PRELIMINARY NOTE ON THE ACTIVATION OF THE EGG IN THE CRUCIAN CARP (*Carassius auratus*).

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1) Introduction

The so-called activation of the unfertilized egg is the first step of the change in the process of fertilization.

This phenomenon is brought about before the entrance of the spermatozoa by the breakdown of cortical alveoli which are evenly embedded in the cortical protoplasm of the unfertilized egg. The breakdown of the cortical alveoli and the subsequent elevation of the chorion can also be induced by picking, heating, electric current and chemical reagents such as sodium taurochorate, sodium glycochorate, sodium oleate, saponin and digitonin etc. (Yamamoto '39, '44, '47). Thus the egg has an activable system in itself which causes the cortical change.

Yamamoto ('49) worked on the same subject in detail in the oryzias egg. Having found minute cortical granules embedded in the surrounding cortical alveoli, he suggests that an esterase-like substance in the granules may participate in the activating reaction.

Ishida ('49, '50) thought a kind of esterase decomposed the cortical lipoid of the egg at the time of activation, and the negative wave caused by the ferment may be absorbed in the plasma surface of the egg in an instant.

As in the above eggs, the cortical alveoli are also observed in the unfertilized egg of the crucian carp.

The present study deals with some experiments on the response of the activating and inhibiting enzymes in the activation. The existence of the lipase (esterase) in the egg is to be confirmed. Though it may be considered unnatural to apply the enzyme contained in the pancreas, stomach, milk etc. directly to the egg as an activator or inhibitor, a series of experiments have been carried out with these enzymes and the following results obtained.

2) The response of the cortical alveoli to the esterase activator.

The ripe unfertilized egg of the crucian carp looks less transparent than that

of the oryzias. When the egg is immersed in water, the cortical alveoli disappear in 13～15 seconds and the perivitellin space appears in 2～3 minutes. The ripe egg of the crucian carp loses its fertilizability within 40 seconds in fresh water. Among various esterase activators, lead-acetate gave good results in the experiment.

When the ripe egg is treated with 0.01% Ringer-lead-acetate solution before the fertilization and returned to isotonic Ringer's solution (M/7.5 NaCl 100 parts + M/7.5 KCl 2 parts + M/11 $CaCl_2$ 2.1 parts, buffered Ph. 7.0), the breakdown of the cortical alveoli and subsequent elevation of the chorion ensue. The breakdown of the cortical alveoli is also caused by 0.005% Ringer-pancreatin solution. However, if the ripe egg is immersed in this solution for 15～18 minutes, the chorion is partly dissolved out.

3) The response of the cortical alveoli to esterase inhibitor.

Physiologically, the response of the cortical alveoli to esterase inhibitor is of more interest than their response to an activator.

The breakdown of the cortical alveoli of the ripe unfertilized egg occurs just at the moment when the egg is treated with distilled water. However, when the egg is immersed in 0.01% Ringer-monoiodo-acetate solution for 5 minutes, then washed with isotonic Ringes's solution and transferred to distilled water, the breakdown of the cortical alveoli does not occur until 60～90 seconds have elapsed.

The effect of the solution of 0.1% Ringer-lead-phosphate is the same as that of the above solution. If the ripe unfertilized egg is first immersed in 0.01% Ringer-monoiodo-acetate, then washed with isotonic Ringer's solution and finally immersed in 0.01% Ringer-lead-acetate, the breakdown of the cortical alveoli occurs after 5～6 minutes and the elevation of chorion ensues.

The same result is also obtained by using lead-phosphate instead of monoiodo-acetate.

4) The response of the cortical alveoli to the decomposed substance of the esterase.

As the decomposed substances of the esterase (especially, lipase and lecithase), fatty acid, alcohol, cholin, lysolecithin, phosphoric acid, glycerophosphoric acid and glycerin are produced.

Of these substances, fatty acid (stearic acid and oleic acid), alcohol, lysolecithin, phosphoric acid and glycerophosphoric acid are examined. Cholin which is physiologically important, was not examined in this experiment. Lysolecithin

was prepared from fresh hen's egg by means of Nikuni's method (Nikuni '32).

An activation did not occur in such solutions as 0.1% Ringer-alcohol, 0.01% Ringer-phosphoric acid and 0.01% Ringer-glycerophosphoric acid. The solution of 0.003% Ringer-lysolecithin has also no effect. The lack of response of the cortical alveoli to the lysolecithin may be accounted for by the low concentration of the solution. However, further detailed research is desirable.

Of fatty acids which affect the egg, oleic acid and stearic acid were used. When the egg is immersed in 0.01% Ringer-oleic acid solution for 2—3 minutes and returned to isotonic Ringer's solution, the cortical alveoli disappear and subsequently the perivitellin space is formed.

On the other hand, if the ripe unfertilized egg is immersed directly in 1% oleic acid solution for 60—90 seconds, the egg shows no change. However, if the same is returned to isotonic Ringer's solution after washing several times with solution, the breakdown of the cortical alveoli takes place subsequently.

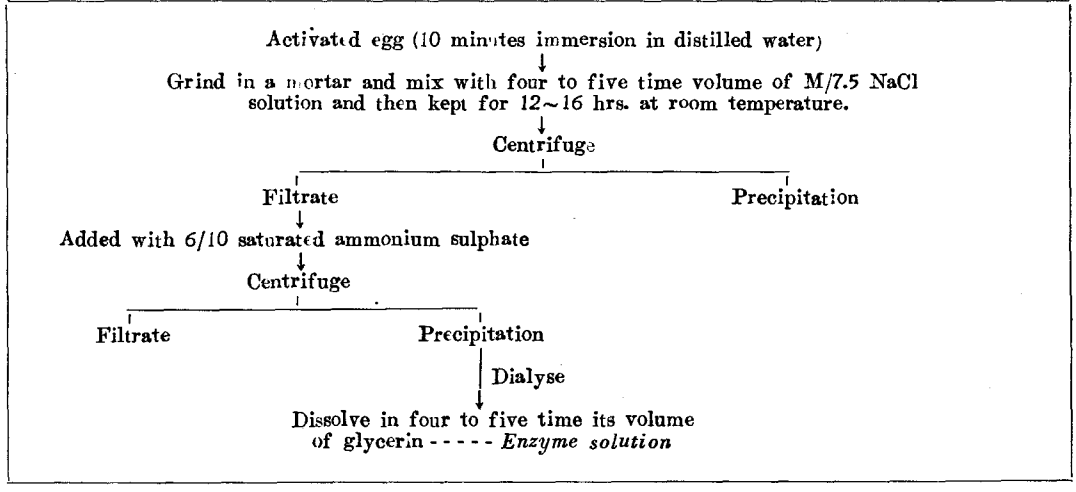
The same result is also obtained with stearic acid. The effect of stearic acid on the egg is stronger than that of oleic acid, as the egg immersed in 1% stearic acid solution for more than 40 seconds, shows cytolysis.

5) The lipase (esterase) of the egg.

The lipase (esterase) from the egg is obtained by modified Rona and Michaelis method ('11, '24).

On account of the immature stage of the ovary of the crucian carp, satisfactory preparation of the enzyme was not possible in this experiment.

The enzyme preparation is as follows;



As the substrate, saturated tributylin was used, added with sodium oleate, calcium chloride, albumin and buffer solution (Ammonium buffer, Ph. 8.5). The mixture was kept at a temperature of 37°C.

The activity of the enzyme was measured by the number of falling drops using the Stalagmometer. The number of drops every 20 minutes is as follows.

Time	At the start	After 20 minutes	After 40 minutes	After 60 minutes
number of drops	57	55	53	51

This is approximately 0.25 butylase units.

From the above results, it has been concluded that some esterase-like substance is contained in the egg.

It is a further question whether the esterase-like substance participates in the activation processes or not. However, taking into consideration Yamamoto's detailed observation as well as Ishida's results ('49), it is highly probable that the esterase-like substance does have connection with activating process.

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