ELECTRIC IMPEDANCE OF SINGLE MARINE EGGS*

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INTRODUCTION

It was first shown by Fricke (1925) that it is possible to calculate the electrical capacity of the membrane and the resistance of the interior of the red blood cell from impedance measurements made on suspensions of the cells. Since then these properties have been determined for many different types of cells with constantly improved methods of handling the suspensions, of measuring their impedance, and of interpreting the results. Although it is now possible to work with as little as 0.1 cc., the use of suspensions has definite limitations.

In its simplest form the analysis of the paths of the current flow, which forms the basis of the technique of interpretation, requires that the cells of the suspension shall be uniformly distributed, and shall all be identical spheres with identical electrical properties. As the theory becomes more complicated, it is possible to deal with cells having spheroidal form and, less satisfactorily, with suspensions in which the capacity and resistance vary from cell to cell. Also, the interpretation should not be expected to be valid above a 60 per cent volume concentration of cells in the suspension, but, on the other hand, the measurements become rather difficult when the volume concentration is less than 10 per cent. There are many cases where it is difficult or impossible to obtain 0.02 cc. of identical cells and to maintain them in a uniform 20 per cent suspension. For them a technique requiring only a single cell would be very valuable, and although a few of the large unicellular plants have been measured individually, (Blinks, 1936; Curtis and Cole, 1937), the methods used are not easily adaptable to smaller cells.

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Probably the simplest procedure for measuring a single cell is to slide a cell into a snugly fitting capillary tube and measure the impedance between electrodes at the two ends of the tube.

Method

To obtain a satisfactory accuracy of measurement it is only necessary that the resistance of the electrolyte between the electrodes and the cell shall be relatively small, but difficulties are to be expected in the interpretation of the data. A complete theoretical picture of the paths of current flow is more difficult to obtain than in the case of a suspension, partly on account of the geometry of the system and partly because it is not easy to determine the separation between the cell membrane and the capillary wall. However, with the method of measurement and rather crude analysis to be outlined, it has been possible to check the method with single unfertilized and fertilized whole *Arbacia* eggs, and obtain preliminary

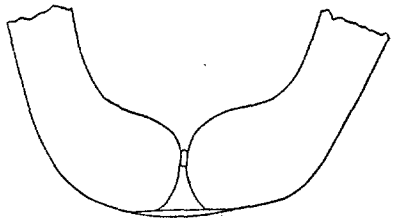

FIG. 1. Line tracing made from a photomicrograph of an unfertilized *Arbacia* egg in a 48μ capillary.

data on unfertilized and fertilized white *Arbacia* halves, whole *Cumingia* and *Chaetopterus* eggs, and unfertilized red *Arbacia* halves.

The end of a clean 2 or 3 mm. thin walled glass tube is heated until it closes down to a short capillary somewhat smaller than the diameter of the egg to be measured. The center portion of such a capillary is usually quite uniform in diameter and, by the proper choice of the initial wall thickness and diameter, almost any desired diameter and length of capillary can be obtained. A flat face is ground and polished on the outside of the tube so that the capillary may be seen without distortion under a microscope.

The outside of the tube is covered with a layer of vaseline to prevent surface losses at high frequencies, filled with sea water, and the capillary end immersed in sea water. An egg is then placed in the tube and when it has settled to the top of the capillary, the outside water level is raised or lowered to push it to the center as shown in Fig. 1. The middle portion of the egg is cylindrical and the ends are nearly hemispherical. When the inside and outside pressures are then equalized, the egg will remain in position and in good condition for long periods. Platinized platinum electrodes are then placed in the sea water inside and outside the tube and the impedance between them is measured.

These measurements have been made at frequencies from 1 to 2500 kc. (kilocycles per second) with the Wheatstone bridge which has been described by Cole and Curtis (1937). At the end of a frequency run the egg was forced out by raising the pressure, and a sea water run was made. From these data, the series resistance and reactance at each frequency were computed and plotted as the impedance locus.

The method is quite good electrically, although these are errors for frequencies above 2500 kc. which prevent the use of higher frequencies. It is often a tedious and tiresome task to get an egg satisfactorily placed in the capillary. Some eggs are quite difficult to handle and, for example, every *Arbacia* red half went into the two cell stage before it could be placed. It is expected that the manipulation can be made more positive and rapid than it is at present.

For the most part the eggs seem to remain in good condition in the capillary. Only one or two cytolyzed, and a fertilized egg will go into the two and four cell stages in the capillary, although it has not been possible to fertilize an egg in place. Furthermore, several of the *Arbacia* eggs showed 90° phase angles which suggests that they, at least, were quite normal.

DATA AND INTERPRETATIONS

Whole Arbacia Eggs

To investigate the possibilities of this single egg method, the first data were taken on whole unfertilized and fertilized *Arbacia punctulata* eggs whose characteristics have been determined from measurements on suspensions.

The impedance loci, for a single unfertilized *Arbacia* egg, shown in Fig. 2, and for a single fertilized *Arbacia* egg, shown in Fig. 3, have the same form as the loci for suspensions of these eggs when they are found in good condition. These loci show that either egg can be represented over a wide frequency range by equivalent circuits having a single capacity and two or more resistances, such as the two shown in Fig. 4. In both of these $\bar{R}$, the resistance of the sea water between the electrodes and the egg, has not been measured directly. It has been eliminated for either circuit by subtracting from the measured resistance of the capillary when filled with sea water, the calculated resistance of a cylinder of sea water having the diameter of the capillary and the volume of the egg. At very high frequencies, where the membrane impedance is negligible, the specific resistance of the egg interior is computed by assuming the egg to have this same cylindrical form. On the other hand, at low frequencies, it will be assumed that

the membrane impedance of the egg is so high that the current density in the exposed hemispherical ends of the membrane is essentially uniform. The effective membrane area at each end will then be that of the hemisphere and the membrane capacity and resistance for a unit area will be computed on this basis.

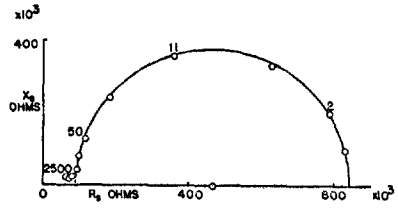

FIG. 2

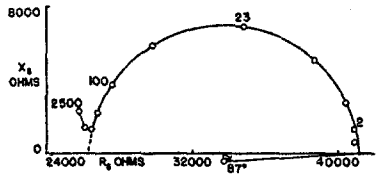

FIG. 3

FIG. 2. Impedance locus, series resistance, R_s, *vs.* series reactance, X_s, for a single unfertilized *Arbacia* egg. Frequencies indicated are in kilocycles per second.

FIG. 3. Impedance locus, series resistance, R_s, *vs.* series reactance, X_s, for a single fertilized *Arbacia* egg. Frequencies indicated are in kilocycles per second.

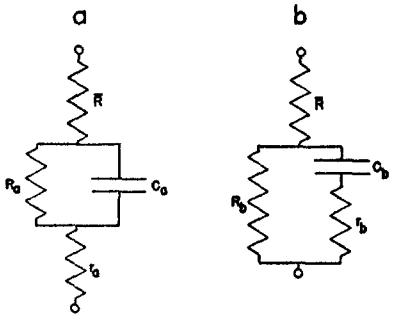

FIG. 4. Equivalent circuits for a single egg. (*a*) Assuming no leakage around the egg. (*b*) Assuming cell membrane to be non-conducting.

If we now assume that there is no current leakage around the egg, in circuit *a* of Fig. 4, R_a and C_a represent the resistance and capacity of the two hemispherical end portions of the membrane in series, and r_a is the resistance of the interior. The computed membrane capacities are 1.4 and 3.15 μf./cm.^{2} for two unfertilized eggs and 16 and 43 μf./cm.^{2} for two fertilized eggs, and the membrane resistances are 1.3 and 13 ohm cm.^{2} for the unfertilized and 0.17 and 0.41 ohm cm.^{2}

for the fertilized eggs. These capacities are much larger than those obtained from measurements on suspensions while the membrane resistances are so low that they should have been detected in suspension measurements.

Going to the other extreme, we may assume that the membrane resistance is so high that at low frequencies all the current flow is around the egg. In this case, which is represented by circuit *b* of Fig. 4, R_b is the resistance of the sea water between the membrane and the capillary wall, C_b the capacity of the membrane, and r_b the resistance of the interior. C_b is then computed from the low frequency parallel resistance R_0 and capacity C_0 by

$$C_b = (R_0^2/\bar{R}^2)C_0.$$

TABLE I

Membrane Capacity and Internal Resistance of Whole Arbacia Eggs Computed on the Basis of Circuit b of Fig. 4

	Membrane capacity		Internal resistance
	μf./cm.²	φ	
Unfertilized	1.08	90°	7.05 × sea water
"	1.17	90°	7.7 × " "
Fertilized	2.7	90°	10. × " "
"	2.9	87°	7.2 × " "

When C_b has a phase angle less than 90°, it is corrected to the characteristic frequency. R_b and r_b are determined by the low and high frequency extrapolations. The results from the calculation of the data on this basis are given in Table I, and they are not only self consistent, but in quite good agreement with those obtained from suspensions. Primarily for this reason we shall assume that, as a first approximation, the current flow at low frequency is entirely around the egg and not through the membrane.

Arbacia Halves, Cumingia, and Chaetopterus Eggs

The few measurements which have been made on cells not previously measured are given in Table II. The *Arbacia* halves were available through the courtesy of Dr. Ethel B. Harvey (1932), and

the *Cumingia* and *Chaetopterus* eggs were obtained by the usual techniques.

DISCUSSION

Without a complete analytical solution for the current flow, it is difficult to determine the errors due to the approximations which have been made. It seems probable that the assumption of the cylindrical form for the estimation of $\bar{R}$ and the resistance of the interior does not

TABLE II

Membrane Capacity and Internal Resistance of Various Marine Eggs Computed on the Basis of Circuit b of Fig. 4

	Membrane capacity		Internal resistance
	μf./cm.²	ϕ	
Arbacia halves			
Red unfertilized	0.62	90°	14.5 × sea water
" "	1.4	80°	17.6 × " "
" "	2.15	89.5°	29.7 × " "
" "	2.29	90°	31. × " "
White unfertilized	0.64	—	—
" "	0.625	87°	5.0 × " "
" "	0.635	90°	5.0 × " "
" fertilized	2.25	87.5°	5.1 × " "
Cumingia			
Unfertilized	2.68	90°	7.25 × " "
Fertilized	2.05	90°	6.58 × " "
"	2.62	—	—
Chaetopterus			
Unfertilized	1.15	82°	7.8 × " "
Fertilized	1.32	—	—
"	1.08	—	—

involve an error of more than 5 per cent. The use of the hemispherical caps for the calculation of membrane capacity and resistance seems quite permissible when the space between the egg and the glass wall is small, but otherwise it is difficult to justify. Since 90° phase angles are found, it is quite probable both that the current density is uniform over the effective area and that the effective area changes relatively little with frequency. At the present time, probably the most satisfactory justification of these approximations is

the fact that they lead to the previously determined values for the *Arbacia* eggs.

The assumption of a high membrane resistance also is more attractive because it leads to reasonable numerical values, and there are other observations which are consistent with it. When an unfertilized egg with some adherent jelly is placed in the capillary, the low frequency resistance is usually quite low. When the egg is run up and down in the capillary several times the resistance becomes progressively higher and it can be seen that the egg comes closer and closer to the capillary wall. Assuming that all the current flows through the membrane, we are forced to the conclusion that such an egg starts with a very low membrane resistance and high membrane capacity, and that the membrane resistance increases and membrane capacity decreases during this process. On the other hand, assuming a non-conducting membrane, the membrane capacity remains constant throughout and the calculated separation between the membrane and the capillary wall decreases. Thus the latter assumption not only leads to a picture which is more reasonable, but also agrees with the microscopic observations.

A membrane resistance of 25 ohm cm.^{2} or less should give a difference between the actual and the non-conducting volume concentration on the suspension measurement of more than 1 per cent. Although the spread of these differences in the preceding paper (Cole and Spencer, 1938) is large, the average is so nearly zero it seems unlikely that any low values of membrane resistance are to be expected. On the other hand, the highest low frequency resistance yet found for a single egg, over 800,000 ohms, would correspond either to a membrane resistance of 13 ohm cm.^{2} or to a layer of sea water about 0.2 μ thick between the membrane and its capillary wall. The former possibility thus seems improbable while the second is very difficult to disprove.

Turning to the fertilized eggs, it was always found that in the capillary they had a relatively low resistance, and behaved and looked like an unfertilized egg with considerable jelly. Assuming a non-conducting plasma membrane and a completely permeable fertilization membrane, we arrive at acceptable values for the thickness of the perivitelline space, and as shown in Table I, for the membrane capacity and internal resistance.

It is fairly certain, on the basis of these single egg observations that the plasma membrane resistance of both unfertilized and fertilized *Arbacia* eggs is at least considerably greater than the 25 ohm cm.^{2} limit justified by the suspension data. It should be understood, however, that the assumption of a completely non-conducting membrane, which has been used in the interpretation of the data, in no way denies an ionic permeability to the membrane. It merely means that at low frequencies for measurements of this particular type on single eggs and for measurements on suspensions, the current flow through the membrane is so small that it is not possible to detect the difference between a sufficiently small ionic permeability and none at all.

Although an equivalent constant phase angle of less than 90° can result from a statistical distribution of static membrane capacities among the cells of a suspension, the occurrence of low phase angles for single eggs demonstrates that a phase angle of less than 90° can be an inherent characteristic of the egg cell membrane.

The data on fertilized eggs are entirely consistent with a complete ionic permeability for the *Arbacia* fertilization membrane and an increased plasma membrane capacity. Comparing the impedance locus for the fertilized egg, shown in Fig. 3, with the corresponding locus in the previous paper (Cole and Spencer, 1938), it will be seen that there is no deviation from the circle at intermediate frequencies as there is on the curve for a suspension. This deviation is discussed at some length there, and it is pointed out that at least part of it could be explained as being due to non-uniformity of the material. If further experiments on single eggs verify this one experiment in showing no deviation at intermediate frequencies, they will lend rather conclusive support to the non-uniformity postulate.

There are sufficient data on the half *Arbacia* eggs to indicate quite definitely that the membrane capacity for the red half is considerably higher, and for the white half somewhat lower than for the whole egg. It also seems that the granules in the red half increase the internal resistance more than does the oil of the white half. It is possible that the cause of the unexplained high frequency effect shown by whole eggs is primarily transferred to the red half on separation, but the highest frequency data are too uncertain to prove this as yet.

The unfertilized *Chaetopterus* egg showed a membrane capacity slightly higher than the average for other forms, and that of *Cumingia* was even higher. In these forms, the apparent equality of the capacities before and after fertilization is of considerable interest, but should be substantiated by further measurements.

SUMMARY

Alternating current impedance measurements have been made on several single marine eggs over the frequency range from 1 to 2500 kilocycles per second. The eggs were placed in the center of a short capillary made by heating the end of a 2 mm. thin walled glass tube until it nearly closed, and electrodes were placed in the sea water on each side of the egg.

When it is assumed that the membrane conductance is negligible, the membrane capacity and internal resistances of unfertilized and fertilized *Arbacia* eggs agree with the values obtained from suspensions. Preliminary data on centrifugally separated half *Arbacia* eggs, and whole *Cumingia* and *Chaetopterus* eggs are given.

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