# ON THE ELECTROPHORETIC DIFFERENCE BETWEEN ACTIVATED AND FERTILIZED EGGS OF THE POND SMELT, *HYPOMESUS OLIDUS* (PALLAS)*

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## Introduction

A large number of biochemical studies on activation and fertilization of sea urchin eggs have been made, mostly from the metabolic standpoint. However, little attention has been paid to changes occurring in protein pattern. These changes were first reported by Mirsky [1] who demonstrated that KCl-soluble protein fraction of the unfertilized sea urchin egg becomes insoluble as a result of fertilization. Recently, the analysis of water extract of sea urchin egg by electrophoresis has shown that a new component appears after fertilization but disappears soon. [2]

As compared with the studies on sea urchin egg, those on fish eggs are too few. The activating reaction is the first step of changes in the process of fertilization. This phenomenon is brought about by the entrance of the spermatozoa (normal fertilization) in nature.

The activating reaction can also be induced artificially (physicaly, chemically and mechanicaly).[3] Unfertilized eggs of the pond smelt are easily activated and lose fertilizability within a short time when they are put into water.[4] The morphologial change of the activated eggs caused either by water or by the entrance of spermatozoa (fertilization) is the same. That is to say, there occur the breakdown of the cortical alveoli which are evenly embedded in the cortical protoplasm of the unfertilized egg and the subsequent elevation of the chorion in succession. Although the external changes of activated egg are similar to those of the fertilized egg, internally some changes of different type may occur. The present paper deals with the difference between the activated and fertilized eggs as shown by electrophoresis.

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## Materials and Method

The materials used in this work are eggs of the pond smelt (*Hypomesus olidus* (*PALLAS*)) obtained in Lake Ōnuma in Hokkaido. The eggs were washed repeatedly in isotonic NaCl solution (M/7.5) at 0°C to remove ovarian fluid. The eggs were broken by pressure, and then centrifuged in order to separate as completely as possible the free lipid in form of a deep red supernatant, including oil and egg-membrane (chorion). In this case, the yolk probably includes all other substances other than the oil drops of egg. After separation of the supernatant oil and chorion, the yolk was diluted by 20 times in volume with phosphate buffer solution (pH 7.0), and was left dialyzed for about 48 hrs at 4°C. After dialysis, the sample was submitted to an electrophoretic analysis.

Unfertilized eggs (as a control), fertilized and activated (by water) eggs were respectively submitted to the electrophoresis. Fertilized eggs used as the material included the eggs 2 minutes after the fertilization and those 15 minutes after. Activated eggs included those 30 seconds, 2 minutes and 15 minutes in contact with water respectively. The above stated time was determined from the loss of fertilizability at the time of activation. That is to say, the fertilization rate of the egg decreased by about 50% in contact with water for 30 seconds and then the egg lost its fertilizability absolutely by 2 minutes immersion in water.

Electrophoretic analysis was carried out with HT-B type Tiselius Apparatus (Hitachi Seisakusho Company) with the size of cell 2 x 15 x 50 mm. All of the experiments were carried out in $Na_2HPO_4$-$KH_2PO_4$ buffer (pH 7.0), with ionic strength approximately 0.3.

## Experimental Results and Discussion

The electrophoretic diagram of the whole yolk of unfertilized egg showed the following components (Fig. 1. A). In the central part of the diagram three components (No. 1, No. 2 and No. 3) are present, components No. 1 and No. 2 did not disappear for 15 minutes after fertilization, but component No. 3 disappeared 2 minutes later (Fig. 1. E and F). On the other hand, in case of activated eggs, component No. 1 disappeared 30 seconds later, while components No. 2 and No. 3 did not disappear for 15 minutes after activation (Fig. 1. B, C and D). Component No. 4 disappeared 2 minutes after fertilization. In the front (fast) part of the diagram the fastest component was present; component No. 6 reacted more rapidly faster as the result of activation and also of fertilization having disappeared 2 minutes later in both cases. In activated eggs, the opposite charged component appeared 30 seconds later and disappeared 2 minutes later (Fig. 1. B, C and D), while this component was not visible in fertilized eggs. Monroy [2] has reoprted that this opposite charged component is discovered neither in unfertilized nor fertilized eggs of sea urchin. In front of component No. 3 a faster moving component was

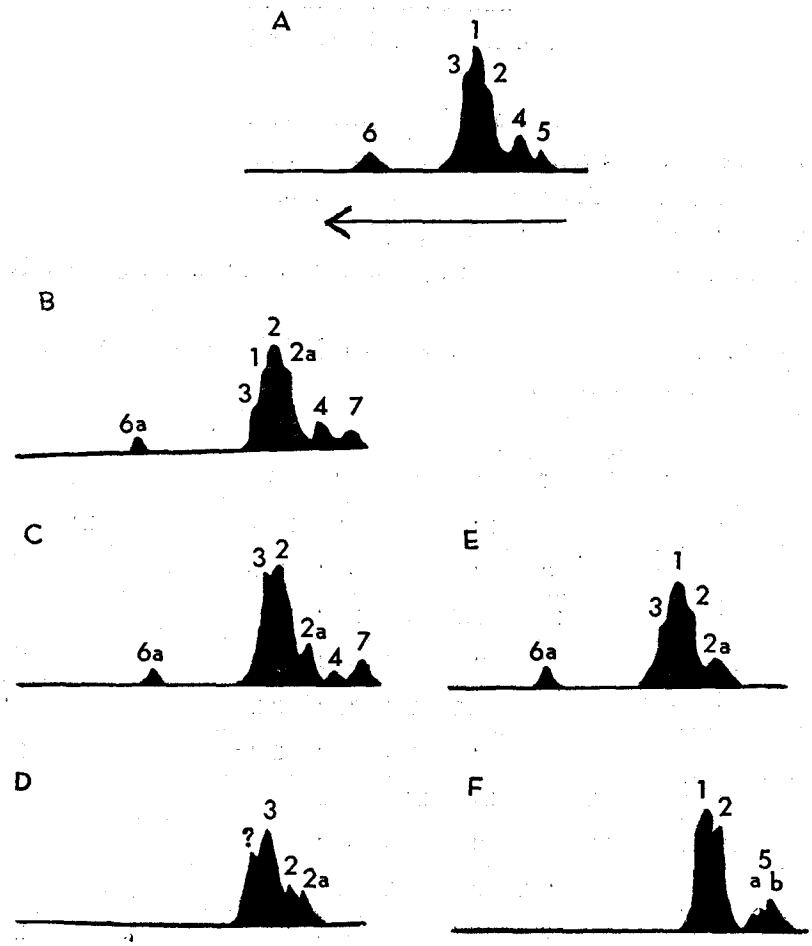


Fig. 1. Electrophoretic diagrams of whole yolk. A) unfertilized eggs; B) eggs 30 seconds after activation; C) eggs 2 minutes after activation; D) eggs 15 minutes after activation; E) eggs 2 minutes after fertilization; F) eggs 15 minutes after fertilization.

Descending limb. pH=7.0; Potential grade, A, B, D, E and F=7.3 volt/cm C=7.4 volt/cm. Period of electrophoresis, A, B, C, E and F=3605 sec. D=3606 sec.←—=Direction of migration.
(Representations of all the components have been prepared by tracing the original photographs.)

present (Fig. 1. D ?). This component must be a new one. Component No. 2a is also considered to be new. This component may be derived from component No. 2. Component No. 2a did not disappear in the activating process; but in fertilized egg, this component had already disappeared 2 minutes later. Furthermore, component No. 5b appeared 15 minutes after fertilization (Fig. 1. F). This component is also to be considered new as it shows a different mobility as compared with the other

ones (Table 1). This component may be derived from component No. 5. From the above, similar reactions characteristic both to activation and fertilization are as follows;

1. The egg diminished the number of components 2 minutes after activation or fertilization as a result thereof. In this time, activated eggs lose the fertilizability absolutely. This fact shows that some great changes occurred in the egg 2 minutes after fertilization (or activation).
2. The fastest component No. 6a may be derived from component No. 6 of unfertilized egg. This component becomes faster and disappeared 2 minutes later. Component No. 6a probably plays an important part in activating process because the egg lost its fertilizability in 2 minutes when in contact with water.
3. The new component No. 2a appeared as the result of activation and fertilization.

Table 1. Mobility (u. $10^{-5}$ $cm^2$/volt. sec.) and ratio. Whole yolk.
Descending limb.

| | | Components | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | ? | 1 | 2 | 2a | 3 | 4 | 5 | 6 | 6a | 7 |
| Unfertilized egg (Control) | Ratio (%) | | 28.5 | 13.5 | | 23.7 | 14.4 | 9.1 | 13.5 | | |
| | Mobility | | 3.9 | 3.1 | | 4.3 | 1.3 | 0.9 | 8.9 | | |
| 30 sec after activation | Ratio (%) | | 11.0 | 27.7 | 26.5 | 10.3 | 11.6 | | | 6.4 | 6.5 |
| | Mobility | | 3.9 | 3.4 | 2.7 | 4.3 | 1.3 | | | 10.3 | -0.5 |
| 2 min after activation | Ratio (%) | | | 47.1 | 7.8 | 31.3 | 3.1 | | | 3.8 | 6.9 |
| | Mobility | | | 3.2 | 2.4 | 4.3 | 1.3 | | | 10.3 | -0.5 |
| 15 min after activation | Ratio (%) | 24.1 | | 14.7 | 13.8 | 47.4 | | | | | |
| | Mobility | 5.1 | | 3.2 | 2.4 | 4.3 | | | | | |
| 2 min after fertilization | Ratio (%) | | 39.1 | 22.7 | 9.1 | 21.8 | | | | 7.3 | |
| | Mobility | | 4.0 | 3.4 | 2.4 | 4.4 | | | | 10.8 | |
| 15 min after fertilization | Ratio (%) | | 42.9 | 37.6 | | | | a 8.1 / b 11.4 | | | |
| | Mobility | | 3.7 | 3.1 | | | | 0.9 / 0.6 | | | |

Essential different reactions between activation and fertilization are as follows;

1. All components of fertilized eggs migrate anodically in pH 7.0 while no component makes cathodic migration. On the contrary, in activated egg the opposite charged component appears.
2. In activated eggs, the new component No. 2a does not disappear in 15 minutes, and then another new component appears (Fig. 1. D ?). On the other hand, in fertilized eggs, component No. 2a disappears 2 minutes after fertilization, and

then a new component different from that of the activated egg appears (Fig. 1. F. 5b).

As stated above, although the electrophoretic diagram in fertilized egg is similar on the whole to that of the activated egg at the same lapse of time, each component shows different mobility. Therefore, the eggs submitted to the two treatments have each shown a different quality of protein level as a result of activation or fertilization respectively. It is hard to say what kind of reaction exists in changes of protein level soon after activation and fertilization. But it seems those changes in eggs treated in both ways may show a week degree of denaturation. The comparison of the two shows that the activating reaction is stronger in the degree of denaturation than the fertilization reaction is. But the denaturation in activated eggs recovers to some extent within a short times, because the opposite charged component disappears in 2 minutes after activation. In this time, the egg has already lost its fertilizability altogether. Therefore, this fact shows that there exists a correlation between the appearance of the opposite charged component and fertilizability of the egg. The first demonstrable change at the time of fertilization or activation may be the occurrence of denaturation of egg. In fact, Rapkine [5] recognized the increase of -SH groups at the time of fertilization in sea urchin egg (*Paracentrotus lividus*). However, according to Mirsky,[1] it is not accompanied by a liberation of the -SH groups, being more like the related phenomenon of the reversible denaturation of myosin during muscular contraction.

On the other hand since activating reactions are fast and of cortical changes, these protein levels at the time of activation or fertilization may be derived from the changes of the cortical lipid of the egg.

## Summary

Investigation has been made of the difference between activated and fertilized eggs in the pond smelt (*Hypomesus olidus* (*PALLAS*)) electrophoretically.

1. The analysis of the whole yolk of activated eggs shows an opposite charged component which appears soon after activation and disappears 2 minutes later. On the contrary, in fertilized egg, this opposite charged component can not be discovered for 15 minutes after fertilization.
2. The great electrophoretic changes, both in activated and fertilized eggs, occur 2 minutes later.

## References

1. Mirsky, A. E. (1936). Protein coagulation as a result of fertilization. *Science* **84** (2180), 333.
2. Monroy, A. (1950). A preliminary electrophoretic analysis of protein and protein fractions in sea urchin egg and their changes on fertilization. *Exptl. Cell Res.* **1** (1), 92.
3. Yamamoto, T. (1944). Physiological studies on fertilization and activation of fish egg. I. Response of the cortical layer of the egg of *Oryzias latipes* to insemination and artificial stimulation.

*Anno. Zool. Jap.* **22**, 109.

4. Yamamoto, T. (1948). The cortical change at the time of fertilization and activation in the eggs of gold fish and pond smelt. *Zool. Mag.* **58** (3, 4, 5), 65. (in Japanese).
5. Rapkine, M. L. (1930). Sur les prccessus chimiques au ccurs de la division cellulaire. *Compt. Rend. Acad. Sci.* **191**, 871.