69. Mechanism of Membrane Elevation in the Egg of *Oryzias latipes* at the Time of Fertilization.¹⁾

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The fertilized egg of teleost has the egg membrane or the chorion which is tough and elastic. The unfertilized egg is so closely surrounded by the chorion that there exists no perivitelline space. Upon fertilization the chorion lifts up from the cortex of the egg and the perivitelline space containing perivitelline fluid is formed. Shortly after the fertilization the chorion is very soft and gradually becomes hard. As to the mechanism of membrane elevation, it has merely been stated that water penetrates from the outer medium by osmotic relations. The present paper deals with some experiments intended to make clear the mechanism of membrane elevation. First I found it necessary to ascertain the properties of the chorion and the perivitelline fluid.

I. Nature of the perivitelline fluid.

The following experiments were performed on the blastulae of *Oryzias latipes*.

a) The chorion has a high tension, and it requires a considerable force to crush the egg. Since the chorion of *Oryzias* egg is permeable not only to water but also to dissolved crystalloids (Yamamoto 1936), this high tension may be due to the existence of impermeable colloid in the perivitelline space which exerts osmotic pressure.

b) When *Oryzias* egg is put into a concentrated solutions of crystalloids, first shrinkage of the chorion takes place, but eventually it recovers (Ikeda 1934, Yamamoto 1936). When eggs which have previously been kept in M/100 balanced salt solution are put into the solution of M/2 NaCl (pH 7.3), the chorion shrinks for about three minutes and then it gradually recovers. In the solutions of M/2 or M/1 sucrose eggs shrink for about one hour or 4 hours respectively and then they recover to the normal shape. This can but mean that, although the chorion is permeable to both water and crystalloids, the rate of passing out of water from the chorion is greater than that of dissolved crystalloids. After the chorion regains its spherical shape, it again becomes to possess a high tension.

The water of perivitelline space can also be drawn out by means of mechanical pressure without injuring the chorion. For this purpose an egg was placed between the points of a drawing pen and was gradually compressed by screwing down the latter, care being taken not to crush the egg. By this means water in the perivitelline space

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can be expressed through the chorion. When the egg is removed from the drawing pen it shows slight shrinkage for a short time, and afterward the egg gradually resumes the spherical shape. The same thing can be done by placing three eggs¹⁾ in a Petri-dish containing M/100 balanced salt solution or tap water, and a small piece of lead weighing 2-4 grams was placed over them. After a few minutes the plate was removed. A slight shrinkage is noticed for a while and then the eggs recover normal shape and tension. These experiments seem to indicate that water is forced to diffuse out of the perivitelline space by mechanical pressure and the chorion recovers its normal shape and tension owing to colloid osmotic pressure.

c) When the egg is kept in distilled water for a number of hours we would expect that crystalloids in the perivitelline space may completely diffuse out through the chorion, since the chorion is permeable to crystalloids. When a slit is made through the chorion of such eggs without injuring the egg proper (*cf.* Yamamoto 1934), the escaping of some substance in the perivitelline space can be seen as 'schlieren.'

d) Loeb (1908) in sea-urchin showed that when the fertilized eggs are put into sea water which contains colloidal solution (*e. g.* the white of egg or blood serum of mammals), the fertilization membrane collapses and comes to lie close to the egg surface. When these eggs are put back into normal sea water the membrane soon assumes its spherical shape. He concluded that the perivitelline liquid contains a colloidal substance which determines the tension and spherical shape of the fertilization membrane.

When the fertilized eggs of *Oryzias* are immersed in the white of hen's egg, shrinkage occurs after a while. Time course of the extent of shrinkage is graphically illustrated in Fig. 1. They are based on the average values taken from ten eggs. It shows that shrinkage stays in the egg white, while it recovers in crystalloids. It may be remarked

Fig. 1. Time course of the shrinkage of the chorion of *Oryzias* eggs (blastula stage) in solutions of crystalloids and in the white of hen's egg. Time is taken in logarithmic scale. Shrinkage is expressed in terms of area of shrunken surface, the total area of chorion being taken as 100.

1) Three because this is the minimum number of eggs to keep the weight horizontal.

that eggs are able to develop in the white of egg inside the shrunken chorion for a number of days. This seems to indicate that permanent shrinkage occurs, because the white of egg is impermeable through the chorion and the colloid osmotic pressure of the medium is greater than that of perivitelline space. From the experiments mentioned above there leaves no doubt that the perivitelline fluid contains a colloidal substance which is impermeable through the chorion.

II. *Mechanism of membrane elevation at the time of fertilization.*

It has been shown that the cortical alveoli embedded in the cortex of the unfertilized *Oryzias* eggs break down on fertilization, beginning at the animal pole and ending at the vegetal pole (Yamamoto 1939). Elevation of the chorion follows the breakdown of the cortical alveoli. The dissolution of the cortical alveoli seems to be the underlying process of elevation of the membrane. It is not clear at present the origin of the colloidal substance in the perivitelline space. One possibility is that it is already present in the unfertilized egg between the chorion and the cortex as a gel. Another possibility is that it may be secreted from the cortex as the cortical alveoli break down. But one thing is certain that some colloidal substance which does not pass through the chorion goes into solution between the chorion and the cortex at the time of fertilization. And water diffuses into the perivitelline space by the colloid osmotic pressure, which determines the high tension of the chorion.

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