THE MEMBRANE CAPACITANCE OF THE SEA URCHIN EGG

BY LORD ROTHSCHILD, PH.D.

(From the Laboratoire Arago, Banyuls-sur-Mer, France, and the Department of Zoology, University of Cambridge, England)

PLATE 16

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INTRODUCTION

The surface or plasma membrane of the sea urchin egg appears to have an unusual and paradoxical electrical property, which Cole (1935) observed in the unfertilized eggs of *Tripneustes ventricosus* (Lamarck) and which Iida (1943 *b*) confirmed, using fertilized eggs of *Pseudocentrotus depressus* (A. Agassiz). This property concerns the membrane capacitance per unit area, c_m, the value of which is about 1 μfd./cm.^{2} When eggs were induced to swell by being put into hypotonic sea water, c_m was found to decrease. Iida showed that the phenomenon was reversible.

A decrease in c_m under such conditions is unexpected, for the following reason: almost every cell is believed to have a plasma membrane, composed of lipides or lipoprotein, at or near its surface. This lipide layer is thought to be 30 to 100 A thick and to constitute one of the barriers to diffusion between the inside of the cell and the external medium. Some such layer or membrane, with a dielectric constant of the order of 3, seems to be required to explain the membrane capacitance of approximately 1 μfd./cm.^{2} which is characteristic of many living cells. In a spherical cell such as a sea urchin egg, we are, therefore, concerned with the capacitance of a spherical shell in which the thickness of the dielectric is small compared with the cell radius, the ratio being of the order of 1:5,000. The capacitance of the plasma membrane is therefore given by

$$c_m = ke_o/d \qquad (1)$$

where c_m = capacitance per sq. cm. of egg surface in μfd./cm.^{2}, k = dielectric constant of the medium between the "plates" of the condenser, e_o is a constant = 0.885×10^{-7} μfd./cm., and d = distance between the "plates" of the condenser in cm.

If the egg is made to swell, the plasma membrane might be expected to become thinner, in which case d would become smaller and c_m larger. Until now, it has been thought that the only conditions in which an increase in

the surface area of the plasma membrane could fail to be associated with an increase in c_m were first, that while expanding, the plasma membrane continually synthesized new membrane material to maintain its normal thickness; or secondly, according to Iida (1943 *b*, p. 171), that "if an assumption is made that the membrane is of a mosaic structure with two intermingling areas, one of which is 'effective' and the other of which is 'ineffective' in manifesting measurable capacitance, and if the latter area alone is extensible on mechanical stretching, the capacitance will vary in a manner represented by C[1]. A scheme like this appears to be a little too artificial, but it may not be altogether physically implausible."

An alternative explanation of the anomalous behaviour of c_m in the sea urchin egg, and one which can be examined experimentally, is that the plasma membrane has folds in it. A similar suggestion has been made about the plasma membrane of muscle (Katz, 1949; Martin, 1954; Hodgkin, 1954). The folded membrane hypothesis also has a bearing on the observations of Cole (1935) and Iida (1943 *a*) that c_m of the sea urchin egg markedly increases at fertilization.

To convert the observed membrane capacitance of an egg to c_m, the actual membrane capacitance is divided by $4\pi a,^2$ where a = egg radius. Suppose that the surface is folded and that ordinary measurements of the egg radius therefore result in an underestimate of the surface area. c_m will then appear to be higher than it really is, because the divisor, $4\pi a,^2$ will be too small. Suppose also that the "error" in estimating the surface area of swollen eggs is less. Given appropriate values for these "errors," c_m will appear to decrease and not increase, when the egg swells. This implies that the plasma membrane of a normal egg is folded, but that when the egg swells, the folds, as might be expected, are partly or completely smoothed out.

Equation (1) applies when measurements are made by inserting a microelectrode into the egg cytoplasm, as in the experiments of Grundfest *et al.* (1955). Cole (1935) and Iida (1943 *a*, *b*) used an A.C. bridge method, with external electrodes, in which case the appropriate equation is of the form

$$c_m = \frac{1}{a\omega r_1^2 x} \cdot \frac{n^2 + q^2}{9\rho} \tag{2}$$

where ω = angular frequency of the A.C. current, r_1 = resistivity of the suspending medium, x = equivalent series reactance of the suspension, $n = 2r(1 - \rho) - r(2 + \rho)$, r = equivalent series resistance of the suspension, ρ = (volume of eggs)/(volume of suspension), $q = 2x(1 - \rho)$, and the membrane resistance is assumed to be infinite. Equation (2) shows that in this case as well, calculation of c_m involves estimation of the egg radius.

[1] C refers to a curve in Iida's paper, showing the capacitance/cm^2. decreasing as the surface area of the egg increases.

The experiments described below were done to investigate the folded membrane hypothesis. When interpreting them, consideration must naturally be given to the question of the location of the plasma membrane and to the question of the identity of the plasma membrane with the osmiophilic or dark layer observed in electron micrographs of the surface of the sea urchin egg and many other cells.

Material and Methods

Unfertilized and fertilized eggs (showing more than 95 per cent normal fertilization and membrane formation) of *Paracentrotus lividus* (Lamarck) and *Psammechinus miliaris* (P. L. S. Müller) were used. The eggs were fixed in 1 per cent OsO_4 in normal sea water for 45 minutes. The observations of Afzelius (1956) that this is a good general fixative for sea urchin eggs were confirmed. After fixation the eggs were washed with sea water or hypotonic sea water and passed through the alcohols with washing at the ethanol stage. After this the procedure was as follows: eggs into (*a*), a 50/50 *v/v* mixture of ethanol and 3 parts butyl + 1 part methyl methacrylate; (*b*), butyl/methyl methacrylate with one wash; and (*c*), butyl/methyl methacrylate + 1 per cent benzoyl peroxide, within gelatin capsules (Parke Davis "OO"). The capsules were maintained at 45°C. for 8 hours after which they were dissolved away from the polymerized plastic. Thin sections were then cut from the blocks in the usual way.

This procedure was applied to normal and swollen eggs, but the latter were washed after fixation in hypotonic sea water instead of sea water. For the swelling experiments, unfertilized eggs were put into sea water diluted 50/50 *v/v* with distilled water for 15 minutes before fixation. Fertilized eggs were left in sea water diluted 60/40 *v/v* with distilled water for the same time.

RESULTS AND DISCUSSION

Unfertilized Eggs.—Electron micrographs show that the surface of the unfertilized sea urchin egg has papillae or folds on it (Cheney and Lansing, 1955; Afzelius, 1956; Rothschild, 1956). The folds are too small to be seen in unfertilized eggs under the light microscope and one cannot, therefore, exclude the possibility that they are artifacts due to the preparative procedures before examination under the electron microscope. Examination of Afzelius' electron micrographs and that of a normal unfertilized egg reproduced in Fig. 1 *a* shows that the osmiophilic or dark layer at the egg surface follows the contours of these folds. The folds can be eliminated by exposing unfertilized eggs to hypotonic sea water (Fig. 1 *b*). When an egg which has been induced to swell by treatment with hypotonic sea water is put back into normal sea water, it returns to its normal size and the folds reappear (Fig. 1 *c*). The average radius of the egg population from which the egg in Fig. 1 *a* was taken was 53.5 μ, making the apparent average surface area $3.597 \times 10^4 \mu.^2$ After swelling in hypotonic sea water, the average radius increased to 63.8 μ and the apparent average surface area therefore increased to $5.115 \times 10^4 \mu.^2$ The average radii of the normal and swollen, unfertilized eggs which had been

kept overnight in the methacrylate were 52.0 and 59.2 μ, a relatively slight shrinkage.

Fertilized Eggs.—The surface of fertilized sea urchin eggs is far more folded than that of the unfertilized egg, as can be seen in Afzelius' electron micrographs (1956), in Fig. 1 *d*, and in a drawing on p. 104 (Fig. 32 *e*) in Gray's Experimental Cytology. The folds, or caves as Afzelius calls them, may be caused by the expulsion of cortical granules which occurs at fertilization. As in unfertilized eggs, the osmiophilic or dark layer follows the contours of the folds. When a fertilized egg is induced to swell by treatment with hypotonic sea water, the folds in the egg surface are reduced. Although the effect is superficially less dramatic in fertilized eggs using a 60/40 *v/v* hypotonic sea water than in unfertilized eggs using a 50/50 *v/v* hypotonic sea water, it is in fact more pronounced when examined quantitatively.

TABLE I

The Ratio (Submicroscopic Length)/(Microscopic Length) of Part of the Periphery of a Section of a Sea Urchin Egg (Paracentrotus lividus)

U_N, normal unfertilized eggs; F_N, normal fertilized eggs; U_S, swollen unfertilized eggs; F_S, swollen fertilized eggs.

Egg	Ratio	Standard error	Percentage difference between
U_N	1.1	0.01	
F_N	2.0	0.26	F_N and U_N, 87*
U_S	1.0	0.005	U_N and U_S, 9‡
F_S	1.6	0.09	F_N and F_S, 23§

* $0.05 > P > 0.01$. ‡ $0.01 > P$. § $0.3 > P > 0.05$ (Fisher-Behrens test).

Analysis.—Is the difference in the degree of surface folding between fertilized and unfertilized eggs sufficient to explain the increase in c_m observed at fertilization; and is the reduction in folding when the egg swells sufficient to explain the fall in c_m observed under these conditions? In the absence of more precise information about the form and distribution of the folds or papillae (ridges in section?) on the egg surface, it would not at this stage be profitable to try to give a quantitative answer to these questions. One way of attempting to obtain more quantitative information is by measuring the "microscopic" length of part of the periphery of an egg section and comparing this measurement, in the form of a ratio, with the "submicroscopic" length[2] of the membrane of the same part of the periphery (Table I). Cole (1935) found that the

[2] In this context, "microscopic" means "when using the light microscope"; a microscopic length is the length, in a straight line, between two points 0.5 μ apart. "Submicroscopic" means "when using the electron microscope"; a submicroscopic length is the actual length (approximately, of course) of the periphery of the egg section.

ratio (c_m, fertilized eggs)/(c_m, unfertilized eggs) was 3.8 in the eggs of *T. ventricosus*; in *Arbacia punctulata* it was 2.3 (Cole and Spencer, 1938); while in *P. depressus* the ratio was 2.7 (Iida, 1943 *a*). The average of these three values is 2.9, which means that, if the folded membrane hypothesis is correct, the ratio (submicroscopic area)/(microscopic area) must be approximately equal to 1.5 × (submicroscopic length)/(microscopic length), which may not be unreasonable. If, as is now known, the surface of the egg is folded and this is not taken into consideration, c_m will obviously appear to decrease as the egg is made to swell. Examination of Iida's data (1943 *b*) on the variation of c_m with surface area shows that the product of c_m and the surface area remains approximately constant. This is what would be expected if the surface area of the egg remained constant in hypotonic sea water and the apparent increase were due to the elimination of folds present in the unswollen state. Table I and the subsequent observations suggest that the folding of the fertilized as compared with the unfertilized egg surface is sufficient to account for the "observed" increase in c_m at fertilization, which obviates the necessity to postulate special structural changes in the plasma membrane, associated with a change in capacitance. Table I may also be consistent with the reduction in c_m observed when fertilized eggs are placed in hypotonic sea water. The case of swollen unfertilized eggs is more marginal, the difference in the ratio (submicroscopic length)/(microscopic length) being 9 per cent.

These results establish an *a priori* case for the membrane capacitance changes observed in the sea urchin egg on swelling or after fertilization being due to a reduction in membrane folding in the first case, and in the second, to an increase in membrane folding, after fertilization. The argument presupposes that the plasma membrane or barrier to diffusion is at, or near, the optical cell surface. The evidence in support of this presupposition is that the egg has an osmiophilic or dark layer, of approximately the right dimensions, very near the optical surface. Apart from the fact that the layer may be osmiophilic, which may mean that it contains lipides or lipoproteins, some slight additional evidence is provided by an electron micrograph of a sea urchin egg in the act of being fertilized, published by Rothschild (1956). The osmiophilic layer appears to have disintegrated in the region of the anterior end of the sperm head. Spermatozoa, and probably their acrosomes, contain haemolytic substances which may be unsaturated fatty acids or compounds with similar properties to those found in snake and bee venoms. Although there is much evidence, of a less direct nature, indicating that virtually all cells are separated from their external environment by a thin lipide or lipoprotein layer, there is some evidence that the plasma membrane is *not* situated at or very near the periphery of the unfertilized sea urchin egg. Some of this evidence is based on examination of eggs with the light microscope. Attempts to identify or distinguish between thin, closely apposed layers at the surface

of a spherical object like an egg are of questionable value, because of the diffraction effects which occur at the surface. Runnström (1949) reproduced a diagram of an unfertilized sea urchin egg in which a structure described as the "plasma surface" (p. 276) is situated under the cortical granules, though in another paper (Runnström *et al.*, 1946), the plasma membrane is shown outside the cortical granules. Parpart and Laris (1954), using the television microscope, adduced more important evidence in favour of the plasma membrane being under the cortical granules in the unfertilized sea urchin egg. Part of their evidence was that though sea urchin eggs are relatively impermeable to such substances as erythritol, their cortical granules disintegrate when the eggs are exposed to isosmotic solutions of this substance.

If the plasma membrane is situated under the cortical granules, the hypothesis that membrane folding and unfolding can explain the anomalous behaviour of c_m in unfertilized eggs is untenable. The hypothesis that the increase in c_m observed after fertilization is due to an increase in membrane folding, and therefore in actual surface area, is reinforced; because the folded external surface of the fertilized egg may have been under the cortical granule layer before fertilization (though there is little evidence in support of the existence of such a membrane in electron microscope studies). The external surface of the fertilized egg is obviously more folded than any visible surface or interphase in the unfertilized egg. The hypothesis which explains the anomalous behaviour of c_m in swollen fertilized eggs, involving a reduction in membrane folding in hypotonic sea water, is unaffected by Parpart and Laris' observations.

SUMMARY

1. The surface of the unfertilized sea urchin egg is folded and the folds are reversibly eliminated by exposing the egg to hypotonic sea water. If the plasma membrane is outside the layer of cortical granules, unfolding may explain why the membrane capacitance per unit area decreases (and does not increase) when a sea urchin egg is put into hypotonic sea water.

2. The degree of surface folding markedly increases after fertilization, which provides an explanation for the increase in membrane capacitance per unit area observed after fertilization.

3. The percentage reduction in membrane folding in fertilized eggs after immersion in hypotonic sea water is probably sufficient to explain the decrease in membrane capacitance per unit area observed in these conditions.

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EXPLANATION OF PLATE 16

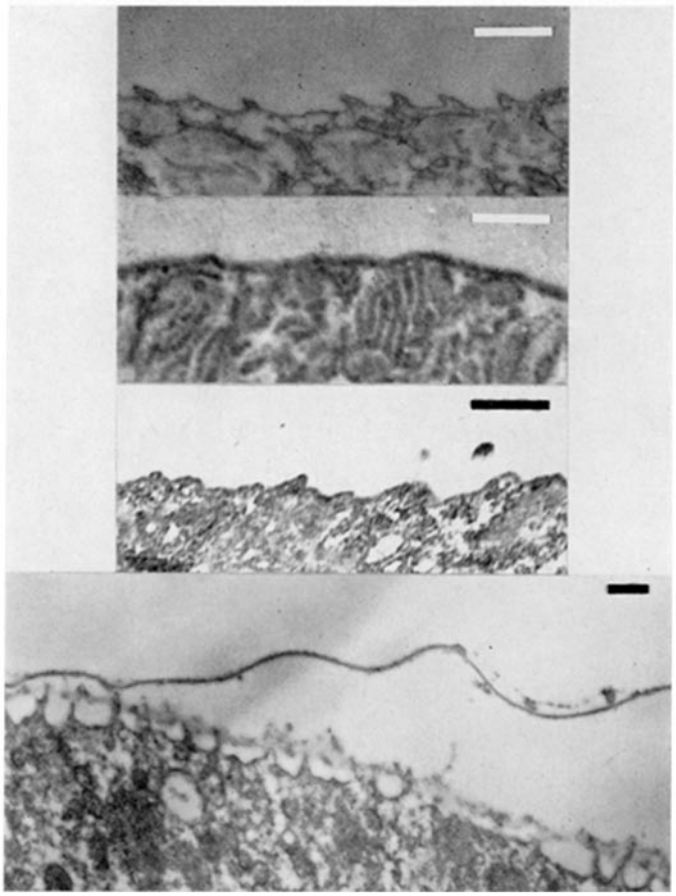
FIG. 1 *a*. Unfertilized egg of *Paracentrotus lividus* in normal sea water. × 20,000.

FIG. 1 *b*. Unfertilized egg of *Paracentrotus lividus* in hypotonic sea water. × 20,000.

FIG. 1 *c*. Unfertilized egg of *Psammechinus miliaris* in normal sea water after having been in hypotonic sea water. × 20,000.

FIG. 1 *d*. Fertilized egg of *Paracentrotus lividus*, in hypotonic sea water. Note fertilization membrane, hyaline layer, and three transverse sections of a sperm tail on the surface of the fertilization membrane. × 10,000.

The line near the top right hand corner in each photograph is 1 μ long.



(Lord Rothschild: Membrane capacitance of sea urchin egg)