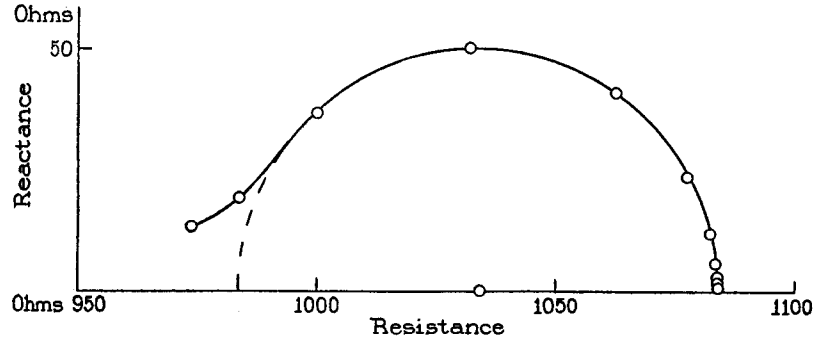

FIG. 2. Resistance R *vs.* reactance X in ohms for a 39.3 per cent suspension of unfertilized *Hipponoë* eggs.

Membrane Capacity

The equivalent series resistance, R, and reactance, X, were computed for each frequency, n, from the observed parallel resistance, R_p, and capacity, C_p, by the formulae,

$$R \doteq R_p;\ X \doteq R_p^2\, C_p\, \omega$$

where $\omega = 2\pi n$ and the $R_p^2 C_p^2 \omega^2$ term is negligible. The complex plane locus (Cole, 1928*a*) for an unfertilized suspension is shown in Fig. 2. It is seen that the phase angle of the variable impedance element is 90° over most of the frequency range, so it is concluded that the membrane impedance is predominantly that of a static capacity. This agrees with the observations at frequencies from 1.08 kc. to 10.8 kc.

that the parallel resistance and capacity of all suspensions were constant within the limits set by temperature variations and electrode polarization errors.

The membrane capacity per unit area, C_0, may then be computed from the formula (Cole, 1928 *a*)

$$C_0 = \frac{2c\,C_p}{(2 + r_1/r_0)\,(1 - r_1/r_0)\,a}$$

where c is the cell constant, C_p the parallel capacity of the suspension, r_1, and r_0, the resistances of sea water and the suspension at low frequency, respectively, and a the egg radius. For the suspension of Fig. 2, $c = 29.5$, $C_p = 70\ \mu\mu f$, $r_1 = 552$ ohms, $r_0 = 1082$ ohms, and $a = 39.5\mu$, so $C_0 = 0.85\ \mu f/\text{cm.}^2$. After standing several hours, the membrane capacity decreased, occasionally as much as 10 per cent. The

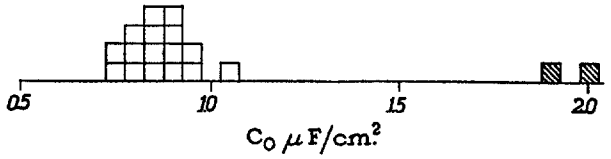

FIG. 3. Frequency distribution of the membrane capacity C_0 of *Hipponoë* eggs. Open blocks for unfertilized eggs and shaded blocks for fertilized eggs.

frequency distribution of the first measurement of C_0 on the eggs of sixteen females is shown in Fig. 3.

For unfertilized eggs, the average $C_0 = 0.87\mu f/\text{cm.}^2$, which is close to the value of 0.81 $\mu f/\text{cm.}^2$ for the red blood cell (Fricke, 1925) and $0.6\mu f/\text{cm.}^2$ for yeast (Fricke and Curtis, 1934). It is to be noticed that there is a rather wide variation between the eggs of different urchins, the largest value being $1.07\mu f/\text{cm.}^2$ and the two smallest, $0.77\mu f/\text{cm.}^2$

Quite by chance, the eggs of the two latter urchins were chosen for fertilized experiments. After insemination, less than 1 per cent of the eggs failed to raise membranes and nearly all underwent normal third cleavage. No measurements were made less than 10 minutes after insemination, but at that time one had a membrane capacity of 1.9 $\mu f/\text{cm.}^2$ and the other 2.0 $\mu f/\text{cm.}^2$ or an average of two and a half times the capacity of the unfertilized eggs. No changes were observed during the half hour the eggs were in the cell.

Internal Specific Resistance

The departure of the high frequency points from the semicircle is believed to be real, although it is just at the limit of the experimental error. Consequently an extrapolation to the infinite frequency resistance is difficult to justify. However, if the measurements are not in error, they strongly suggest the entrance of another reactive element into the picture. The divergence is more marked and extends to lower frequencies in the complex plane locus of the fertilized eggs shown in Fig. 4 which lends considerable weight to the hypothesis. From this viewpoint, the internal resistance, r_2, calculated by Equation 1 from $r = r_\infty$, the high frequency intercept of the semicircle on

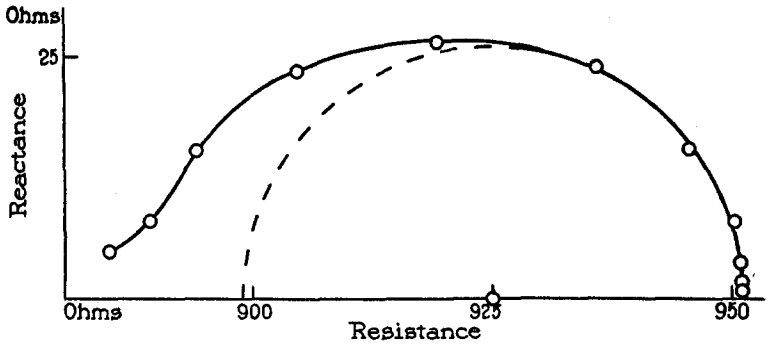

FIG. 4, Resistance R *vs.* reactance X in ohms for a 29.7 per cent suspension of fertilized *Hipponoë* eggs.

the resistance axis, should be the equivalent low frequency resistance of the egg interior. Then the values of r_2/r_1 range from 9.5 to 13.7. The average low frequency internal specific resistance is eleven times that of sea water for the unfertilized egg and eighteen times for the fertilized egg.

Swelling Experiments

Low frequency measurements were made on two lots of unfertilized eggs in equilibrium with successive dilutions of sea water. The eggs were measured in 100 per cent sea water and then in 80 per cent, 60 per cent, and 40 per cent sea water with a half hour allowed for them to reach equilibrium after each change. The egg diameters were not

measured, but were computed on the basis of an 11 per cent osmotically inactive volume as found for *Arbacia* (McCutcheon, Lucké, and Hartline, 1931). The membrane capacity per cm.^{2} *vs.* per cent increase of surface area is plotted in Fig. 5 for one series. It is very interesting that the specific capacity should be a linear function of the surface. Since the capacity per unit area increases as a macroscopic dielectric is made thinner, the observed decrease is surprising and puzzling, and suggests a constitutional change in the membrane. A single high frequency run in 44.5 per cent sea water showed a 37 per cent increase of the equivalent internal specific resistance.

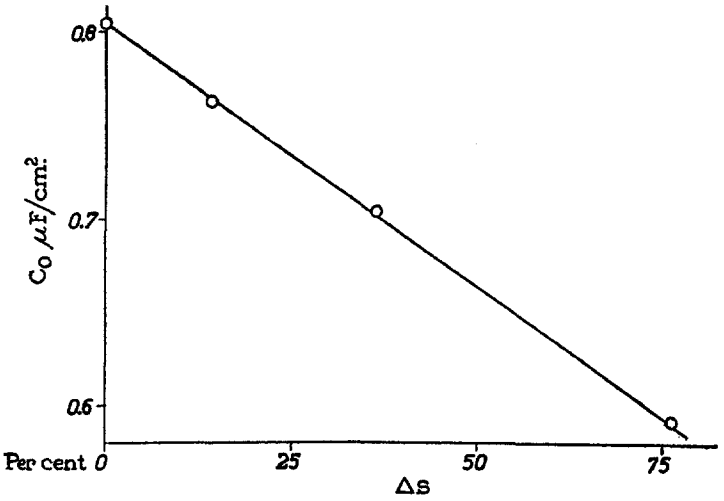

FIG. 5. Increase of surface area ΔS in per cent of normal *vs.* C_0 capacity per cm.^{2} of egg membrane for swollen unfertilized *Hipponoë* eggs.

DISCUSSION

The reactance of many tissues and of *Arbacia* eggs seems to be largely due to a membrane impedance of the type $z(\omega) = z_1(j\omega)^{-\alpha}$ (Cole, 1934) found in electrolytic electrode polarization and the selenium barrier layer photocell. Although there is no adequate theoretical basis for these phenomena or the empirical equation, the effect may be qualitatively explained as due to a back electromotive force resulting from a selective transfer of ions across the surface of discontinuity. For zero permeability of all ions, $\alpha = 1.0$, for complete

permeability, $\alpha = 0$, and for perfect permeability for one ion and zero for the other ion of a binary electrolyte, $\alpha = 0.5$ on the Warburg (1899) theory. Thus for the case $\alpha < 1.0$, some ionic permeability is to be expected. If then there is any permeability, we should expect a finite membrane resistance at zero frequency, and if there is a selective permeability we should expect α to be greater than zero and less than unity.

Thus it has been difficult to understand the relation of the ion permeability postulated from other considerations to the observed high resistance and static capacity of the red blood cell membrane. However, the experiments of Fricke and Curtis on blood (1935) and yeast (1934) suggest that the static capacity which predominates at high frequencies is of relatively slight physiological importance and that it is the small increments of resistance and capacity at low frequencies which seem to have the characteristics of a polarization impedance and may be correlated with the physiological condition of the cells. On the other hand the increase of the static capacity of *Hipponoë* eggs on fertilization and the decrease on swelling indicate that the seat of this capacity is not entirely inert. When both a static capacity and a polarization impedance are present, the former may predominate when the membrane is relatively impermeable and the latter when the permeability is greater. To predict the frequency range for the appearance of each would require a justifiable equivalent membrane circuit.

There is no obvious reason to suspect a large difference in the ion permeabilities of the *Hipponoë* and *Arbacia* eggs so it was quite surprising to find the predominance of the static capacity in the former when the latter indicated a polarization impedance. As careful measurements as the conditions would permit down to 1.08 kc. gave no definite evidence of the existence of a polarization impedance for the *Hipponoë* eggs. Even if present it might be very difficult to detect because of the low resistance of sea water and the high internal resistance of the eggs.

The internal specific resistance of eleven times that of sea water seems singularly high since *Arbacia* gave 3.5 times sea water, red blood cells twice plasma, and frog sartorius 3.5 times Ringer. If the nuclear membrane enters at the high frequencies, then it would be effectively

non-conducting at the frequencies for which this resistance is calculated. If the cytoplasm has a specific resistance 3.5 times that of sea water then from the Maxwell equation for a two phase sphere (Cole, 1928 *a*) the nucleus would occupy 60 per cent of the unfertilized egg and 73 per cent of the fertilized egg. Again, the nuclear membrane may not be involved and the internal resistance may be low, but the high frequency divergence due to a polarization impedance at the plasma membrane in such relation to the static capacity that it appears at high frequency rather than low. It is not possible at present to say whether the pronounced effect in the fertilized egg is due to a change in the postulated unknown element or whether it is the same as in the unfertilized egg, but unmasked by the increase in the static capacity on fertilization.

The author is particularly indebted to Dr. H. J. Curtis of the Biophysics Laboratory, Cold Spring Harbor, for his assistance and cooperation in this work.

SUMMARY

Alternating current resistance and capacity measurements have been made from $1.08\ 10^3$ to $2.32\ 10^6$ cycles per second on suspensions of unfertilized, fertilized, and swollen unfertilized eggs of the echinoderm *Hipponoë esculenta*. A simple method has been developed for measuring the volume concentration of eggs in a suspension.

The membrane of the unfertilized egg is practically non-conducting at low frequencies and shows a static capacity of 0.87 $\mu f/\text{cm.}^2$ except perhaps at the highest frequencies. The equivalent specific resistance of the egg interior is 11 times that of sea water.

The membrane of the fertilized egg is practically non-conducting at low frequencies and shows a static capacity 2.5 times that of the unfertilized egg except at the higher frequencies where another reactive element produces a marked effect. The internal resistance is apparently higher than that of the unfertilized egg.

The static capacity per unit area of the membrane decreases as a linear function of the surface area when the eggs are swollen in dilute sea water. In 40 per cent sea water, the capacity falls to about 75 per cent of normal.

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