ELECTRIC IMPEDANCE OF FERTILIZED ARBACIA EGG SUSPENSIONS*

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In previous work on the alternating current impedance of *Arbacia punctulata* egg suspensions (Cole and Cole, 1936*b*), it was found that the unfertilized egg had a static membrane capacity of 0.73μf./cm.^{2} which was independent of the frequency. On fertilization, the low frequency membrane capacity increased to 3.1μf./cm.^{2} and at intermediate frequencies there was evidence of another capacity element. It was suggested that the fertilization data could be interpreted either by an increased plasma membrane capacity which varied with frequency or by an added high capacity of the fertilization membrane. The latter picture was considered the more probable one, and diagrams of the current flow were subsequently published (Cole, 1937). The present work was undertaken to determine whether or not this explanation was correct.

Measurements

The impedance of the suspensions was measured over the frequency range from 1 kc. (kilocycle per second) to 10 mc. (megacycles per second) with an alternating current Wheatstone bridge which has been described (Cole and Curtis, 1937).

For the first eggs available in June, the method of preparation of the suspensions and the impedance cell in which they were measured were the same as had been used in the previous work. The membranes did not consistently have 90° phase angles, which would indicate static capacities, but varied from 87° to 90° for the unfertilized eggs and from 85° to 90° for the fertilized eggs. Furthermore, the

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intermediate frequency effect which had been at its height at 512 kc. (see Fig. 2, Cole and Cole, 1936 *b*) was much less pronounced.

It was then thought that the eggs might have been injured by handling and a new impedance cell was designed to minimize this possibility. This cell, shown in Fig. 1, is essentially a glass centrifuge tube with two platinized platinum electrodes near the bottom. Its total volume is 13.3 cc. and the volume of the impedance cell proper, with a cell constant of 12.58 $cm.^{-1}$, is 0.8 cc. Since the diameter of the electrodes is somewhat larger than the internal diameter of the tube

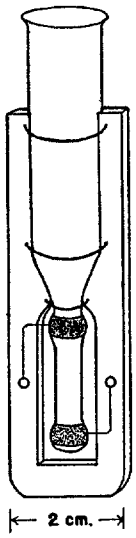

FIG. 1. Impedance cell described in text

between them, the electrode polarization correction is considerably diminished at the cost of an increased volume. The cell is wired and cemented into a Victron holder which allows it to fit into a standard centrifuge cup. Washed dilute suspensions of the eggs were transferred to the cell, allowed to settle, and then centrifuged very lightly to minimize the time required for steady low frequency resistance and capacity values to be attained.

Although suspensions were used when inseminated samples showed over 90 per cent fertilization membranes, they were nearly always over 95 per cent. After measurement, the suspensions were diluted and a

similar viability and satisfactory development found. As noted before, in the volume concentrations usually used, 30 to 50 per cent, the fertilized eggs will remain in the single cell stage for a considerable period and yet proceed normally to at least the 16 cell stage when diluted at the end of a run.

After this change in technique, the membrane phase angles of both the unfertilized and fertilized eggs were, with a single exception, found to be 90° at low frequencies as shown in Figs. 2 and 3. It will be seen that for the fertilized suspension of Fig. 3, although the points corresponding to frequencies above 100 kc. still show a definite departure from the semicircle which corresponds to a single capacity element, the divergence is much less than had been found previously (see Fig.

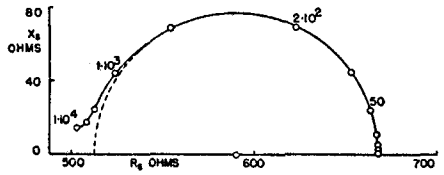

FIG. 2

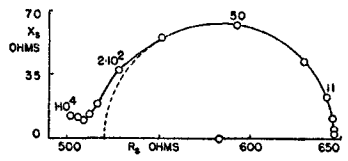

FIG. 3

FIG. 2. Impedance locus, series resistance, R_s *vs.* series reactance, X_s, for a 52.1 per cent volume concentration suspension of unfertilized *Arbacia* eggs in sea water. Frequencies indicated are in kilocycles per second.

FIG. 3. Impedance locus, series resistance, R_s *vs.* series reactance, X_s, for a 50.3 per cent volume concentration suspension of fertilized *Arbacia* eggs in sea water. Frequencies indicated are in kilocycles per second.

2, Cole and Cole, 1936 *b*). Thus it seems that the interpretations which were based on this characteristic in the earlier data are probably not applicable. In a more thorough investigation, the principal problem is the definite localization of the high capacity membranes in the unfertilized and fertilized eggs. Such membranes may be expected to have relatively high electrical resistances and, with this initial assumption, their positions can be determined from volume concentration and low frequency measurements of the suspensions.

Volume Concentration of Suspensions

When the suspended eggs are enclosed by a poorly conducting membrane, the low frequency resistance of the suspension, r_0, is the same

as it would be if the eggs were solid non-conductors, and is given by the Maxwell formula, $\rho_0 = 2\ (1 - r_1/r_0)/(2 + r_1/r_0)$, where r_1 is the resistance of the medium and ρ_0 is the non-conducting volume concentration. For a cubic centimeter of suspension containing n_0 eggs, $\rho_0 = n_0 v_0$, where v_0 is the volume enclosed by the non-conducting surface of a single egg. If the volume concentration, ρ, of this same suspension is determined by another method, $\rho = n_0\, v$ where v is the volume defined by the method, and then of course, $\rho/\rho_0 = v/v_0$. For the dextrose method, used in the work on *Hipponoë* eggs (Cole, 1935), ρ/ρ_0 was very close to unity, so that the volume enclosed by the ion impermeable membrane was practically identical with that enclosed by the dextrose impermeable membrane. Although it is generally believed that the fertilization membrane is freely permeable to both ions and dextrose, and that the plasma membrane is not, it is obvious that the dextrose method alone does not give pertinent information, and that one depending upon microscopic observations on the eggs must be used. The following procedure was used. After the impedance measurements had been made, the suspension was weighed and its volume computed with an assumed egg density of 1.09 gm./cc. (Harvey, 1932). The suspension was diluted with 250 cc. of sea water and the number of eggs in small fractions counted as suggested by Parpart (Shapiro, 1935) to give n_0. The average plasma membrane enclosed volume, v_p, and fertilization membrane enclosed volume, v_f, were calculated from measurements of the respective diameter in fifty eggs, and expressed in terms of the non-conducting membrane enclosed volume, ρ_0, by v_p/v_0 and v_f/v_0. The rather unsatisfactory sampling technique is probably largely responsible for the wide spread of the results which are given in Fig. 4. The plasma membrane enclosed volume averaged 1.6 per cent less than the non-conducting membrane enclosed volume in ten unfertilized suspensions, and 2.6 per cent less in twenty fertilized suspensions, while the fertilization membrane enclosed space averaged 32 per cent greater than the non-conducting membrane enclosed volume.

Membrane Capacity

The membrane capacities, C_M, have been determined for eleven suspensions of unfertilized and seventeen suspensions of fertilized eggs by the equation (Cole, 1928 *a*),

$$C_M = \frac{2C_0 k}{\left(2 + \frac{r_1}{r_0}\right)\left(1 - \frac{r_1}{r_0}\right) a}.$$

Here k is the cell constant of the impedance cell, r_1 the resistance of the sea water, and r_0 the low frequency parallel resistance of the suspension, a, the radius of the plasma membrane. C_0, the low frequency parallel capacity of the suspension due to the membrane was found by the method used previously (Cole and Cole, 1936 *a*). The distribution of these capacities is shown in Fig. 5. We are quite

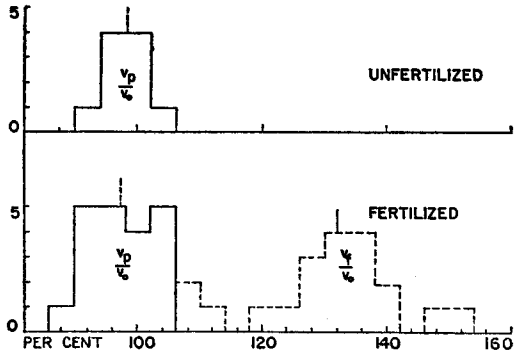

FIG. 4

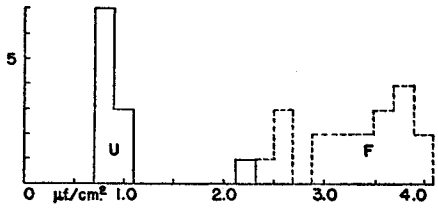

FIG. 5

FIG. 4. Frequencies per 4 per cent interval of the ratios of the plasma and fertilization membrane enclosed volumes, v_p and v_f, to the non-conducting membrane enclosed volume v_0, for unfertilized and fertilized suspensions. The mean values are indicated above each group.

FIG. 5. Frequencies per 0.2 μf./cm.² interval of the membrane capacities of unfertilized, *U*, and fertilized, *F*, eggs.

unable to explain the high capacity of one unfertilized batch of eggs which was the last of the season. However, ignoring this value, we obtain average membrane capacities of 0.86 μf./cm.² before fertilization and 3.3 μf./cm.² after fertilization.

Internal Resistance

The internal resistances of the eggs may be estimated as has been done previously (Cole, 1935) from the high frequency extrapolation obtained by neglecting the highest frequency points as indicated by the dotted lines in Figs. 2 and 3.

For the unfertilized eggs an average value of 5.7 times the resistance of sea water is obtained from ten suspensions. Twenty fertilized suspensions gave 7.2 times sea water.

Interpretation and Discussion

The non-conducting volumes of both the unfertilized and fertilized eggs were slightly larger than the plasma membrane volumes even though the outermost visible edge of the latter membranes was measured. This fact and a statistical analysis both give us reason to believe that the differences involved are within the experimental limits, and one may then say that the high resistance membrane is either at, or very close to, the plasma membrane in both the unfertilized and fertilized egg. Both because the fertilization membrane volume is significantly larger than the non-conducting volume and because the latter is little, if any, different from the plasma membrane volume, it is quite improbable that the resistance of the fertilization membrane and perivitelline space is appreciably different from sea water. As a consequence, it seems practically impossible for the fertilization membrane to show a measurable capacity. The capacity change on fertilization must then take place at or very near the plasma membrane, and the picture previously presented is untenable. On fertilization it might be assumed either that one of two closely adjacent membranes became completely permeable or that the structure of a single membrane so altered as to increase its capacity. But there seems to be no basis for a choice between these two and probably several other possibilities.

We are still faced with the unpleasant necessity of explaining the large intermediate frequency effect in the earlier fertilized egg data, and, for that matter, the smaller effect which still persists. The simplest and most obvious explanation is that the fertilized suspensions were not homogeneous, but contained unfertilized eggs or eggs having unfertilized membrane capacities. Some support is given to this explanation by the data on a single fertilized *Arbacia* egg (Cole and Curtis, 1938, Fig. 3), which showed no intermediate frequency effect. Calculation shows that in the earlier work on *Arbacia* and *Hipponoë*, it would have been necessary for 25 to 35 per cent of the eggs to have had unfertilized membrane capacities. Unfortunately, there are no

data available on the development of these eggs, but it is believed that the percentages of unfertilized eggs were considerably lower than these figures.

On the same basis we calculate from 90 to 100 per cent of fertilized eggs from the present data on suspensions which showed better than 90 per cent fertilization and early development. There is not a complete agreement between the two sets of values as is illustrated by the suspension of Fig. 3, which showed better than 99 per cent development in the 16 cell stage. The impedance locus shows the largest intermediate frequency effect which was found in the present work, corresponding to only 90 per cent with fertilized membrane capacities. We are then left with the possibility that a few eggs may show normal early development but still retain the "unfertilized" membrane capacity after fertilization.

It is difficult to interpret either the absolute values or the change of the internal resistance on fertilization until it is possible to make measurements at considerably higher frequencies than have been used in this work. There is an indication that the highest frequency phenomenon is not dependent upon the integrity of the cell.

It should be pointed out that the 90° phase angle which is found at lower frequencies means that there is no wide variability of membrane capacities among the eggs from a single female. It is found that apparent phase angles less than 90° can be obtained when there is a statistical distribution of the product $C_M a$ among the eggs in the suspension (Cole and Curtis, 1936). We may conclude that the statistical variation of membrane capacities is no greater than the variation of diameters of the eggs from a single female.

There is as yet no certain explanation for the low phase angles which were first found in this work. It may be that these are characteristic of early season eggs, but it seems more likely that they are a result of injury. In either case it is not possible to say for certain either that every egg had a membrane phase angle less than 90°, or that every egg had a 90° phase angle, while the membrane capacities and diameters varied from one egg to another so that the apparent phase angle of the "average" egg was less than 90°. The fact that single eggs with phase angles less than 90° have been measured (Cole and Curtis, 1938) argues against the latter explanation. If injury does cause the

membrane phase angle to fall below 90°, the results in the first *Arbacia* paper (Cole, 1928*b*) may in part be explained as the result of injury due to stirring the suspensions by bubbling.

SUMMARY

From the low frequency alternating current impedance and the volume concentrations of suspensions of *Arbacia* eggs, it is shown that the high resistance membrane is either at or very near the plasma membrane for both unfertilized and fertilized eggs, and that the specific resistances of the perivitelline space and fertilization membrane are not greatly different from that of sea water. The effect of the capacity element which appears after fertilization at intermediate frequencies is considerably less than in the earlier experiments on *Arbacia* and *Hipponoë* eggs.

These findings indicate that the fertilization membrane does not have the high capacity previously attributed to it and that the increase in membrane capacity takes place at or near the plasma membrane.

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