33. **Activation of the Unfertilized Eggs of the Fish and the Lamprey with Synthetic Washing Agents.**

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It has been shown that the ripe unfertilized eggs of the fish (*Oryzias latipes*) can be activated with surface active substances such as sodium taurocholate, sodium glycocholate, sodium oleate, saponin and digitalin (Yamamoto 1939, 1944 a). The unfertilized eggs of the brook lamprey (*Lampetra planeri*) can also be activated with sodium taurocholate, sodium oleate and saponin (Yamamoto 1944 c). An unpublished study of the writer indicates that they can also be activated with digitalin. The process of the activation by these agents is essentially similar to that of the normal fertilization. The breakdown of the cortical alveoli begins near the animal pole and ends at the vegetal pole. The separation of the chorion follows the wave-like breakdown of the cortical alveoli. It has been postulated that the activation of the unfertilized eggs by these surface active substances may be associated with their emulsifying power for lipoids which are believed to be rich in the cortex of eggs.

Recently very surface active substances have been synthetized in order to meet a need in textile industry. They have powerful wetting and washing capacity and are termed as synthetic washing agents, new detergents or wetting agents and are used as textile assistants.

It may be interesting to see whether or not the synthetic agents have the activating capacity in the unfertilized eggs of the fish and the lamprey, since they are very surface active and have emulsifying capacity for fats.

The materials used are the eggs of the orange-red type of the medaka (*Oryzias latipes*) and the brook lamprey (*Lampetra planeri*). The ripe unfertilized eggs have been kept in the isotonic Ringer's solution (pH 7.3) before used in experiments since they keep their capacity for fertilization and activation for long in the solution. For *Oryzias* eggs M/7.5 NaCl 100 parts + M/7.5 KCl 2.0 parts + M/11 CaCl₂ 2.1 parts + M/10 NaHCO₃ 0.25 parts and for *Lampetra* eggs M/7 NaCl 100 parts + M/7 KCl 2.0 parts + M/10 CaCl₂ 2.1 parts + M/10 NaHCO₃ 0.25 parts were used as the isotonic Ringer's solutions and they are designated as M/7.5 and M/7 Ringer's solutions respectively.

1) Aided by a grant from the Hattori Hokokai to which my thanks are due.
Aerosol OT

The chemical constitution of the Aerosol OT is sodium octyl sulphosuccinate and it has following chemical formula,

\[ \text{C}_8\text{H}_{17}\text{OOCCH}_2 \text{C}_8\text{H}_{17}\text{OOCCHSO}_3\text{Na} \]

When the ripe unfertilized Oryzias eggs are treated with 0.01 per cent solution of Aerosol OT dissolved in M/7.5 Ringer's solution and then returned to the Ringer's solution, they undergo changes essentially similar to normal fertilization whereas the control kept in the Ringer's solution remains unchanged.

### TABLE 1

<table>
<thead>
<tr>
<th>Activation of unfertilized eggs of Oryzias latipes with 0.01% Aerosol OT-M/7.5 Ringer's solution. Temperature 23-29°C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Time of exposure in Min.</td>
</tr>
<tr>
<td>-------------------------</td>
</tr>
<tr>
<td>0(Control)</td>
</tr>
<tr>
<td>2</td>
</tr>
<tr>
<td>4</td>
</tr>
<tr>
<td>8</td>
</tr>
</tbody>
</table>

The breakdown of the cortical alveoli and the subsequent separation of the chorion begin at the animal pole and end at the vegetal pole as in normal fertilization. These changes occur while the eggs are in the solution. A few Oryzias eggs activated with Aerosol OT showed cleavage.

When the ripe unfertilized eggs of the lamprey (*Lampetra planeri*) are immersed in 0.01 per cent solution of Aerosol OT dissolved in M/7 Ringer's solution for a few minutes and then returned to the Ringer's solution, they showed typical activation. The colloid secretion and the separation of the chorion begin at short distance from the animal pole and the changes propagate towards the vegetal pole. The reason why the first separation of the chorion does not take place at the middle of the animal pole is due to the absence of the cortical alveoli at this region. As has been pointed out elsewhere (Yamamoto 1944 c) the breakdown of the cortical alveoli and the elimination of the colloid from the cortical alveoli at the time of fertilization and activation is the cause of the separation of the chorion in the lamprey egg. Formation of the polar cone and other changes are essentially similar to normal fertilization.

1) American Cyanamid & Chemical Corp.
Monogen

Since Monogen is not pure, alcoholic extract was used. The solution was then evaporated in order to remove alcohol. The residue was dissolved in the isotonic Ringer’s solution. The chief constitution of this agent is the mixture of sodium cetyl sulphate $\text{C}_{16}\text{H}_{31}\text{OSO}_3\text{Na}$ and sodium oleyl sulphate $\text{C}_{18}\text{H}_{35}\text{OSO}_3\text{Na}$.

When the ripe unfertilized eggs of *Oryzias* are treated with 0.5 per cent Monogen extract for a few minutes and then put back into M/7.5 Ringer’s solution,

**TABLE 2**

Activation of unfertilized eggs of *Oryzias latipes* with 0.5% “Monogen” extract-M/7.5 Ringer’s solution. Temperature 23-29°C.

<table>
<thead>
<tr>
<th>Time of exposure in Min.</th>
<th>Number of experiment</th>
<th>Total number of eggs</th>
<th>Number of activated eggs</th>
<th>% of activation</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 (Control)</td>
<td>5</td>
<td>30</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>2</td>
<td>&quot;</td>
<td>29</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>4</td>
<td>&quot;</td>
<td>30</td>
<td>6</td>
<td>20</td>
</tr>
<tr>
<td>8</td>
<td>&quot;</td>
<td>33</td>
<td>21</td>
<td>64</td>
</tr>
<tr>
<td>16</td>
<td>&quot;</td>
<td>31</td>
<td>31</td>
<td>100</td>
</tr>
</tbody>
</table>

they are activated and show typical breakdown of the cortical alveoli and the separation of the chorion. The results are shown in table 2. These changes occur while the eggs are in the solution of the Monogen extract. When the time of exposure is long (16 minutes) most eggs become cytolyzed immediately after activation. Eggs treated with this agent for a short time showed typical bipolar differentiation. When the ripe unfertilized eggs of the lamprey (*Lampetra planeri*) are treated with 0.5 per cent Monogen extract dissolved in M/7 Ringer’s solution for 1-4 minutes and then returned to Ringer’s solution, they are activated.

Nekal BX

The chemical constitution of Nekal BX is a salt of alkyl naphthalene sulfonic acid and is used as wetting and emulsifying agent. The ripe unfertilized eggs of *Oryzias* were immersed in 0.05 per cent solution of Nekal BX dissolved in M/7.5 Ringer’s solution and then returned to the Ringer’s solution. The typical activation ensues when they are immersed for 4-8 minutes. A longer exposure of the eggs to the solution leads to cytolyis.

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1) Daiiti-kogyo-seiyaku Kaisya.
2) I. G. Farbenindustrie A.G.
The breakdown of the cortical alveoli and the subsequent separation of the chorion begin at the animal pole and end at the vegetal pole as in normal fertilization. Activated eggs showed typical bipolar differentiation and most of them showed cleavage. In one series of experiments the cleavage was relatively regular and most of eggs reached as far as blastula stage. The rate of development, however, was very slow as compared with the fertilized eggs which had been kept at the same temperature.

When the ripe unfertilized eggs of the lamprey (Lampetra planeri) are treated with 0.05 per cent solution of Nekal BX in M/7 Ringer’s solution for 2–4 minutes and then put back into M/7 Ringer’s solution, they show typical activation. The colloid secretion and the separation of the chorion ensue as in normal fertilization.

Labolan is a quaternary ammonium base and has the following structural formula

\[
\text{C}_{16}\text{H}_{33}-\text{N}^+\text{CH}_3\text{CH}_2\text{C}_2\text{H}_4\text{C}_6\text{H}_{15} X^-
\]

where X is an anion. While the soap and most surface active substances are anion active, Labolan is cation active as indicated in the structural formula. So that this is an “inert soap” and has strong surface activity as well as germicidal action. In the solution of Labolan Oryzias eggs becomes very adhesive and adhere each other. This may be due to the reduction or neutralization of the negative charge of the chorion by the active cation of Labolan. Previous studies on Oryzias eggs indicates that the chorion is negatively charged in ordinary media and the charge is neutralized or reversed by polyvalent cations such as Al\(^{+++}\) and

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1) Shionogi & Co.
When the ripe unfertilized eggs of *Oryzias* are treated with 0.05 per cent solution of Labolan dissolved in the M/7.5 Ringer's solution for a few minutes and then returned to the Ringer's solution, they are activated as is shown in table 4. The breakdown of the cortical alveoli and the subsequent separation of the chorion ensue as in normal fertilization. Labolan, however, is very toxic. A short exposure (0.5-1 minute) to the solution leads to typical bipolar differentiation, whereas a longer exposure (2-4 minutes) entails cytolysis immediately after activation.

It was found that 0.02-0.05 per cent solution of Labolan is too toxic to the unfertilized eggs of the lamprey (*Lampetra planeri*). When the unfertilized eggs are immersed in these solution for more than 30 seconds and then returned to M/7.5 Ringer's solution, all eggs show sign of activation near the animal pole and then cytolosed. When the unfertilized eggs are treated with 0.005 per cent solution of Labolan for 1-4 minutes and then returned to M/7 Ringer's solution, they are activated. The perivitelline space is very small and most eggs showed partial activation, namely, the separation of the chorion is not complete. Generally speaking separation of the chorion occurs at the animal hemisphere while the vegetal hemisphere shows no separation.

**Discussion**

It has been shown in previous papers (Yamamoto 1939, 1943, 1944), that the most prominent feature in fertilization of eggs of the fish and the lamprey is a wave-like breakdown of the cortical alveoli which begins near the animal pole and ends at the vegetal pole. The separation of the chorion subsequently followed the breakdown of the cortical alveoli. These changes are also induced by agents of artificial parthenogenesis. As has been shown in previous papers the for-

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**TABLE 4**

Activation of unfertilized eggs of *Oryzias latipes* with 0.05% Labolan-M/7.5 Ringer’s solution.

<table>
<thead>
<tr>
<th>Time of exposure in Min.</th>
<th>Number of experiment</th>
<th>Total number of eggs</th>
<th>Number of activated eggs</th>
<th>% of activation</th>
</tr>
</thead>
<tbody>
<tr>
<td>0(Control)</td>
<td>4</td>
<td>35</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>0.5</td>
<td>&quot;</td>
<td>34</td>
<td>26</td>
<td>76</td>
</tr>
<tr>
<td>1</td>
<td>&quot;</td>
<td>35</td>
<td>34</td>
<td>97</td>
</tr>
<tr>
<td>2</td>
<td>&quot;</td>
<td>35</td>
<td>35</td>
<td>100</td>
</tr>
<tr>
<td>4</td>
<td>&quot;</td>
<td>35</td>
<td>35</td>
<td>100</td>
</tr>
</tbody>
</table>
mation of the perivitelline space or the separation of the chorion is the consequence of the breakdown of the cortical alveoli. As the result of the breakdown of the cortical alveoli a colloid is eliminated from the alveoli between the chorion and the cortex of egg proper. Since the chorion is impermeable to the colloid, water is imbied from the medium by colloid osmotic pressure. Thus the separation of the chorion follows the breakdown of the cortical alveoli.

The fact that surface active substances have powerful activating power favors the Loeb's lysin theory (cf. Loeb 1913) which emphasizes an hypothetical substance (lysin) of spermatozoa. Loeb's theory is not complete, since it ignores the fact that the egg itself is a highly irritable system. According to our experiments (Yamamoto 1943, 1944) the ripe unfertilized eggs of Oryzias and Lampetra are highly irritable system with a gradient of excitation-conduction which is the highest at the animal pole and the lowest at the vegetal pole.

In the activation with the synthetic washing agents the breakdown of the cortical alveoli begins near the animal pole and ends at the vegetal pole. This may partly be due to rapid diffusion of chemicals through the micropyle and partly due to the high sensitivity of the animal pole. As has been pointed out elsewhere (Yamamoto 1944 a) electric and pricking experiments indicate that any point of the cortical layer is potentially capable of response to stimulation and that the sensitivity is the highest at the animal pole and the lowest at the vegetal pole.

The breakdown of the cortical alveoli in activation with the surface active substances is not due to the direct action of these substances on the alveoli. The previous studies indicate that stimulation of alveoli-free area of the cortex in centrifuged unfertilized egg brings about the breakdown of the cortical alveoli in the cortex. Primary action of these synthetic washing agents may be due to their emulsifying power for lipoid at the protoplasmic layer of the animal pole though they may potentially be capable to act at any part of the cortex. This evokes some sort of change or "impulse" at the cortical layer and it propagates in wave-like fashion as the cause of the breakdown of the cortical alveoli.

The present study has been enabled through the kindness of Prof. F. Egami of the Chemical Institute, Faculty of Science and Prof. Z. Mikumo of the Department of Organic Chemistry, Faculty of Technology, Nagoya Imperial University who generously gave me chemicals used. To both Professors I take pleasure in expressing my hearty thanks for their kindness. My thanks are also due to Mr. K. Takahashi who helped me in collecting the brook lampreys.
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