CHANGING ELECTRICAL CONSTANTS OF THE FUNDULUS EGG PLASMA MEMBRANE*

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INTRODUCTION

Much valuable knowledge concerning the nature of the plasma membrane has been obtained by combining information acquired from kinetic permeability studies with that resulting from various physical measurements (*cf.* reference 7). In general, it is now believed that the plasma membrane is a mosaic structure composed of lipides and proteins with interposing pores throughout (*cf.* reference 18). Electrically, the plasma membrane resembles a resistive-capacitative circuit, a fact which itself indicates the mosaic nature of its structure. By its very nature, the capacitative component is considered to be the ion-impermeable elements (3). Data obtained from a variety of cells (4) indicate that most cells have a membrane capacity of *circa* 1 μF/cm.^{2}, suggesting that the ion-impermeable portions probably are due to geometrically prominent fractions of the plasma membrane with relatively similar thicknesses and compositions. On the other hand, the resistive component varies markedly among different cell types, and analogous cells of different species. In contradistinction to the capacitative elements, the resistive component has been thought to be due to small but crucial sites for the penetration of charged particles.

In the eggs of some teleosts, a profound change in the permeance characteristics of the plasma membrane occurs during activation (*cf.* reference 12). In the fully activated stage, these eggs are impermeable to water and ions. On the basis of an absence of D_2O exchange, Ussing (25) suggested that the fertilized trout egg had a plasma membrane which was composed of a continuous lipide phase without any pores. This suggestion would require the

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accompaniment of some other unusual properties which can be detected by measuring the electrical constants of the plasma membrane. Previously, Rothschild (20) had shown that the membrane capacity of the trout egg was 0.58 μF/cm.^{2}, a value rather close to that of other cells which were known to be permeable to water and ions.

The purpose of this paper is to present the results of some experiments with the *Fundulus* egg toward this end. The electrical constants have been measured and their significances are discussed. Furthermore, sequential measurements during the activation process contribute some information with regard to the alterations within the plasma membrane, which are perhaps responsible for its ultimate impermeability.

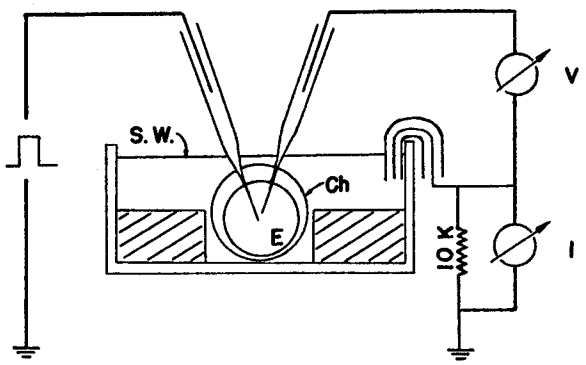

FIG. 1. Scheme of experimental arrangement. The egg is held in a shallow hole cut in some paraffin wax in the bottom of a dish. The outer shell is the non-elastic chorion (*Ch*). The microelectrodes are in the egg proper (*E*). *V* is for recording potential; *I*, current. The whole egg is covered by sea water (S.W.) or sucrose in sea water.

Material and Method

Mature eggs stripped from female *Fundulus heteroclitus* or on rare occasions from *F. majalis* were used in the experiments. No apparent difference was noted for the two species. The general experimental arrangement and technique were similar to those employed for inserting a micropipette into the interior of the egg for measuring hydrostatic pressure (13). Individual eggs were held in a small depression in some paraffin wax, and were impaled with two microelectrodes from the top at a slight angle (Fig. 1). The microelectrodes (16), filled with 3 M KCl (17) were made by a method of prefilling (11). One of the electrodes was used for passing a square pulse which could be varied in amplitude and duration. The other electrode was used for recording the membrane potential changes produced by such a pulse. It was connected to a high impedance, negative capacity input amplifier[1]. The indifferent elec-

[1] Designed by Mr. E. Amatinek (Department of Neurology, Columbia University, College of Physicians and Surgeons) whose expert assistance also facilitated the progress of this work.

trode in the bath was connected to a 10,000 ohm resistor, the other end of which was grounded. Voltage was recorded differentially between the microelectrode and the indifferent electrode. The current recording amplifier was connected across the resistor.

RESULTS

A. Membrane Potential.—With a capillary electrode of 70 μ diameter in the perivitelline space and another in the surrounding fluid, Sumwalt (21) had measured a potential difference of several millivolts across the chorion, when the external fluid had a concentration of K^+ close to that of sea water. In the presence of a non-diffusible colloid which may be charged, a Donnan potential may well be expected. The perivitelline colloid may be amphoteric, and its charge subject to change depending on the pH of the medium. This factor may be contributory to the reversal of polarity of the potential as observed by Sumwalt (22) to occur at pH 3.5, in addition to an alteration of the charges of the chorionic components. These observations, while not pertaining to the plasma membrane, are interesting because they show that the chorion, a non-viable structure, resembles a colloidion membrane with large pores, a conclusion which has also been reached *via* osmotic studies (14).

In the present experiments, the microelectrodes were inserted deep into the interior of the egg proper. The current and the potential were passed and measured across the plasma membrane and the chorion. The microelectrodes were always introduced into the unactivated egg which had a rather soft chorion and no perivitelline space. Throughout the experiments, no consistent membrane potential was observed. There were occasional changes of a few millivolts which could not be distinguished from experimental error. When activation proceeded and a distinct perivitelline space was formed, there were still no appreciable changes in membrane potential. The apparent absence of a membrane potential raises some interesting questions which must remain in abeyance until the cationic content of the interior of the *Fundulus* egg is known. As will be shown in the next section, real entry into the cell has been achieved, and the absence of a membrane potential cannot be attributed to non-penetration as has occurred with earlier reports on the echinoderm eggs (*cf.* reference 24). Neither can the absence be due to short-circuiting paths around the shaft of the electrode because of the high membrane resistance measured.

B. Membrane Resistance and Capacitance.—Penetration of the microelectrode through the plasma membrane could be observed visually. To verify this fact, the recorded form of the current pulse was invaluable especially since no distinct change in potential could be used as an index of entry into the cell proper. Fig. 2 shows the current (lower) and voltage (upper) traces as recorded on the oscilloscope. In *A*, both the polarizing and recording microelectrodes were in sea water which bathed the indifferent electrode. The cur-

rent trace recorded a long square pulse of 70 msec. duration. Except for two short lived capacitative surges at the make and the break of the pulse, the voltage line was undisturbed. In *B*, both microelectrodes have been inserted into the center of an egg proper. Under this condition, the current path included the resistance of the plasma membrane and the chorion. As is to be expected, there was a large IR drop observed on the voltage trace. Owing to the capacity of the plasma membrane, the voltage transients also exhibited

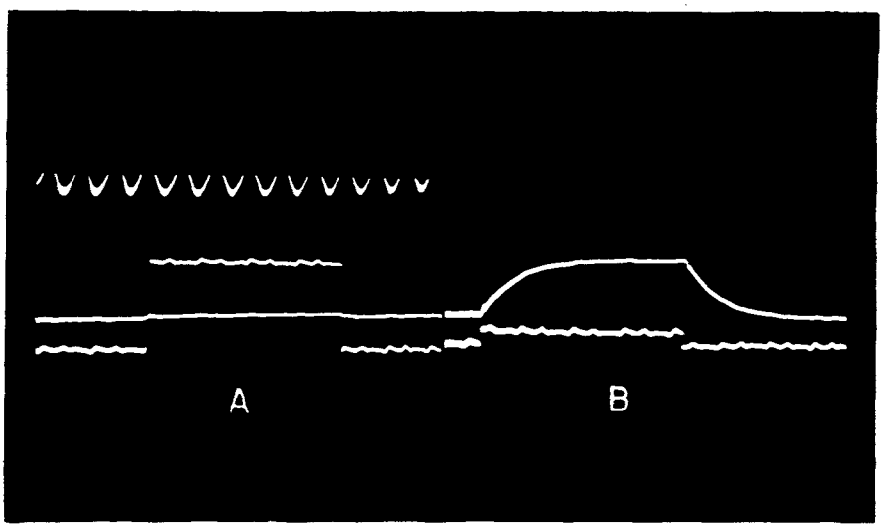

FIG. 2. Current and voltage traces before (*A*) and after (*B*) penetration of microelectrodes into the egg proper. Upper trace, voltage; lower trace, current. Amplification for current trace is higher, accounting for the noise present. Time 100 cycles/sec. The current strengths are not the same. Before entry, current pulse, even when strong, does not produce any significant change in the voltage line. After entry, small current pulses produce marked voltage changes which also show the exponential rise and decay.

the characteristic exponential rise and decay. Almost all these features were due to the electrical properties of the plasma membrane because the contributions of the chorion, the yolk and cytoplasm, and the perivitelline contents were negligible. This was evidenced by the fact that after the entire egg proper was disrupted by mechanically stirring with the microelectrodes, the current and voltage traces reverted back to the forms observed when the microelectrodes were in sea water.

As shown in Fig. 3, D.C. resistance is determined from the slope of current-voltage relationship dV/dI. To avoid possible polarization of the current microelectrode, inward (anodal) and outward (cathodal) pulses were alter-

nated during the experiment for each current strength used. The oscilloscopic traces were photographed and the records enlarged approximately 10 times for examination. The resistance so obtained is the total resistance (r_m). In order to compare the state in the unactivated eggs with that of the activated eggs which differ in volume and surface area, r_m is converted to R_m which is

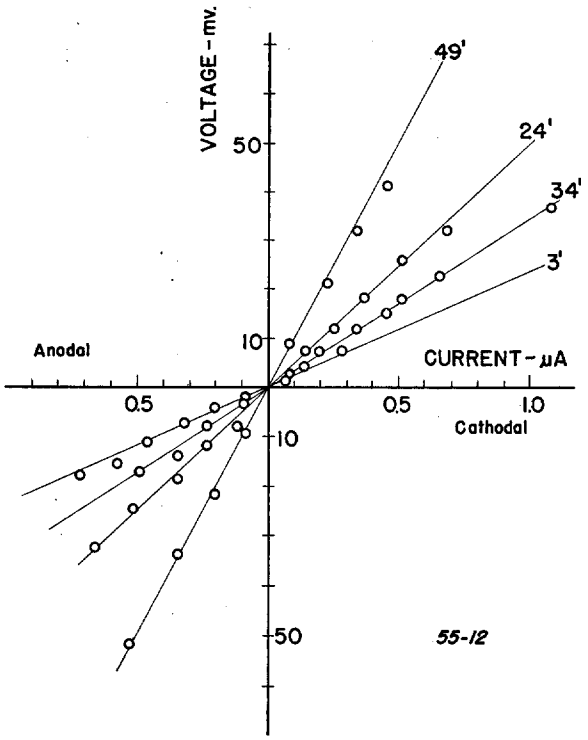

FIG. 3. Current-voltage relationship of an activating egg. At 3′, some platelets were still visible; the egg was considered unactivated. At 34′, current-voltage relation was determined within 1 minute after immersion in a hypertonic sucrose solution. Note that the slope decreased from that at 24′, but later again increased. The values for the various parameters are given in Table I.

membrane resistance in ohm-cm.^{2}, surface area having been obtained from diameter measurement. From the enlarged records, the time constant (τ) of the voltage transients, *i.e.* the time at which $1/e$ of the final stable value is attained, can be determined. From the time constant and R_m, membrane capacity C_m in μF/cm.^{2} is obtained from $\tau = RC$. Sometimes, the polarizing electrode was of high resistance, and a long pulse was needed to allow attainment of an ultimate steady state. In such cases, the sweep was necessarily

TABLE I

Electrical Constants of the Fundulus Egg Plasma Membrane

Time	Condition	Area	r_m	R_m	τ_m	C_m
min.		*cm.²*	*ohm*	*ohm-cm.²*	*msec.*	*μF./cm.²*
Exp. 55-10						
1½–2½	Unactivated	0.108	28200	3040		
26–27	Activated	0.104	57800	6010	2.48	0.41
48–49	"	0.099	57800	5720	2.48	0.43
53–54	0.25 M sucrose	0.108	65800	7100	2.48	0.35
66–67	0.25 M "	0.108	82300	8870	3.42	0.39
78–80	0.25 M "	0.108	82300	8870	3.60	0.40
85–87	0.38 M "	0.108	82300	8870	3.42	0.39
Exp. 55-11						
10–11½	Activated	0.096	73700	7090	5.0	0.71
34–36	"	0.091	108000	9850	4.70	0.48
44–45½	"	0.080	127000	10100	3.92	0.39
50½–53	0.25 M sucrose		40100		3.34	
94–96	0.25 M "		126000		3.72	
100½–102	0.38 M "		126000		5.40	
103–105	0.38 M "		94400		3.52	
121–123	0.38 M "		108000		3.81	
Exp. 55-12						
3–4	Unactivated	0.118	25200	2580	2.10	0.81
9–11	Activated	0.095	55200	5350	3.36	0.63
22–24	"	0.096	55100	5300	2.66	0.50
34–35	0.25 M sucrose		33700		0.97	
36	0.25 M "		38200		1.03	
42	0.25 M "	0.096	83000	7960	3.18	0.39
45½–46	0.38 M "	0.104	92300	9600	3.04	0.32
49	0.38 M "	0.107	92300	9880	4.28	0.43
Exp. 55-13						
1½–2½	Unactivated	0.116	15500	1860	4.75	2.56
9–10	Activated	0.078	158000	12300	10.00	0.82
30	"	0.086	158000	13600	5.33	0.39
Exp. 55-15						
3	Unactivated	0.116	35700	4140	2.49	0.60
9–10	Activated	0.095	71400	6790	4.18	0.62
21–24	"	0.096	93800	9000	5.66	0.63
49–50	"	0.093	132000	12300	7.09	0.58
Exp. 55-16						
3–4	Unactivated	0.111	51700	5750	10.00	1.74
20–23½	Activated	0.105	127000	13300	10.00	0.75
40–42	"	0.105	167000	17500	8.29	0.47
54–56	"	0.105	167000	17500	6.57	0.38
58–60	0.20 M sucrose	0.107	167000	17900	9.90	0.57
68–69	0.20 M "	0.105	192000	20200	9.45	0.57
89–90		0.105	238000	25000	10.06	0.43

reduced, a maneuver which affects the accuracy of the τ determination, and consequently C_m.

Unactivated Egg.—The microelectrodes were inserted into mature eggs within 1 to 2 minutes after the latter were stripped from female fish. Square pulses were repeated at 1/sec. while the electrodes were being inserted. Upon penetration, a sudden change in the voltage trace served as definite proof of entry. Current-voltage relationships were determined for different currents and were completed within another 1 to 2 minutes. Occurrence of activation was also verified by the persistence or absence of cortical platelets by direct observation. All the values in Table I for unactivated eggs are for those eggs in which the platelets were still present, at least in part, at the end of the first series of determinations. As shown, R_m for unactivated eggs range from 1860 to 5750 ohm-cm.^{2}, with a mean of 3450 ohm-cm.^{2}

Activated Egg.—As described previously (10, 13), puncture induces activation in the *Fundulus* egg, resulting in a sequence of changes which are indistinguishable from those initiated by successful insemination. Such activated eggs always reached the blastodisc stage, and sometimes might even undergo the first cleavage. Because of such similarity, the present results may perhaps be applied to the fertilized egg as well. The time resolution of the present experiments was limited by the necessity of alternating inward and outward current pulses; and a continuous time course could not be obtained. But it is clear that total resistance (r_m) began to increase almost immediately upon the complete disappearance of the platelets. Subsequent determinations carried out at intervals show a progressive increase of r_m which tended to reach some steady level at about an hour following the beginning of activation. Throughout this time the perivitelline space has also been enlarging, and, as has been shown, a hydrostatic pressure was developing (13). All these phenomena were accompanied by a reduction in the volume and surface area of the egg proper.

With a reduction in surface area, R_m, membrane resistance in ohm-cm.^{2}, has to be evaluated. From Table I, it can be seen that not only r_m, but also R_m increases as activation proceeds. In the ultimate steady state, R_m of activated eggs ranges from 8870 to 25,000 ohm-cm.^{2}, with a mean of 13,290 ohm-cm.^{2} Although a threefold range is present among these values, for any individual egg the change of membrane resistance upon activation is consistently in the increasing direction, the increase being 2 to 7 times that of the unactivated egg. Fig. 3 illustrates the current-voltage relationships of one egg at different times after activation, and shows the progressive increase of the slope of dV/dI.

In Figs. 4 and 5 are shown the relationship of surface area to r_m and to R_m respectively. Except for a few points in the right upper corner, all the others follow a distinct distribution which indicates an increased r_m or R_m with decreasing surface area. The aberrant points are all from one egg,

volume changes of which were small and subject to much inaccuracy of measurement. The points plotted include both the ones obtained during the activation process, and those present when the egg proper had been induced to

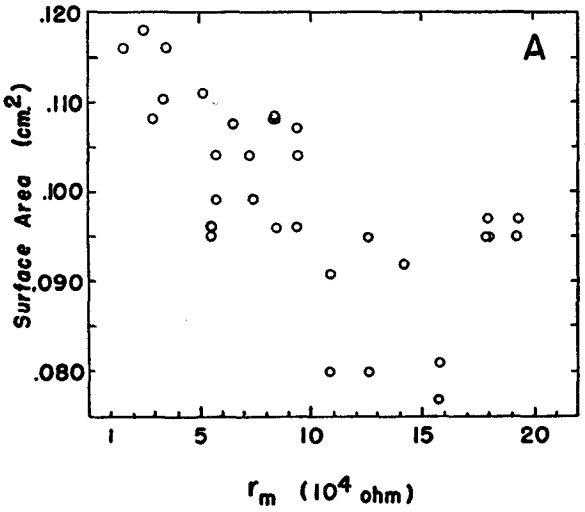

FIG. 4. Relation of surface area to r_m. The distribution indicates an increased r_m with decreased surface area.

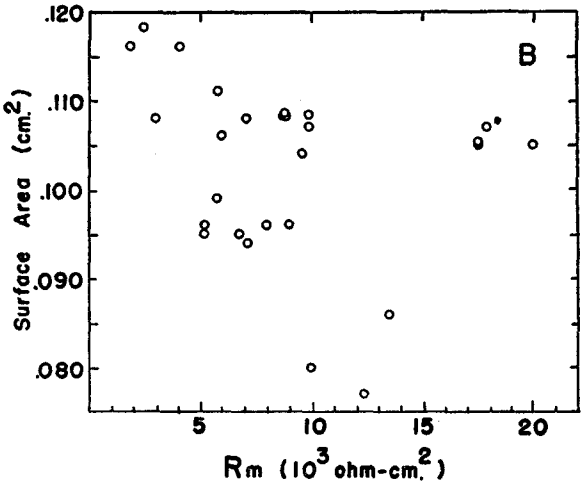

FIG. 5. Relation of surface area to R_m. See text for discussion.

swell by a hypertonic sucrose solution (*cf.* p. 115). If the increase of total resistance (r_m) is only caused by a reduction in the surface area through which the applied current is dissipated, membrane resistance evaluated on a basis

of equal area (R_m) should not be affected. Fig. 5 shows that increase of resistance is not attributable completely to a decrease of surface area. It further emphasizes that a more subtle and more permanent alteration takes place within the plasma membrane during activation (12).

Reversal of the Membrane Resistance Changes.—As shown in a previous paper (12) a hypertonic sucrose solution when added to the external environment releases the perivitelline pressure and causes the egg proper to swell, swelling being accompanied by an increase in the surface area. This phenomenon has been employed in the present experiments. During the increase of r_m and R_m accompanying the shrinkage of the egg proper, highly hypertonic solutions of sucrose were applied to the environment. The egg proper promptly enlarged. Within the first minute of swelling, both r_m and R_m dropped markedly, and approached those values present in the unactivated egg. They returned rapidly and usually high values were again attained before any significant secondary changes in the size of the egg proper could be detected. The effect is illustrated in Fig. 3 in which the line marked 34′ indicated the current-voltage relationship 1 minute after immersion in 0.25 M sucrose in sea water. r_m dropped from 5.51×10^4 ohms measured at 24 minutes after activation to 3.37×10^4 ohms upon immersion in sucrose. The rapid return of resistance is shown by the fact that at 36 minutes r_m is already 3.82×10^4 ohms, and at 42 minutes, r_m has attained 8.30×10^4 ohms. Such a rapid return of the membrane resistance accounts for the apparent absence of changes on some occasions in which the egg proper had visibly swelled. In all such cases, measurements could not be carried out within the first 2 minutes after immersion in sucrose.

Capacity.—The determination of membrane capacity in the present experiments is not of a high order of accuracy, since slight errors in estimating the time constant may seriously affect the capacity value. The average C_m for both unactivated and activated eggs is 0.62 μF/cm.^{2}, a value which corresponds well to the value for many other types of cells, *viz.*, 1 μF/cm.^{2} (4). From the best experiments in which the rise and decay portions of the voltage transients were expanded on a fast sweep, no significant alteration of C_m could be detected during the volume and surface area changes.

DISCUSSION

Based on the lack of D_2O exchange across the fertilized trout egg, Ussing (25) suggested that the plasma membrane of the trout egg is composed of a complete lipide phase with no pores. As a result, water molecules cannot traverse it either by solution in the lipide phase or by filtration through pores under osmotic or hydrostatic gradients. The similarity between the *Fundulus* and trout eggs with regard to their water and ion impermeability has been discussed previously (13, 14). Probably the structure of the plasma membrane of these two eggs is also similar. Although the absence of a membrane po-

tential in the *Fundulus* egg cannot as yet be fully accounted for, the acceptance of the above suggested nature of the plasma membrane casts some doubt on the hypothesis of phase-boundary potentials as the basis of membrane potentials in biological systems (1). In various excitable tissues (8) and in an echinoderm egg (24), the existence of a membrane potential is associated with a membrane resistance of *circa* 1000 ohm-cm.^{2}, suggesting that higher ionic fluxes must underlie the membrane potentials, although the exact mechanism is still unclear (6). This reasoning, however, cannot account for the absence of a membrane potential in the unactivated *Fundulus* egg.

As illustrated in Fig. 3, the current-voltage relationship of the *Fundulus* egg plasma membrane remains linear even up to current densities of 10 μA/cm.^{2} of inward as well as outward directions. With a membrane capacity of *circa* 0.5 μF/cm.^{2}, the charge displaced is about 5×10^{-8} coulombs/cm.^{2} at the highest current. This quantity is one order of magnitude larger than the threshold value for exciting the squid giant axon (8). A similar linear current-voltage relationship is also observed (24) for the starfish egg at even higher current densities. This linearity indicates clearly that two electrically inexcitable animal ova do not possess specialized cationic transport mechanisms as do nerves and muscles.

The hypothesis that a complete lipide phase accounts for the permeance peculiarities of these plasma membranes (25) however, appears to be at odds with the values of membrane capacity, 0.58 μF/cm.^{2}, and 0.63 μF/cm.^{2} for the trout and the *Fundulus* eggs respectively. Membrane capacity varies directly as the dielectric constant, an indication of the composition, and inversely as the thickness of the plasma membrane. Taking the lipide-protein ratio of 1:1.7 of the erythrocyte plasma membrane (18) as representative of cells with a membrane capacity of *circa* 1 μF/cm.^{2}, two alternative interpretations can be offered for the plasma membrane of the teleost eggs. First, it has a similar composition to that of erythrocytes and many other cells, in which case, the unique impermeable properties must be explained by lack of pores in the plasma membrane. Second, if a complete lipide phase prevails, the dielectric constant would be high, and could only be counterbalanced, for the membrane capacity, by an increase in the thickness of the plasma membrane, possibly to within the resolution of present experimental methods. Both alternatives can be experimentally investigated, but at present, the observations during the activation process favor the former.

As has been shown, during activation membrane resistance increases markedly while membrane capacity remains unchanged. Categorically, the alterations in the plasma membrane which lead to an increased electrical resistance may come about in two ways. It may be due to the formation of additional lipides in the plasma membrane, increasing the thickness, and further slowing the diffusion of molecules and ions through it. Or alternatively, it may be

due to a decrease in the effective pore size, with the tacit assumption that under a potential gradient most of the ionic movements occur within the continuous aqueous phase which is present in the pores and their channels. The first is made unlikely by the finding that the membrane capacity does not change significantly during activation, because a change in the lipide nature or content should be manifested in the capacity measurement. In addition, if no carrier systems are present within the membrane phase, a membrane of hydrophobic lipides would behave like parallel plate condensers, and would provide infinite D.C. resistance. In any event, it seems that diffusion of aqueous particles through a lipide phase should depend more on its solubility in the lipide than on the thickness of the latter. Constancy of membrane capacity was also observed by Rothschild (20) in unfertilized and fertilized trout eggs. The observation, however, was weakened because no mention was made of whether or not the unfertilized eggs were activated.

The second possibility of the fundamental changes in the plasma membrane during activation depends on the assumption that ionic movements occur partly through pores in the plasma membrane. No serious objections to this assumption can be raised since the ions are in an aqueous phase which is continuous through the channels provided by the pores. Despite the numerous carrier mechanisms postulated for active ion transport across membranes (*cf.* reference 2), it is implicitly agreed that aqueous diffusion plays an appreciable role in the passive transfer of ions across the plasma membrane (23, 25). The linearity of the current-voltage relationship indicates that ions can be moved across the plasma membrane of the *Fundulus* egg under a potential gradient of either polarity. Although it measures the resistance offered by the membrane components to movement of charged particles, the ohmic nature of the relationship does not favor the presence of any active transport. Thus, the ionic movement which occurred under the conditions of the experiment is most likely passive in nature. Another indication that some passive ionic movement occurs through pores is shown by the modifying effects produced upon it by concomitant water filtration; facilitated if water moves in the same direction as the ions, and retarded if water moves in the opposite direction. In the case of the isolated frog skin, Na^+ movement has been shown to occur in part through pores in the presence of neurohypophyseal extract which increases water filtration (15) and, simultaneously, Na^+ influx (26).

For the *Fundulus* egg, previous osmotic studies (12, 14) suggest that upon activation the effective pore size in the plasma membrane is reduced, the decrease accounting mainly for the water impermeability. This has been verified with D_2O exchange for the salmon egg (19). The present experiments further support this view. The lower membrane resistance values of the unactivated egg and eggs induced to swell by hypertonic sucrose solution are

accompanied by a larger surface area, which must in some way affect the pores available for aqueous exchange. In the eggs that were induced to swell, the rapid drop of membrane resistance is also consistent with the swelling of the egg proper which has been attributed to entry of water into the egg proper. The lower membrane resistance indicates that ionic movement through pores is 2 to 7 times easier than through the membrane phase itself which predominates in the activated egg. For the latter, absence of an active transport system does not exclude the operation of a thermodynamically reversible ferrying mechanism in the membrane phase such as that responsible for exchange diffusion (25).

Ionic movement through pores differs from water filtration *en masse* in that Poiseulle's law should not affect the former. The relationship between the electrical resistance and effective pore size, however, remains complex. Obviously, changes in the pore size cannot be obtained simply from changes in the total surface area. Electrical resistance, while providing some information concerning the sum total of the resistances of all the pores, does not indicate the number or the size of individual pores. The latter information can only be provided by kinetic permeability studies with substances of varying molecular volumes (*cf.* references 5 and 7). Nevertheless, it is clear from Table I that less than 20 per cent, or even as little as 5 per cent, of the surface area of plasma membrane is involved in the drastic changes of the permeance properties.

SUMMARY

Electrical constants of the plasma membrane of the *Fundulus* egg have been measured with microelectrodes by the transient method. No consistent and significant membrane potential was measured. Membrane capacity averages 0.63 μF/cm.^{2} for both unactivated and activated eggs. Membrane resistance averages 3450 ohm-cm.^{2} in the unactivated eggs, but increases 2 to 7 times to an average of 13,290 ohm-cm.^{2} in the fully activated state. In a hypertonic sucrose solution, the swelling of the egg proper is accompanied by a rapid fall of membrane resistance towards that in the unactivated state. The changes of the membrane resistance are interpreted as probably caused by alterations in the effective pore size in the plasma membrane.

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