

ELECTRIC IMPEDANCE OF ARBACIA EGGS

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In the first paper on this subject (Cole, 1928), the absolute value of the impedance of suspensions of *Arbacia* eggs was determined as a function of frequency of the measuring current and the following conclusions were given:

“The specific resistance of the interior of the egg is about 90 ohm cm. or 3.6 times that of sea water and the impedance of the surface of the egg is probably similar to that of a polarization capacity. The characteristics of this surface impedance can best be determined by measurements of capacity and resistance of suspensions of eggs. No specific change has been found in the interior resistance or the surface impedance which can be related either to membrane formation or to cell division.”

The finding of a static capacity, *i.e.* a capacity which does not vary with frequency, for the membrane of the unfertilized *Hipponoë* egg, two and a half times this capacity for the fertilized egg, and a much higher equivalent internal resistance (Cole, 1935) made it desirable to measure both the resistance and the capacity of the *Arbacia* egg over as wide a frequency range as possible. The results on the unfertilized *Asterias* egg (Cole and Cole, 1936) further emphasized the necessity for such measurements.

Material

Live *Arbacia punctulata* were shipped from Woods Hole packed in seaweed and on ice. The ovaries were removed and placed in sea water or the injured animal was allowed to shed into sea water. The eggs were washed, centrifugalized lightly, and drawn into the conductivity cell, as was done for the *Hipponoë* and *Asterias* eggs. When a fertilized run was to be made, a few of the lower frequency points were measured on the unfertilized eggs. The suspension was then withdrawn, diluted with sea water, inseminated, washed to remove excess sperm, centrifugal-

ized lightly, and returned to the conductivity cell. Over an hour was required for a complete frequency run and it was anticipated that the first cell division might complicate the experiments. It was found, however, that the electrical characteristics remained constant and that the eggs did not undergo first cleavage while in the cell. After diluting such a suspension with sea water most of the eggs went into the two cell stage but many stopped there and those which did proceed further developed abnormally. The material was not in especially good condition, but there was very little cytolysis.

The conductivity equipment of the Biophysics Laboratory, again available to us through the courtesy of Dr. Hugo Fricke, the other apparatus, corrections, and calculations are the same as for the *Asterias* work and are described in the preceding paper.

Data

The data for a typical unfertilized suspension are given in Table I and the complex plane locus plotted in Fig. 1. This locus is much the same as that of *Hipponoë* (as far as that went) and that of *Asterias*, except that the frequencies for corresponding points are higher. This is due in part to the smaller size of the egg and in part to the smaller capacity. The membrane capacity is again independent of frequency. The low frequency phenomena found for the *Asterias* egg are again present and the low frequency capacity is extrapolated in the same manner as before. In the data given, $C_0 = 140 \mu\mu\text{f.}$, so the membrane capacity $C_M = 0.74 \mu\text{f./cm.}^2$ by the usual formula. The average value of C_M is $0.72 \mu\text{f./cm.}^2$. A divergence similar to that for *Asterias* is sometimes found at the high frequency end of the circle. The calculation of the equivalent low frequency internal specific resistance leads to a value of 184 ohm cm. The average value is 186 ohm cm. or about 6 times sea water. The behavior of the curve at the highest frequencies is similar to that of *Asterias* except that it does not go so far, which makes an extrapolation to infinite frequency even more dangerous. Without attempting to assign any especial importance to it, this extrapolation leads to an average equivalent high frequency internal specific resistance of 120 ohm cm.

The data for a fertilized *Arbacia* suspension are given in Table II and Fig. 2 is the complex plane locus. It is much the same as for the fertilized *Hipponoë* eggs, but with the added high frequency tail. The extrapolated value of the low frequency capacity $C_0 = 560 \mu\mu\text{f.}$, so the static membrane capacity $C_M = 3.1 \mu\text{f./cm.}^2$. These eggs gave

TABLE I

Suspension of Unfertilized Arbacia Eggs of Diameter 72.4 μ

Resistance of sea water, $R_W = 440.0$ ohms. Volume concentration, $\rho = 53.7$ per cent. Extrapolated capacity, $C_0 = 140\mu\text{f}$. Cell constant, $K_2 = 14.53$. Temperature, 21.7°C .

Frequency		R_S	X_S
No.	n		
		<i>ohms</i>	<i>ohms</i>
1	$1 \cdot 10^3$	1209.2	1.562
2	2	1209.3	2.843
3	4	1209.4	5.375
4	8	1209.3	10.64
5	$1.6 \cdot 10^4$	1208.4	20.60
6	3.2	1202.6	40.55
7	6.4	1190.0	75.45
8	$1.28 \cdot 10^5$	1146.0	125.0
9	2.56	1052.0	150.1
10	5.12	967.5	125.9
11	$1.024 \cdot 10^6$	916.0	79.15
12	2.048	893.0	49.15
13	4.1	882.8	39.90
14	8.2	871.9	48.15
15	$1.64 \cdot 10^7$	857.0	50.30

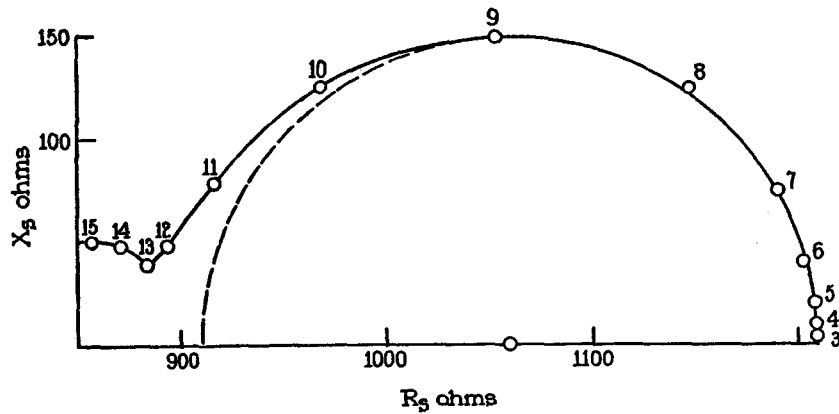


FIG. 1. Complex plane locus, series resistance, R_S , vs. series reactance, X_S , for a suspension of unfertilized *Arbacia* eggs. Frequencies given in Table I.

TABLE II

Suspension of Fertilized Arbacia Eggs of Diameter 72.4 μ

Resistance of sea water, $R_W = 436.2$ ohms. Volume concentration, $\rho = 42.8$ per cent. Extrapolated capacity, $C_0 = 560\mu\text{f}$. Cell constant $K_2 = 14.53$. Temperature, 21.5°C.

Frequency		R_S	X_S
No.	n		
		<i>ohms</i>	<i>ohms</i>
1	$1 \cdot 10^8$	1025.0	4.01
2	2	1024.3	7.63
3	4	1023.4	14.83
4	8	1020.0	28.90
5	$1.6 \cdot 10^4$	1005.0	54.50
6	3.2	977.5	84.30
7	6.4	919.6	98.60
8	$1.28 \cdot 10^6$	866.0	83.60
9	2.56	826.0	57.22
10	5.12	808.0	47.45
11	$1.024 \cdot 10^6$	790.0	34.35
12	2.048	781.0	22.20
13	4.1	772.1	35.40
14	8.2	762.9	35.45
15	$1.64 \cdot 10^7$	752.4	32.95

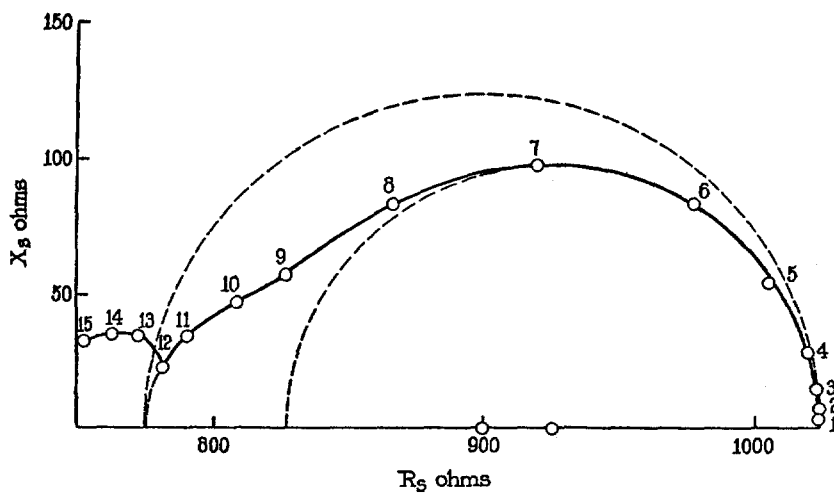


FIG. 2. Complex plane locus, series resistance, R_S , vs. series reactance, X_S , for a suspension of fertilized *Arbacia* eggs. Frequencies given in Table II.

a value of $C_M = 0.73 \mu\text{f./cm.}^2$ before fertilization, so the low frequency capacity increased about 4 times on fertilization. There was no change in the surface capacity effect at the lowest frequencies which could be correlated with fertilization. The low frequency data lie quite well on a circle up to the maximum reactance as was found for the fertilized *Hipponoë* eggs. The extrapolation of the high frequency end of this circle leads to an equivalent low frequency internal specific resistance of about 11 times that of sea water. The high frequency data are approximately the same as for the unfertilized egg.

DISCUSSION

Almost everything that was said of the unfertilized *Hipponoë* and *Asterias* eggs is applicable to the unfertilized *Arbacia* eggs. The static membrane capacity is about $0.72 \mu\text{f./cm.}^2$ and the low frequency surface capacity effect is not qualitatively different from that in *Asterias*. The low frequency internal specific resistance is about the same: 6 times that of sea water, and on the assumption of a highly conducting nucleus, its volume would be about 2 per cent of the egg volume. If the internal nuclear resistivity were the same as that of the cytoplasm, the nucleus would have a volume about 7 per cent that of the egg volume, and the resistivity of the cytoplasm would be 120 ohm cm. or about 4 times that of sea water. The order of magnitude of such a "nuclear" membrane capacity is probably again $0.1 \mu\text{f./cm.}^2$. The data of Harvéy, 1932, give the nuclear volume as 0.4 per cent, so that on the basis of the above extrapolations the nucleus can only be a fraction of the material contributing to the high frequency effect.

The increase of static membrane capacity on fertilization seems to be somewhat larger for *Arbacia* than for *Hipponoë*, and the extrapolated value of the equivalent low frequency internal specific resistance, although high, is somewhat less for *Arbacia* than for *Hipponoë*. There seems to be no reasonable explanation of this increase of internal resistance on fertilization and no attractive interpretation of the course of the curve after it leaves the circle, so it will now be assumed that the internal resistance remains unchanged on fertilization. If then the membrane were non-conducting and had a static capacity independent of frequency, we would obtain the large dotted semicircle of Fig. 2. This may be taken as an indication that the internal conduct-

ance is unchanged and the departures from the unfertilized circle are due solely to the membrane. It is then possible to calculate the membrane capacity as a function of frequency, the result of which is shown in Fig. 3. The marked similarity of the form of this curve to a dielectric dispersion due to a dipole relaxation time is shown by the comparison in the figure with the theoretical curve calculated from the Debye theory (Debye, 1929). However, it has not been possible to take into account the energy dissipation which accompanies such a phenomenon, so this hypothesis cannot be taken too seriously until a mechanism can be proposed which will give a change of dielectric

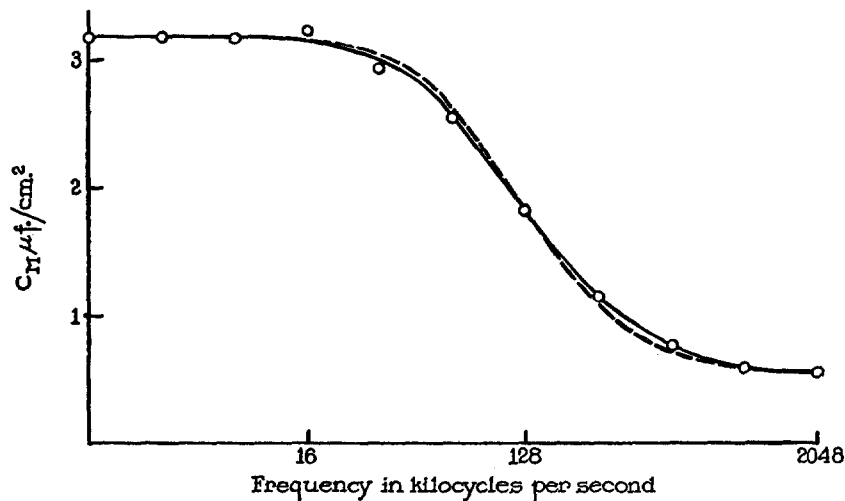


FIG. 3. Log frequency, n , vs. membrane capacity, C_M , for fertilized *Arbacia* eggs.

constant without dissipation. Another possibility will therefore be considered.

The outer portion of the fertilization membrane may be postulated to have a high static capacity, and be laid down at an appreciable distance from the plasma membrane of the unfertilized egg, while the interspace has the conductivity of sea water. Then at very low frequencies the current which does pass through the fertilization membrane will follow the layer of sea water rather than go through the plasma membrane and the capacity will be that due to the fertilization membrane alone. At higher frequencies, most of the current will

cross the plasma membrane and the cytoplasm, so the capacity will be that of the two membranes in series. Although a detailed analysis is not yet available, approximate calculations make this picture a very attractive one. The separation between the membranes would have to be 1.5μ for the *Arbacia* egg and somewhat less for the *Hipponoë* egg on this basis. The capacity of the plasma membrane is then $0.73 \mu\text{f./cm.}^2$ and that of the fertilization membrane is $3.12 \mu\text{f./cm.}^2$, so that high frequency capacity of the membranes in series should be $0.59 \mu\text{f./cm.}^2$ whereas $0.55 \mu\text{f./cm.}^2$ is the observed value.

The incomplete form of the data at the highest frequencies does not warrant any conclusions other than that there seems to be no qualitative difference between the unfertilized and the fertilized egg in this respect, and the speculations as to the cause of this effect are thus equally applicable.

In view of the wide disagreement between these conclusions and those drawn in the earlier paper, it is interesting to inquire as to the cause. Assuming an homogeneous egg interior, our present infinite frequency extrapolations give it an average specific resistance of 4 times sea water which is comparable with the value of 3.6 previously obtained. It is of course obvious that in the former work the frequency intervals were too great and the accuracy of the impedance measurement (1 per cent) not high enough to recognize the existence of the high frequency element. Even this is not sufficient to account for the rather highly dissipative polarization impedance previously found. The discrepancy has been investigated in some detail and on the basis of the present data it is not possible to explain the previous data in any way except to blame the material. This seems very unreasonable, since there is little doubt but that the material in the earlier work was in better condition than in the present case. It has occasionally been found that eggs which were in very poor condition gave a polarization impedance, but the *Hipponoë* eggs, which were in excellent condition, gave results consistent with the present ones.

On the other hand, it is comparatively easy to understand how the effect of fertilization was missed. In the first place, the series reactance is so small that there is never more than a 2 per cent difference between the series resistance and the absolute value of the impedance. Second, the frequency range where the effect of a change of capacity would be most marked occurs where the impedance is changing most

rapidly. A large capacity increase would have been observed except for the fact that in this particular frequency range the effective membrane capacity has fallen to about the same value as that of the unfertilized membrane.

We are very much indebted to Dr. Hugo Fricke and Dr. Howard J. Curtis of the Walter B. James Laboratory for Biophysics for the courtesy and cooperation which made this work possible.

SUMMARY

The alternating current resistance and capacity of suspensions of unfertilized and fertilized eggs of *Arbacia punctulata* have been measured at frequencies from 10^3 to 1.64×10^7 cycles per second.

The unfertilized egg has a static plasma membrane capacity of $0.73 \mu\text{f./cm.}^2$ which is practically independent of frequency. The fertilized egg has a static membrane capacity of $3.1 \mu\text{f./cm.}^2$ at low frequencies which decreases to a value of $0.55 \mu\text{f./cm.}^2$ at high frequencies. The decrease follows closely the relaxation dispersion of the dielectric constant if the dissipation of such a system is ignored.

It is considered more probable that the effect is due to a fertilization membrane of $3.1 \mu\text{f./cm.}^2$ capacity lifted 1.5μ from the plasma membrane, the interspace having the conductivity of sea water.

The suspensions show a frequency-dependent capacity at low frequencies which may be attributable to surface conductance.

The equivalent low frequency internal specific resistance of both the unfertilized and fertilized egg is about 186 ohm cm. or about 6 times that of sea water, while the high frequency data extrapolate to a value of about 4 times sea water.

There is evidence at the highest frequencies that the current is penetrating the nucleus and other materials in the cytoplasm. If this effect were entirely due to the nucleus it would lead to a very approximate value of $0.1 \mu\text{f./cm.}^2$ for the capacity of the nuclear membrane. The measurements do not indicate any change in this effect on fertilization.

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