109. *The Change in Volume of the Fish Egg at Fertilization.*¹⁾

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It has been shown that the cortical alveoli embedded in the cortex of the unfertilized egg of *Oryzias latipes* break down on fertilization, beginning at the animal pole and ending at the vegetal pole. This is the first visible change of fertilization and the elevation of the chorion comes next (Yamamoto 1939 a). It has also been shown that the perivitelline space of the fertilized *Oryzias* egg contains a colloidal substance to which the chorion itself is impermeable. It was postulated that this colloidal substance goes into solution between the chorion and the cortex at the time of fertilization and the chorion is elevated by the colloid osmotic pressure (Yamamoto 1939 b). As to the origin of the colloidal substance in the perivitelline space, one possibility is that it may be secreted from the egg-cortex as the cortical alveoli break down. In this connection it is important to know whether or not the volume of the egg proper decreases at the time of fertilization.

The materials used were the eggs of the orange-red type of the fresh-water fish (*Oryzias latipes*). It has been found that the unfertilized eggs of this fish are fertilizable for a number of hours when kept in the isotonic Ringer's solution (M/7.5), while they lose this capacity within a few minutes when kept in tap water (Yamamoto 1939 a). The fertilization processes go on quite normally in the isotonic Ringer's solution. This method of insemination has many advantages over the ordinary one for the study of the fertilization processes of fish eggs.

The ripe eggs obtained from female were kept singly in vessels containing M/7.5 Ringer's solution (pH 7.3). The size of individual eggs was measured by an ocular micrometer under a binocular microscope. After each was seminated its size was measured again. This method of measurement of the identical eggs before and after fertilization gives more reliable results than the "wholesale" method. The eggs before and shortly after fertilization are not spherical but oblate spheroidal in shape, the apico-basal axis running through the animal and vegetal poles being shorter than the lateral axis. These two axes of the chorion and the egg proper were measured before and after fertilization. Eggs were measured five to twenty minutes after fertilization. By this time the conical elevation of the blastodisc has not yet formed and the egg proper assumes a spheroidal shape. Egg-holder was made of a loop of glass rod whose diameter was slightly larger

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than the egg so that under a binocular the egg can be rolled in any direction by means of a small glass rod.

The average values with probable errors based on forty-one eggs obtained from four females are tabulated (table 1). The volume was calculated as solid formed by the rotation of an ellipse about its minor (apico-basal) axis: $V = \frac{4}{3} \pi ab^2$ where a is the apico-basal semi-axis, and b the major (lateral) semi-axis.

TABLE 1.

Size of the Oryzias eggs before and after fertilization. The data are averages with probable errors based on 41 eggs. The average volume of eggs and its probable error were calculated from volumes of individual eggs and not from the average diameter of eggs.

		UNFERTILIZED EGGS	FERTILIZED EGGS	DIFFERENCE (Δ) WITH P.E. OF Δ	$\frac{\Delta}{\text{P.E. OF } \Delta}$	% CHANGE
Egg proper	Lateral axis	1.16 $\pm$ 0.0044 ^{mm}	1.12 $\pm$ 0.0044 ^{mm}	0.04 $\pm$ 0.0060 ^{mm}	6.7	- 3%
	Apico-basal axis...	1.07 $\pm$ 0.0069 ^{mm}	1.07 $\pm$ 0.0054 ^{mm}	0.0 $\pm$ 0.0094 ^{mm}	0	0%
	Volume.....	0.76 $\pm$ 0.0091 ^{cu. mm}	0.71 $\pm$ 0.0084 ^{cu. mm}	0.05 $\pm$ 0.012 ^{cu. mm}	4.2	- 7%
Chorion	Lateral axis	1.24 $\pm$ 0.0044 ^{mm}	1.28 $\pm$ 0.0044 ^{mm}	0.04 $\pm$ 0.0062 ^{mm}	6.5	+ 3%
	Apico-basal axis...	1.14 $\pm$ 0.0067 ^{mm}	1.20 $\pm$ 0.0069 ^{mm}	0.06 $\pm$ 0.0096 ^{mm}	6.2	+ 5%
	Volume.....	0.92 $\pm$ 0.011 ^{cu. mm}	1.04 $\pm$ 0.010 ^{cu. mm}	0.12 $\pm$ 0.015 ^{cu. mm}	8.0	+13%

It will be seen from the table that the lateral axis of the egg proper decidedly decreases after fertilization, whereas the apico-basal axis remains unchanged. This means that egg proper has a tendency to assume spherical shape after fertilization. The volume of the egg proper of the fertilized egg shows a decrease of 7 per cent of the original volume. Both axes of the chorion, on the other hand, increase after fertilization. The volume of the chorion or the egg as a whole increase in volume by 13 per cent after fertilization. The fifth column of table 1 shows the difference with the probable error of the difference and the sixth column the ratio of the difference and the probable error of the difference. Since all the values of the sixth column excepting the apico-basal axis of the egg proper are greater than three, the differences are statistically significant. It can not be doubted that both the elevation of the chorion and the decrease in volume of the egg proper together bring about the formation of the privitalline space after fertilization.

It need hardly be mentioned that the question of fertilization and egg-volume is closely bound up with the mechanism of the elevation of

fertilization membrane. Loeb (1908) postulated in sea urchin egg that the fertilization membrane is elevated by absorption of water by a colloidal substance which may be secreted from the egg-cortex. He, however, failed to demonstrate any loss of volume after fertilization in the egg of *Strongylocentrotus*. The problem was repeatedly investigated by a number of workers on echinoderm eggs. Glaser (1914, 1924) has shown in *Arbacia* and *Asterias* eggs that the volume of egg definitely decreases after fertilization. The results were subsequently verified by Snyder (1925). Chambers (1921) claimed that there was no change in volume after fertilization. These discrepancies may be due to such factors as change in form before and after fertilization or the flattening of egg by its own weight. Péterfi (1927) has shown that in *Paracentrotus* egg the volume definitely decreases after fertilization.

In vertebrates, Okkelberg (1914) has shown that the egg proper of the brook lamprey, *Entosphenus wilderi*, decreases by 13 per cent in volume after fertilization. Gilchrist and Pincus (1932) has shown in rat eggs that there is a shrinkage in volume of the egg of about 13 to 17 per cent after fertilization. Krassovskaja (1935) demonstrated that volume of rabbit's egg shrinks after fertilization.

It may be said in conclusion that, as the present paper indicates, the egg proper of *Oryzias* does shrink after fertilization. Since the egg of such size and consistency as that of *Oryzias* can hardly change the shape by its own weight, it is certain that the size diminution after fertilization is not an illusory phenomenon. Evidently something escapes from the egg concomitant with the breaking down of the cortical alveoli at the time of fertilization. It seems highly probable that the substance in question is a colloid to which the chorion is impermeable, and that elevation of the chorion is due to the colloid osmotic pressure of this substance.

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