The Surface of the Sea-urchin Egg

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With one plate (fig. 1)

Summary

Unfertilized eggs of the sea-urchin *Echinus esculentus* were fixed in potassium permanganate, sectioned, and examined under the electron microscope. A membrane consisting of an outer electron-opaque layer 84 Å wide, an electron-transparent layer 67 Å wide, and an inner electron-opaque layer 66 Å wide was observed at the egg surface. No other membrane or electron-opaque layer could be found anywhere within 4 μ of the egg surface.

Introduction

A PLASMA membrane is often assumed to exist at the surface of cells and to act as an active or passive barrier to the passage of certain substances between the inside of the cell and the external environment. Evidence for the existence of the plasma membrane has been obtained by studying the chemical and electrical properties of cells. The electrical resistance of the surface of a nerve-fibre, for example, is some 100,000,000 times greater than that of the cytoplasm or axoplasm. Moreover, all cell surfaces so far examined have been found to have an electrical capacitance of about 1 μF/cm^2, while no capacitative elements of this size exist in the cytoplasm.

The electrical and chemical evidence suggests that the cell surface or plasma membrane consists of a lipid or lipo-protein layer, 30–100 Å thick, with a dielectric constant of 3–6 (Huxley and Stämpfli, 1949; Fernández-Morán, 1950; Hodgkin, 1951; Davson and Danielli, 1943).

There was no direct evidence for the existence of the plasma membrane until the thin-section electron micrograph technique was developed. Since then, electron-dense layers of about the right thickness have been found at the surfaces of Schwann cells and axons in peripheral nerve-fibres (Robertson, 1957). A similar membrane has also been seen in muscle, liver, skin, kidney, pancreas, endothelial, and endoneurial cells. These cells are all bounded by a 'double' membrane about 75 Å thick (Robertson, personal communication).

The surface or plasma membrane of the sea-urchin egg has usually been assumed to be composed of lipid or lipo-protein; but in 1954, Parpart and Laris produced indirect evidence that the plasma membrane of this egg was not at the surface, but between 1 and 2 μ within, at the inner margin of what is sometimes called the gel-cortex. Parpart and Laris's evidence was twofold. First, they observed that the cortical granules in the unfertilized egg of *Arbacia punctulata* swelled and exploded a few seconds after the eggs were exposed

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to isosmotic solutions of erythritol and glycerol, though the eggs are relatively impermeable to these substances and do not increase in volume in their presence. Secondly, Parpart and Laris observed under the television microscope that the cortical granules ceased to refract light when the eggs were exposed to isosmotic sucrose solutions and that this could be reversed by replacing the eggs in sea-water. This implies that the sucrose solution came into contact with the granules and had a similar refractive index to them, though sucrose does not penetrate sea-urchin eggs.

Though Parpart and Laris's experiments have only been published as a brief note, it is difficult to conceive of any interpretation of their results other than their own, i.e. that the plasma membrane is beneath the gel-cortex and not at the egg surface. Experiments have therefore been undertaken in the hope of identifying the plasma membrane at the inner surface of the gel-cortex and to re-examine what membrane or membranes are present at the egg surface.

Material and Methods

Unfertilized eggs of *E. esculentus* were fixed in 1% OsO_4, after which they were washed with sea-water. Other unfertilized eggs of the same species were put into 0·6% $KMnO_4$ in sea-water at 0° C for 3 h, after which they were washed three times in ice-cold 25% alcohol. Eggs fixed in both ways were dehydrated and embedded in 85% butyl / 15% methyl methacrylate. The plastic was polymerized in the usual way. Ultra-thin sections were then cut.

Results and Discussion

Fig. 1, A, B shows the surface of the unfertilized sea-urchin egg after $KMnO_4$ fixation. There is a clearly defined double membrane at the egg surface. The average thickness of the outer element of this membrane (measured on enlarged positives of the plate from which A was derived) is 84 ($\pm$2) Å, of the inner element 66 ($\pm$3) Å, and of the space between the two elements 67 ($\pm$3) Å. (The figures within brackets are standard errors of the means.) In spite of the difference in their thickness, the two elements are so similar and the space between them so constant in width, that it seems reasonable to assume that they constitute one membrane and not two separate ones. Trypsin, which inhibits the elevation of the fertilization membrane, may not interfere with both elements. This may explain the fact that the surfaces of trypsin-treated unfertilized sea-urchin eggs do not seem to be different from untreated ones (Mitchison, 1956; unpublished observations of the author). The double membrane, or part of it, is probably the vitelline membrane, the precursor of the fertilization membrane.

OsO_4-fixed eggs only have one osmiophil or electron-opaque layer, 100 Å wide, at their surface (Afzelius, 1956; Rothschild, 1957), though Afzelius

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Fig. 1 (plate). A, part of the surface of an unfertilized sea-urchin egg (*E. esculentus*), fixed in $KMnO_4$. Cortical granules and a double membrane at the egg surface are visible. $\times 7 \times 10^4$. B, part of the membrane of the egg surface. $\times 1{\cdot}4 \times 10^6$.

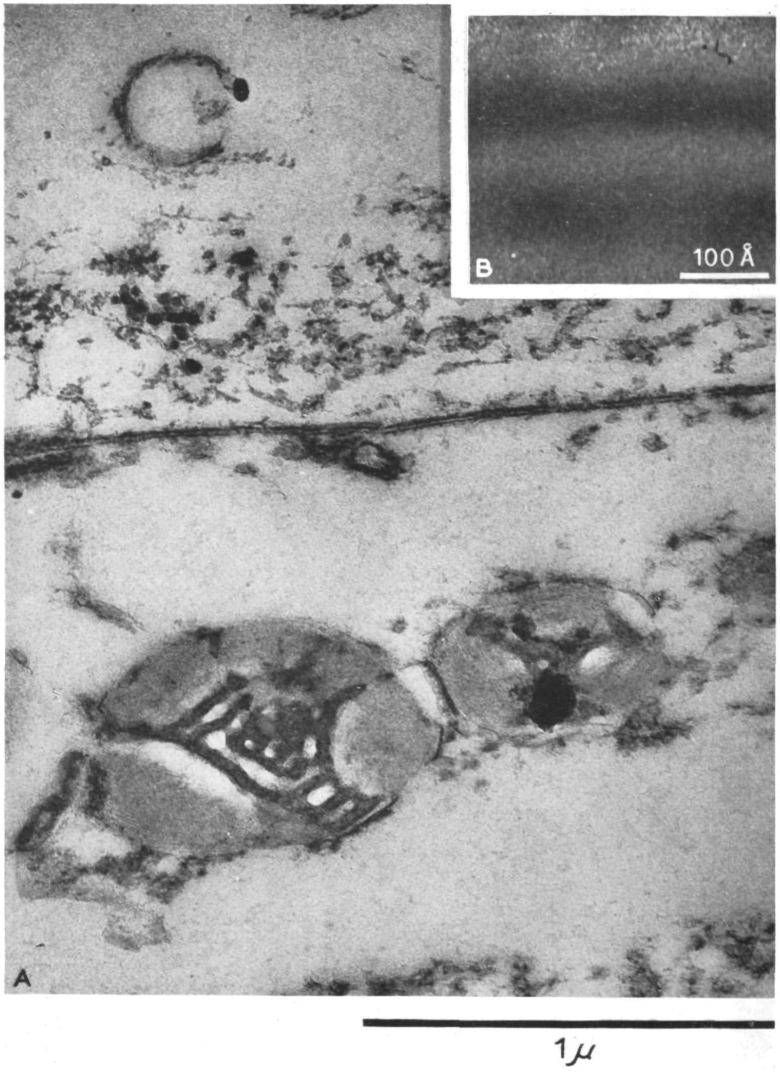

FIG. 1
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says that this membrane sometimes seems to be double. The angle at which the sections are cut will, of course, affect the appearance of the membrane under the electron microscope.

Despite a careful search, no sign of another membrane, between 0 and 4 μ in from the surface of the unfertilized sea-urchin egg, has so far been found, either in OsO_4- or $KMnO_4$-fixed eggs.

In a previous paper (Rothschild, 1957) Runnström was said to have published a diagram of an unfertilized sea-urchin egg in which the plasma membrane was situated under the layer of cortical granules, in 1949, while in 1946 Runnström, Monné, and Wicklund stated that the plasma membrane was outside the cortical granules in the unfertilized sea-urchin egg. Professor Runnström has drawn my attention to the fact that the diagram in the first of these two papers was not of an unfertilized sea-urchin egg but of a just-fertilized one in which the cortical granules had already passed through the plasma membrane. When pointing out this error, Professor Runnström told me he had always been of the opinion that the plasma membrane was outside the layer of cortical granules in the unfertilized sea-urchin egg. I apologize for having unintentionally misrepresented him.

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REFERENCES

AFZELIUS, B. A., 1956. Exp. cell Res., **10**, 257.
DAVSON, H., and DANIELLI, J. F., 1943. *The permeability of natural membranes.* Cambridge (University Press).
FERNÁNDEZ-MORÁN, H., 1950. Exp. cell Res., **1**, 309.
HODGKIN, A. L., 1951. Biol. Rev., **26**, 339.
HUXLEY, A. F., and STÄMPFLI, R., 1949. J. Physiol., **108**, 315.
MITCHISON, J. M., 1956. Quart. J. micr. Sci., **97**, 109.
PARPART, K., and LARIS, P. C., 1954. Biol. Bull., **107**, 301.
ROBERTSON, J. D., 1957. J. biophys. biochem. Cytol., **3**, 1043–8.
ROTHSCHILD, LORD. 1957. Ibid., **3**, 103.
RUNNSTRÖM, J., 1949. Adv. Enzymol., **9**, 241.
—— MONNÉ, L., and WICKLUND, E., 1946. J. colloid Sci., **1**, 421.