Some Experiments on the Chemical Changes in the Membrane of Salmon Eggs Occurring at the Time of Activation[1)]

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I

In recent years, investigations on the physiology of fertilization in fish eggs have made clear the changes of the egg cortex occurring one after the other at the time of fertilization. According to the studies made by T. YAMAMOTO in *Oryzias*-egg[2)], the cortical alveoli embedded in the cortical protoplasm of the unfertilized egg break down at the time of fertilization and the membrane is elevated from the egg proper by a colloidal osmotic pressure of the perivitelline fluid which is derived from the content of the cortical alveoli.

Although some changes of the membrane in fish eggs at the time of activation have long been known, only a little information is available on the cause of this change. When the egg of salmon is activated by immersion in tap water (KANOH '50), its membrane becomes quite opaque and increases in elasticity. AOKI ('41), comparing the behavior in acid of the membrane of unactivated salmon egg with that of activated egg, found that particular change of solubility had occurred at the time of activation. KUSA ('49 a, b) pointed out the importance of Ca ions in this change.

In the sea urchin egg, the change of the vitelline membrane of unfertilized eggs occurring at the time of fertilization has been studied by many authors (RUNNSTRÖM '52). MOTOMURA ('41, '50) has made clear the important role of the cortical granules extruded from the egg cortex into the perivitelline space in this change of the vitelline membrane. According to him, the cortical granular substance adheres to the inner surface of the vitelline membrane in the presence of Ca ions and the so-called MOTOMURA's third factor. In this way, the vitelline membrane is transformed into the tough fertilization membrane. Judging from the similarity of behavior of the cortical alveoli in fish eggs in the process of fertilization, there is a possibility that the cortical alveolar substance is related to the change of the membrane of fish egg as in the case of the sea urchin egg.

The present paper deals with some information on the chemical changes of the egg membrane occurring at the time of activation in the salmon egg.

II

1) Contribution No. 400 from the Zoological Institute, Faculty of Science, Hokkaido University, Sapporo, Japan.

2) For a review of the problems concerning the physiology of fertilization in fish eggs, see T. YAMAMOTO ('56).

Eggs of dog salmon, *Oncorhynchus keta*, were used as materials. As has been reported by KANOH ('50) and K. YAMAMOTO ('51), the unfertilized and the fertilized eggs of salmon remain unchanged in RINGER's solution[3] but are activated and begin to develop when they are immersed in tap water. The activation of the eggs used here was obtained by immersion in tap water for 30 minutes without insemination. To isolate the membrane from the egg proper, the eggs immersed in RINGER's solution were dissected with scissors. The membrane thus obtained was repeatedly washed with RINGER's solution, and then used for experiments.

Studies were made on the solubility of the membrane of activated eggs in antiformin and the effectiveness of double treatment with acidulated RINGER's solution and pancreatin for the removal of the membrane. The double treatment was performed in the following manner : the eggs were treated, in the first place, with acidulated RINGER's solution (with HCl, pH 1.8) for 15 minutes and then transferred into 0.2 % pancreatin dissolved in RINGER's solution (pH 11.0). As reported in a previous paper (cf. KANOH and YAMAMOTO '57), the membrane of unactivated eggs of salmon is dissolved by this double treatment. All the experiments were performed at room temperature (12°—18°C).

III

As has been stated already, the membrane of activated eggs differs considerably from that of unactivated eggs in its optical and mechanical natures. Specifically, the former is more opaque and more elastic than the latter. As reported in the previous paper (KANOH and YAMAMOTO '57), the membrane of unactivated eggs is dissolved by the double treatment with acidulated RINGER's solution and pancreatin. However, the membrane of activated eggs is not dissolved by this double treatment. It is dissolved only in antiformin with concentrations from 100 to 5 % but in these cases the eggs undergo cytolysis. These facts apparently indicate, therefore, that the chemical nature of the membrane is considerably changed at the time of activation. Similar results have been obtained by AOKI ('41), who studied the behavior of the egg membrane in acid solution. According to him, the acid-soluble property of the inner layer of the membrane isolated from the unactivated eggs is lost as a result of the activation of the egg. Recently, NAKANO ('56) observed the hardening in the membrane of *Oryzias*-egg at the time of fertilization and pointed out that the hardening proceeds parallel with the breakdown of the cortical alveoli.

IV

What is the cause of the solubility change of the membrane occurring at the time of activation? When the membrane isolated from the unactivated egg was immersed in tap water for 30 minutes and was subjected to the double treatment, it was dissolved as in the intact unactivated eggs. Thus it is evident that the change of membrane is induced by the substance extruded from the egg proper at the time of activation. According to KANOH ('50, '54, '56), the cortical alveolar content, Ca ions and ammonium ions are extruded

3) RINGER's solution=M/6.5 NaCl 100 parts+M/6.5 KCl 2.8 parts+M/10 CaCl 3.4 parts (pH 7.2).

from the egg proper at the time of activation. In the present study, the membrane was changed within about 5 minutes after the immersion in tap water and became insoluble by the double treatment. Deducing from the observations of many kinds of fish eggs (cf. YAMAMOTO, K. '51, KANOH '52 b, etc.), the breakdown of the cortical alveoli of the salmon egg is expected to be completed within such short range of time as was actually observed. To test the validity of this expectation, a study was made of the nature of the membrane of an egg which had initiated development without the breakdown of the cortical alveoli. As reported by KANOH ('52 a), KUSA ('53) and T. S. YAMAMOTO ('57), the ooplasm of the egg immersed in M/10 $CaCl_2$ solution begins to accumulate at the animal pole and to form the blastodisc without the breakdown of the cortical alveoli. The membrane isolated from the Ca-treated egg was, however, not dissolved by the double treatment. This finding seems to indicate that the insolubility of the membrane of activated egg may be due to Ca ions ; but this conclusion was controverted by the results of the following experiment : Unactivated eggs washed thoroughly with Ca-free RINGER's solution and with M/10 Na_2-oxalate solution are activated by immersion in M/190 Na_2-oxalate solution for 30 minutes. Probably by this procedure, Ca ions adhered to the egg surface and those extruded from the egg proper at the time of activation might have been precipitated with oxalate contained in the environmental medium. The membrane of the egg thus activated was, however, not dissolved by the double treatment. This result seems to indicate, therefore, that Ca ions have no relation with the change of the membrane ; it seems to be inconsistent with the result obtained in Ca-treated eggs. However, the membrane of Ca-treated eggs differs distinctly from that of the eggs activated with tap water. In the eggs of which the cortical alveoli are broken down by immersion in tap water, the opacity of the membrane is considerably increased in comparison with that of unactivated eggs. This holds true in the eggs which are immersed in tap water for 1 or 2 minutes for inducing activation and then returned to the RINGER's solution. On the other hand, in the Ca-treated egg of which the cortical alveoli remain unbroken, the increase of opacity of the membrane is scarcely visible. From these facts, the insolubility found in the membrane of the Ca-treated eggs may differ in its nature from that found in the membrane of the eggs of which the cortical alveoli have already been broken down. The present author ventures to conclude, therefore, that the insolubility of the membrane which occurred at the time of activation may be caused naturally by the substance which is extruded from the egg proper but not by Ca ions, and that Ca ions are able to induce the change of the membrane in some different way. It is known in the case of sea urchin eggs that the cortical granular substance extruded from the egg cortex at the time of fertilization adheres to the inner surface of the vitelline membrane in the presence of Ca ions and the so-called MOTOMURA's third factor ; that substance then forms the tough fertilization membrane of the developing egg (MOTOMURA '50). Considering the similarity of the behavior of the cortical alveoli in fish eggs and of the cortical granules in sea urchin eggs (KUSA '56), it may be thought that Ca ions play some role in the change of the membrane of fish eggs. Though the present results seem to indicate that Ca ions do not play naturally an active role in the solubility change of the membrane, there still remains a pos-

sibility that Ca ions extruded from the egg proper at the time of activation are not completely precipitated with oxalate before inducing the change of the membrane.

V

As stated already, the membrane of activated eggs was dissolved in antiformin but in this case the eggs underwent cytolysis. The present author attempted the removal of the membrane of activated eggs without inducing cytolysis. It has been reported in the eggs of sea urchin (KOPAC '41) and *Oryzias* (NAKANO '56) that the egg membrane is easily attacked by various agents at the transitional period of its elevation but shows a certain resistance prior to or after this period. Thus, in the present study the activation of the eggs was performed in tap water having various pH values. Probably the membrane is subjected to the effect of pH of the environmental medium during its elevation. After 30 minutes' immersion in these solutions, the eggs were subjected to the double treatment. The results of this experiment are presented in Table 1. It is obvious that the membrane of the egg

Table 1. Removal of the membrane of the activated egg by the double treatment. Eggs were activated by the immersion in tap water having various pH values for 30 minutes, then were subjected to the double treatment.

Tap water		Time required for removal of the membrane by the double treatment
pH	Egg-activation	
2.0	−	?
3.0	−	−
4.0	+	−
5.8	+	−
6.8	+	−
7.2	+	−
8.0	+	−
9.5	+	−
10.0	+	−
11.0	+	−*
12.6	+	18 hours

* Egg membrane was attacked considerably.

activated with strongly alkalized tap water is dissolved by the double treatment. In this experiment, the removal of the membrane was not attained without the treatment with acidulated RINGER's solution. The membrane of the egg, immersed in alkalized tap water for 30 minutes after activation had been induced in neutral tap water, was not dissolved by the same treatment. It is clear, therefore, that the removal of the membrane of the activated egg by the double treatment is possible only when the membrane is effected at the transitional period of its elevation.

VI

The results presented in this paper may be summarized as follows : At the time of activation, the chemical nature of the egg membrane is considerably changed by the substance extruded from the egg proper. The content of the cortical alveoli extruded from the egg cortex seems to be related to this change of the membrane. If the activation of the

egg is induced in strongly alkalized tap water, in other words, if the membrane is effected by alkali in the transitional period of its elevation, the membrane of the activated egg is dissolved by the double treatment with acidulated RINGER's solution (with HCl, pH 1.8) and with 0.2% pancreatin dissolved in RINGER's solution (pH 11.0). Probably alkali inhibits the reaction between the membrane and the substance extruded from the egg proper at the time of activation.

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