ELECTRIC IMPEDANCE OF SUSPENSIONS OF ARBACIA EGGS.

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In work done several years ago on the heat production of the *Arbacia* egg at fertilization and during early development (11) the suggestion was made that the phenomena observed might be due to changes in the cortex. Inasmuch as the heat data can give no clue to the action of component parts of the egg, another means of analysis was sought which might give an answer to the various questions raised. The work of Fricke and Morse (3) and Philippson (10) suggested that the impedance of suspensions of eggs to various frequencies of alternating current might yield valuable facts regarding the electric capacity and resistance of the surface of the egg, and the resistance of the interior, and the changes of these three quantities at fertilization and during development.

Apparatus.

Although it was first planned to use a capacity bridge to measure both the resistance and the capacity of the suspensions of *Arbacia* eggs it was found after considerable work that the bridge had several very serious defects for this particular work. In the first place it became very complex as the range of frequencies to be investigated was extended (3). This made it necessary to devote an undue amount of time to the taking of each reading and the changing from one frequency to another. In the second place, unless one were willing to go to the very ultimate in amplification it was necessary to use an undesirably high current density in the electrolytic cell. In this work the aim was to keep the potential difference across each egg so low that the

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current probably would in no way affect it. From these and other considerations it was decided to use a variation of the "ammeter-voltmeter" method of measuring resistances. This method, while less sensitive and giving less data, is on the other hand direct reading and can be made independent of the frequency over a wide range. At first glance it would appear that this was the method used by Philippson (9), but it soon becomes evident that what he used was essentially a comparison method which involved many of the inaccuracies and troubles of the bridge method and gave only the results and accuracy of the direct reading method which has been used in the present work. A small alternating current of the desired frequency was sent through an electrolytic cell containing the suspension and simultaneous measurements were made of the current through the cell and the potential difference across it. The ratio of these two gave the magnitude of the impedance of the cell at that frequency but did not, however, give the phase angle.

Oscillator.—The source of supply of alternating current was a vacuum tube oscillator. This oscillator is of the type known as "tuned plate circuit" and while the type is well known (2) and very commonly used, this particular instrument has several unusual features of design and operation. Although its range can probably be extended in both directions, it has been used entirely between the frequencies of one thousand and fifteen million cycles per second. It is completely shielded electrostatically and is controlled only by switches on the panel over the entire range of frequencies. It has a readily changed but constant output and its frequency calibration has remained remarkably constant. While it is usual to take the output from such an oscillator by means of a coil loosely coupled to the tuned circuit, it was not found advantageous to do so in this oscillator. In addition to the necessity of switching both the grid and plate coils as the frequency was changed this output coil would have to be changed and it would also be a decided advantage in such a case to be able to vary the coupling. These needs would have made the oscillator so much slower and less flexible that it was decided to take the output from the main oscillatory circuit as is shown in Fig. 1. The inductance L' is very small as compared with the main oscillator inductance L but carries the oscillatory current flowing through the condenser.

Over the frequency range of any single inductance L the potential difference across L' remains comparatively constant. This output is connected to the electrolytic cell through a resistance sufficiently high to prevent reactions of the output back on the oscillatory circuit.

Frequency.—A two-turn coil was loosely coupled to the coil L'

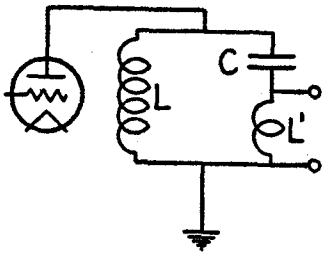
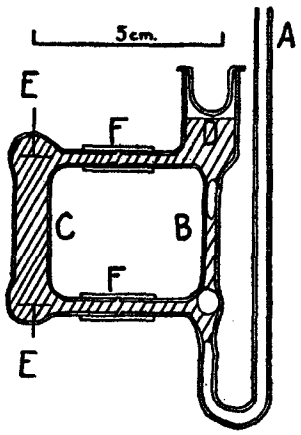


FIG. 1.



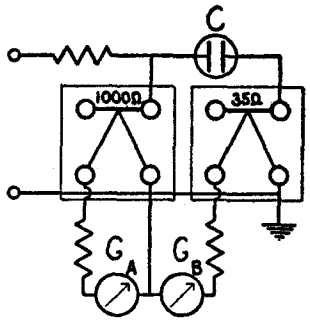


FIG. 2. FIG. 3.

FIG. 1. Method of taking output from vacuum tube oscillator.
FIG. 2. Electrolytic cell and stirrer.
FIG. 3. Circuit for measurement of impedance.

within the oscillator. When the frequency was to be measured, connections from this coil were made through the shielding to a similar coil which was coupled to a General Radio Precision Wavemeter. The resonance point of the wavemeter was determined by means of a vacuum tube voltmeter connected directly across the variable condenser.

Electrolytic Cell.—The factors governing the design of the cell were several from both the biological and the electrical standpoints. In order to prevent settling of the suspended eggs it was necessary to have continuous but gentle stirring. It was also necessary to provide for adequate oxygenation of the suspension. The total volume of the cell should be small in order that a sufficient volume concentration may be obtained with the eggs from a single specimen. From the electrical standpoint it was desirable to have the cell constant such that the impedance would be a convenient value. It was also necessary to have the design such that at low frequencies the error in the impedance caused by the polarization capacity should be small while at high frequencies the error in the impedance caused by the static capacity should be small. For the first case, a comparatively simple analysis assuming a straight line current flow shows that for any given frequency, specific resistance of electrolyte, and polarization capacity per unit area of electrode, the error in the impedance is independent of the area of the electrodes but varies inversely as the square of their separation. On the other hand, a somewhat similar analysis of the error introduced by the static capacity shows that it is entirely uninfluenced by the shape of the cell—as long as the lines of current flow are straight—but is proportional to the product of the specific resistance and the frequency. For any given suspension the cell constant is fixed by the measuring apparatus and the volume of the cell is determined by the amount of material available. These two quantities then determine the dimensions of the cell and it remains to be seen if the error introduced by polarization capacity at low frequencies is negligible. If it is not then the apparatus must be modified, other material used, or corrections introduced. If the error due to the static capacity is not small enough to be neglected at the high frequencies there is only one alternative, that of lowering the specific resistance of the suspension, which may demand the use of other material in certain cases. From these considerations the cell shown in Fig. 2 was designed and found to fullfil all of the requirements

A supply of saturated air at constant pressure was connected to tube A. The bubbles rising in B forced the suspension in that tube before them and gave rise to a circulation in a counter-clockwise direction, giving an entirely adequate stirring and at the same time allowing

a thorough oxygenation of the suspension. Considerable difficulty was experienced from persistance of bubbles; the surface tension of suspension was considerably different from that of sea water as was shown both by this persistance of bubbles at D and by increased size of the bubbles rising in tube B, Fig. 2. If these bubbles were not broken as soon as they appeared at the surface a froth was formed which contained a sufficiently large number of eggs to change the volume concentration noticeably. The only satisfactory method of breaking these bubbles was to place just above the surface of the suspension, at D, a "U"-shaped piece of glass rod which had previously been rubbed on the oily nose of the observer. The electrolytic cell proper C was connected by short pieces of rubber tubing at F F. The electrodes E E were of platinum covered with a very light and very fine deposit of platinum black plated on at as low a current density as possible. It was rather important that these electrodes be placed with their planes containing the axes of the side tubes, for otherwise there was a tendency for the eggs to pile up either above or below them. The cell used in this work has a constant of 5.85. No difficulty was experienced from either polarization capacity or static capacity, a perfectly constant impedance being obtained with sea water between the frequencies of 1000 cycles per second and 15 million cycles per second, D, Fig. 4. The pressure of the air supply was so regulated that there was always at least one bubble in the tube B. It was found that under these conditions about 10 cc. of air at atmospheric pressure passed through the cell per minute. The lowering of the impedance when there was no bubble in tube B was only 4 per cent, so it was assumed that the error introduced by the presence of the film of solution between the bubble and the glass wall of B was entirely negligible. The volume of suspension required was 4 cc.

Measuring Apparatus.—The measuring apparatus consisted of two Western Electric vacuum thermocouples connected to two Leeds and Northrup type *R* low resistance galvanometers. The deflections of both galvanometers were read by light spots thrown on the same scale.

In the vacuum thermocouples there is a small filament of metal or carbon known as the heater through which the current to be measured passes. Attached to the center of this heater element are two wires of dissimilar materials forming a thermocouple to measure the tem-

perature rise in the heater wire resulting from the heat produced by the current to be measured. This whole arrangement is placed in a glass bulb which is then evacuated. The evacuation greatly increases the sensitivity and at the same time makes the arrangement independent of small changes of room temperature. It is found that for small currents the E.M.F. of the thermo-junction is directly proportional to the square of the current flowing through the heater. For this work a couple having a 1000 ohm heater was used to measure the potential drop across the cell and the other thermocouple. This second thermocouple was used to measure the current through the cell and had a resistance of 34 ohms. It was soon found that this arrangement would not work at high frequencies, due to the fact that both galvanometers had a considerable capacity to ground while neither one was at ground potential. This state of affairs led to a more and more effective short circuiting of one-half of each of the heater elements as the frequency was increased beyond a certain point. The solution was to use only one-half of each heater element, Fig. 3—one of the thermocouple terminals serving for the other heater connection. In this way both galvanometers were at the same potential as the grounded side of the output from the oscillator. The high resistance thermocouple then served as an alternating current voltmeter and the low resistance thermocouple as an alternating current ammeter When but one-half of a heater element is used the sensitivity of the device is multiplied by a factor of $\sqrt{2}$ when used as a voltmeter and divided by the same factor when used as an ammeter. From the constants of the thermocouples and the galvanometers it was found that

$$|Z| = 150 \sqrt{s_A/s_B} \text{ ohms.}$$

Experimentally it was found that

$$|Z| = 151 \sqrt{s_A/s_B} \text{ ohms,}$$

where s_A and s_B are the deflections of the voltmeter and ammeter galvanometers respectively, and $|Z|$ is the absolute value of the impedance.

The apparatus was checked over the entire range of frequencies by means of a specially designed shielded resistance whose impedance

was constant up to 10 million cycles, being in error by only 1.6 per cent at that frequency. It was found that the impedance of the electrolytic cell containing sea water, curve D, Fig. 4, was constant up to the highest frequency used, about 15 million cycles. The apparatus has also been checked by measuring the impedance of condensers and inductances.

In actual practice, the square root of the ratio of deflections of the two galvanometers was multiplied by a factor of 151 to give the impedance of the electrolytic cell plus the ammeter heater. This latter was a pure resistance of 18.4 ohms. At both the very high and the very low frequencies, the cell filled with a suspension of eggs had a zero phase angle and the resistance of the series heater could be subtracted directly to give the resistance of the cell suspension, but at the intermediate frequencies the phase angle was not zero and this fact had to be taken into account in the determination of the impedance of the cell suspension.

With the apparatus in its final form it was possible to take readings every 40 seconds over long periods and half of this time interval was consumed in recording the data. The actual impedance measurements had an accuracy of about 1 per cent. For full scale deflections the current through the cell was 0.002 ampere, and the potential drop across it 0.3 volt.

Data.

Of the living animal cells which were available, the eggs of the Echinoderm *Arbacia punctulata* seemed the most satisfactory because of their uniformly spherical shape, their hardiness, and the possibility of using large numbers of them and having them keep "in step" for a considerable period after fertilization. The amount of investigation to which these eggs have been subjected and the availability of a moderate volume of eggs from a single specimen added to their desirability.

Over a thousand measurements of the impedances of suspensions of biological material were made at the Marine Biological Laboratory at Woods Hole during the summer of 1927. Although all of the analysis has been confined to the results on *Arbacia* eggs some data were taken on red blood corpuscles and on the eggs of *Asterias* and *Fundulus*.

Procedure.

The filament of the oscillator vacuum tube was usually turned on for at least 15 minutes in order to allow it to reach equilibrium before any readings were taken. Then a run was taken with the standard resistance to check the constancy of calibration of the thermocouples and galvanometers. A second run was taken with the electrolytic cell filled with sea water to determine the conductivity of the sea water and make certain that the error due to polarization capacity of the electrodes was negligible.

The ovaries of the *Arbacia* were removed entire from the specimen and placed in a finger bowl half filled with sea water. After standing for several minutes they were gently shaken with forceps and removed. The suspension was then carefuly poured through wet unbleached muslin and allowed to settle. After settling the clear sea water was either pipetted or poured off, or else the eggs were brought to the center of the finger bowl by moving it in a circle and then pipetted into fresh sea water. In order to get rid of as much jelly as possible the suspension was gently poured from one finger bowl to another several times at each washing. Sometimes the eggs were centrifuged by hand just enough to throw them down to get the concentration desired before removing them to the electrolytic cell, but this procedure was avoided wherever possible. As soon as the suspension was placed in the cell the air line was connected and the stirring and aeration continued without interruption until the end of the experiment.

A set of readings usually consisted of the zero readings of both galvanometers with the oscillator disconnected and then the readings of both galvanometers at seven or eight different frequencies. After half a dozen or so sets of readings, the eggs were fertilized by the thorough stirring in of a small amount of "dry" sperm and readings continued until after first cleavage. In one or two runs the observations were continued up to 3 hours after fertilization but never longer than that. Very small samples were frequently removed from the cell for microscopic examination. At the end of the run the suspension was removed from the cell and centrifuged to determine the volume concentration of the eggs.

An entirely satisfactory technique for the handling of the eggs has

not been worked out as yet and while normal development was obtained in not over half of the runs made it was considered expedient to take some data on eggs—normal or abnormal—rather than to have normal development and no data. Polyspermy was probably the most common cause of abnormality and was not recognized as such

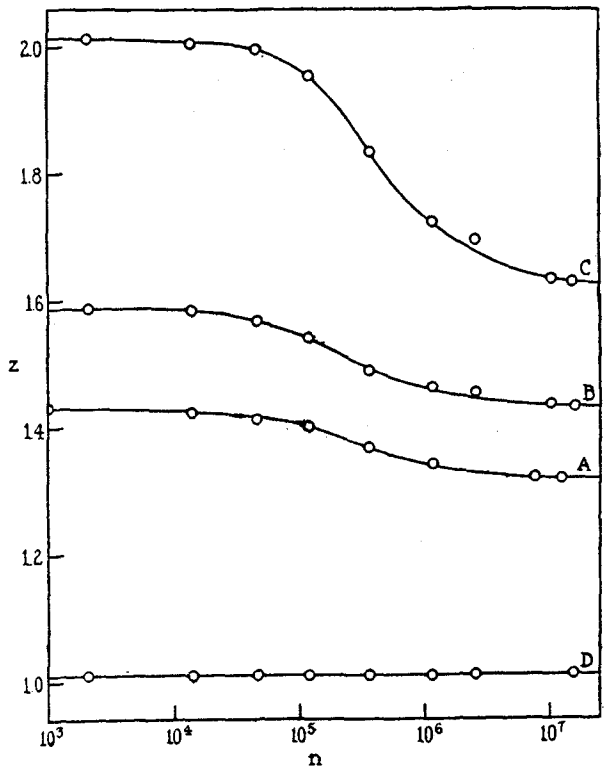


FIG. 4. Impedance (Z, arbitrary units) *vs.* log frequency (n, cycles per second) for sea water and *Arbacia* egg suspensions.

for some time due to the failure of the eggs to raise membranes although fertilized. The reason for this may be that in the concentrated suspensions used a slight cytolysis would elevate the osmotic pressure to such a degree that the membranes would not lift until the suspension was diluted with sea water.

Computations.

The general type of impedance variation with frequency is shown in the three curves of Fig. 4 for suspensions of *Arbacia* eggs in sea water. This change in impedance with frequency can mean but one thing, namely, some element or elements in the egg have an impedance which decreases as the frequency of the measuring current is increased. For the present it will be assumed that the *Arbacia* egg consists of a homogeneous electrolytically conducting interior having a very thin surface layer of vastly different electrical properties, which may be functions of frequency. Since the impedance approaches constant values at both the low and the high frequencies, R_0 and R_∞, it is suggested that at the low frequencies the impedance of the surface is so high that it renders negligible the effect of the internal conductivity, whereas at high frequencies its impedance is so low as to be itself negligible. Having R_w, R_B, and ρ—the resistances of the sea water, B thermocouple heater, and the volume concentration of the eggs—as well as R_0 and R_∞, it is possible to apply the Eq. (1) given in the previous paper[1] for the determination of an average specific resistance of the egg at both high and low frequencies,

$$\frac{r_1/r - 1}{r_1/r + 2} = \rho \frac{r_1/r_2 - 1}{r_1/r_2 + 2} \tag{1}$$

where r, r_1, and r_2 are the specific resistances of the suspension, the suspending medium, and the suspended spheres respectively. For convenience the following notations have been employed,

$$\frac{\alpha_0 - 1}{\alpha_0 + 2} = \rho \frac{\beta_0 - 1}{\beta_0 + 2} \ (2), \quad \frac{\alpha_\infty - 1}{\alpha_\infty + 2} = \rho \frac{\beta_\infty - 1}{\beta_\infty + 2} \ (3),$$

where

$$\alpha_0 = \left(\frac{r_1}{r}\right)_0 = \frac{R_w - R_B}{R_0 - R_B}, \ \beta_0 = \left(\frac{r_1}{r_2}\right)_0,$$

$$\alpha_\infty = \left(\frac{r_1}{r}\right)_\infty = \frac{R_w - R_B}{R_\infty - R_w}, \ \beta_\infty = \left(\frac{r_1}{r_2}\right)_\infty.$$

[1] Cole, K. S., *J. Gen. Physiol.*, 1928, xii, 29.

For rapid calculation a curve of α *vs.* $\frac{\alpha-1}{\alpha+2}$ has been found very useful. From the α given by the data as shown above, the corresponding $\frac{\alpha-1}{\alpha+2}$ is found from this curve. When divided by the observed ρ, this gives $\frac{\beta-1}{\beta+2}$ from which β is found by using the same curve in the reverse direction. Since r_1 is known (usually about 25 ohm cm.) the absolute value of r_2 can be found.

By the above means β_0 and β_∞ have been found for all of the data taken and their values for the runs shown in Fig. 4 are given in Table I. The variations in β_0 and β_∞ are quite typical of all the data taken and are far greater than could be accounted for by errors in the electrical measurements. This conclusion is in accord with the observation

TABLE I.

	ρ	α_0	β_0	α_∞	β_∞	ρ'
	per cent					*per cent*
A	22.2	.733	.08	.804	.282	19.6
B	38.0	.676	.275	.763	.452	24.2
C	44.3	.477	.028	.647	.308	42.5

that the variations in both β_0 and β_∞ were always in the same direction from one run to another but that both were relatively constant during any one run. Eliminating ρ from Eq. (2) and Eq. (3) we have

$$\frac{\alpha_0 - 1}{\alpha_0 + 2} = K \frac{\alpha_\infty - 1}{\alpha_\infty + 2} \tag{4}$$

where K involves only β_0 and β_∞ and should thus be independent of ρ. A plot of $\frac{\alpha_0-1}{\alpha_0+2}$ against $\frac{\alpha_\infty-1}{\alpha_\infty+2}$ for all of the data gave a straight line passing through the origin with $K = 1.41$. This was the final check which verified the electrical measurements and placed all of the blame for the variations on the measurements of ρ, the volume concentration of eggs.

In Table I, ρ' is the volume concentration calculated from the

electrical data on the assumption of Eq. (6) of the previous paper[1] that $\beta_0 = 0$ or that at low frequencies the eggs are very poor conductors. The more reliable values of ρ indicate that this assumption is very close to the truth. For this case, from Eq. (4) we have

$$K = -\frac{1}{2}\frac{\beta_\infty + 2}{\beta_\infty - 1} \tag{5}$$

from which $\beta_\infty = r_1/r_2 = 0.28$. The specific resistance of the interior of the egg is then about 3.6[2] times that of the sea water or 90 ohm cm. As said before, ρ was obtained by centrifuging the suspension at the end of the run. This was never very satisfactory as it was desired to allow the eggs to develop further in order to determine their condition. Consequently the suspensions were centrifuged only enough to give what was thought to be "constant volume." The results show that this was not the case and several other methods of determining the volume concentration have been suggested and will be tried in future work. The formula has never been accurately checked with *Arbacia* simply because of this difficulty in the determination of ρ.

As a first approximation it has been assumed that the surface of the egg acts like a pure capacity, with $r_3 = 0$—corresponding to Fricke's conclusion for the red blood corpuscle and Philippson's assumptions for tissue. On this hypothesis, take Eq. (12) of the previous paper[1]

$$\frac{x_3}{\gamma} = \sqrt{\frac{|z|^2 - r_\infty^2}{r_0^2 - |z|^2}} = s, \tag{6}$$

and s may now be computed directly from the impedance on suspensions of *Arbacia* eggs, as has been done in Table II and Fig. 5 for the runs plotted in Fig. 4. The straight lines are drawn arbitrarily with an intercept of 1.0 at $n = 3.10^5$ and a slope of -0.5 in each case. It will be noticed that the slope of each of these lines is about -0.5 and that s is not greatly affected by the volume concentration.

$|Z|$ has been computed from the two sets of data of Fricke and Morse

[2] Fricke and Morse (3) find the resistance of the interior of the red blood corpuscle to be 3.5 times that of the plasma.

(3) and the values of s are given in Table III and plotted in Fig. 6. The slope of the line is -0.93.

Philippson's data (10) gives slopes of about -0.5 for animal tissues and -0.75 for vegetable tissues.

TABLE II.

A			B			C		
n	$\|Z\|$	s	n	$\|Z\|$	s	n	$\|Z\|$	s
$1.0 \cdot 10^3$	1.433		$2.0 \cdot 10^3$	1.589		$2.0 \cdot 10^3$	2.015	
$1.35 \cdot 10^4$	1.425	3.84	$1.38 \cdot 10^4$	1.586		$1.38 \cdot 10^4$	2.008	
$4.45 \cdot 10^4$	1.414	2.3	$4.55 \cdot 10^4$	1.571	2.64	$4.55 \cdot 10^4$	1.99	5.1
$1.15 \cdot 10^5$	1.403	1.71	$1.16 \cdot 10^5$	1.542	1.56	$1.16 \cdot 10^5$	1.955	2.83
$3.48 \cdot 10^5$	1.368	.865	$3.59 \cdot 10^5$	1.491	.77	$3.59 \cdot 10^5$	1.834	1.08
$1.15 \cdot 10^6$	1.343	.51	$1.13 \cdot 10^6$	1.465	.51	$1.13 \cdot 10^6$	1.723	.56
$7.33 \cdot 10^6$	1.322		$2.5 \cdot 10^6$	1.454	.41	$2.5 \cdot 10^6$	1.695	.44
$1.2 \cdot 10^7$	1.317		$1.0 \cdot 10^7$	1.436		$1.0 \cdot 10^7$	1.634	
			$1.5 \cdot 10^7$	1.429		$1.5 \cdot 10^7$	1.626	

TABLE III.

n	$\|Z\|$	s	$\|Z\|$	s
$.087 \cdot 10^6$	191		332	
.833	180	2.03	317	2.04
1.17	172	1.43	306	1.435
1.52	166	1.155	297	1.135
2.04	157	.875	286	.88
3.04	146	.618	271	.613
3.82	140	.507	261	.456
4.52	136	.419	256	.371
	124		244	
	ρ = 46.0 per cent r_1 = 89.4 ohm cm.		ρ = 17.9 per cent r_1 = 291.0 ohm cm.	

If x_3 were due to a perfect dielectric capacity c_3

$$x_3 = s\,\gamma = \frac{1}{c_3\,\omega}$$

and the slope of the log s *vs.* log ω curve would be -1.0. This is in fair agreement with the data for red blood corpuscles but in no way

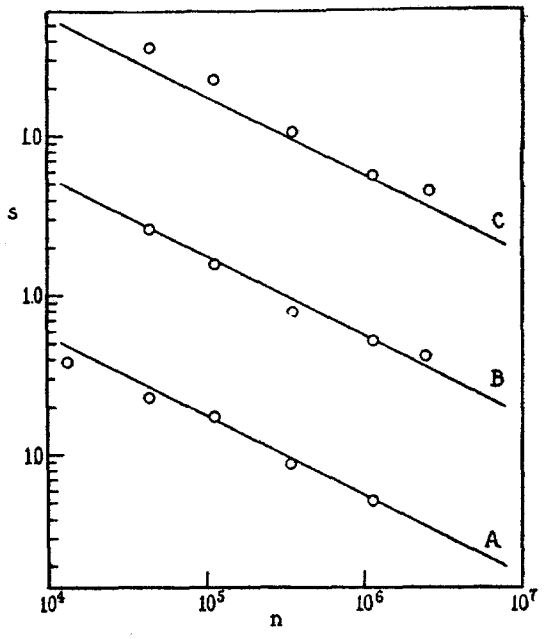


FIG. 5. Log s (Eq. 6) *vs.* log frequency (n, cycles per second) for *Arbacia* egg suspensions.

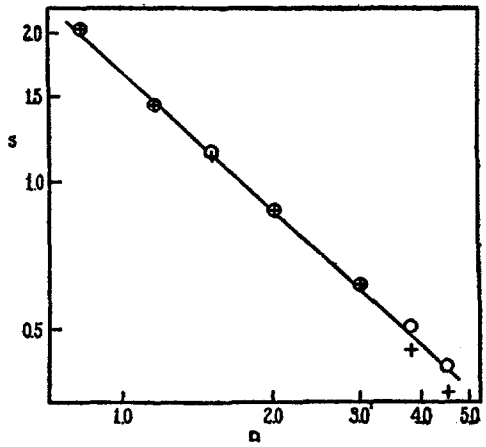


FIG. 6. Log s (Eq. 6) *vs.* log frequency (n, megacycles per second) for red blood corpuscle suspensions; data of Fricke and Morse.

agrees with that on tissues or on eggs—these giving capacities which vary inversely as the -0.25 and -0.5 power of the frequency. As stated in the previous paper, such variable capacities found in physical electrolytic systems have been classed as polarization capacities, and have associated with them an equivalent series polarization resistance. That the same is true for living systems is borne out by computations made on Gildemeister's data (4) for frog skin and on Blinks' unpublished data (1) for *Valonia*. For a single set of data for frog skin it was found that $m = 0.755$ and that the capacity varied as the -0.57 power of the frequency. For three sets of data it was found for *Valonia* that m was fairly constant for each case and varied from 0.63 to 0.75. Also the capacities varied with frequency[3] as a power having values from -0.13 to -0.23. From the biological point of view we should expect $r_3 > 0$, since it may be expected to be more or less proportional to the permeability for ions—being an equivalent *series* resistance. In such cases, the initial assumption that $r_3 = 0$ is not valid, and it is necessary to go to the more general expression, Eq. (10),[1] for $|z_3|$. When impedance data alone are available it is impossible to use this relation without the assumption that m of Eq. (13) is constant. Even then, no convenient method of computation has as yet been found. It has however, been possible to show empirically by the calculation of hypothetical data that as m is allowed to take on different values in different cases—but assumed constant in each case—,

$$\frac{|z_3|}{\gamma} = s^{\rho},$$

where ρ varies from one to two as m goes from zero to infinity. If

$$s = s_0 \omega^q$$

where $s_0 = s$ when $\omega = 1$, then

$$\frac{|z_3|}{\gamma} = s^{\rho} = s_0{}^{\rho}\, \omega^{\rho q}.$$

It follows therefore that if we assume $r_3 > 0$, and consequently $m > 0$, we find that $|z_3|$ instead of varying as the -0.5 or -0.75 power of ω, will vary as a power of ω more nearly -1.0.

[3] *Cf.* Osterhout (8).

Since there is good reason to believe that $m \neq 0$, and no certainty that it is constant, unsupported impedance measurements have no immediate decisive value. It may still be interesting, however, to calculate what this capacity might be if it were a capacity alone. Since the measurements of volume concentrations have been so very unsatisfactory it is convenient to use an expression for γ which does not involve ρ explicitly. It can be shown that

$$\gamma = \frac{r_0^2 (2 + r_1/r_0) (1 - r_1/r_0) a}{2 (r_0 - r_\infty)}.$$

This relation has been used in the calculation of x_3 in place of γ of Eq. (9) of the previous paper.[1] The values c_3 for n about $3.5 \cdot 10^5$ cycles per second are given in Table IV.

TABLE IV.

	r_0	r_∞	r_1	n	s	c_3
A	33.8	30.8	24.8	$3.48 \cdot 10^5$	.865	1.02 μf/cm.^{2}
B	37.8	33.7	25.6	3.59	.77	1.03
C	52.3	38.8	25.0	3.59	1.08	.86

$2a = 75 \cdot 10^{-4}$ cm.

If we extrapolate to 10^3 cycles per second on the assumption that c_3 varies inversely as the 0.5 power of the frequency, we find $c_3 = 18$ μf/cm.^{2}

For red blood corpuscles Fricke found 0.8 μf/cm.,^{2} which he computed would be the capacity of a monomolecular layer of oil if the static capacity formula used was assumed to hold for such dimensions. The value of 18 μf/cm.^{2} would thus lead to a layer only one-twentieth of this thickness at 1000 cycles and it would become "thinner" still at lower frequencies. This line of reasoning suggests that the barrier to ions at the surface of the cell—if a dielectric—must be of less than atomic dimensions, and leads one to the interesting possibility that the restriction on ionic transfer may be due largely to repulsive and attractive electrostatic forces of absorbed ions at the cell surface.

Fertilization.

In almost every run it was noticed that before insemination the values of the impedance for any given frequency were quite variable, giving rise to similar variations in the average specific resistance of the egg at both the high and the low frequencies. Immediately upon fertilization however, these quantities became quite constant and did not change noticeably thereafter. While it is possible that the temperature control was not adequate, it is more probable that the variations were due to some change in the average condition of the eggs in the suspension. It has been suggested that the cause may lie in the differing states of maturation of the eggs taken from different ovaries or different parts of the same ovary, and that the initiation of development completely masked these prefertilization irregularities. As a result it can only be said that there was found no specific change in the conductivity of the interior of the egg, or the impedance of the surface which can be definitely ascribed to the membrane formation. Previous work by Gray (6) and McClendon (7) has shown that there is a change in the low frequency conductivity of a centrifuged mass of eggs upon fertilization.[4] It may very well be that the change is so small that it is beyond the limits of the present apparatus. It should, however, be pointed out that at low frequencies almost all of the current flows through the intercellular spaces since the surface of the egg offers a very high impedance to low frequency currents. Under these conditions a small change in the size of the eggs (5) or in the conductivity of the intercellular liquid would cause a considerable change in the conductivity of the centrifuged mass, as has been pointed out by others. At low frequencies, $|z_3|$ may be so large that—even when it varies considerably—Eq. (6) of the previous paper[1] should be true. While this equation cannot be expected to hold except for small values of ρ, it is worth noticing that if ρ is near unity, as it would be for a centrifuged mass of eggs, r_0 varies rapidly with both ρ and r_1.

[4] In conversation, Dr. Hugo Fricke said that he had found a change in the capacity of a frog egg upon fertilization but gave no further details. Dr. Sterne Morse stated, also in a conversation, that he had found a change in the capacity of a suspension of *Arbacia* eggs upon fertilization that he thought could be measured. He was working with a capacity bridge at 1000 cycles.

SUMMARY.

Apparatus has been designed and constructed for the measurement of the electric impedance of suspensions of *Arbacia* eggs in sea water to alternating currents of frequencies from one thousand to fifteen million cycles per second. This apparatus is simple, rugged, compact, accurate, and rapid.

The data lead to the conclusions that the specific resistance of the interior of the egg is about 90 ohm cm. or 3.6 times that of sea water, and that 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 the 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.

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