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Citation	北海道大學理學部紀要, 16(2), 186-196
Issue Date	1967-12
Doc URL	https://hdl.handle.net/2115/27442
Type	departmental bulletin paper
File Information	16(2)_P186-196.pdf



Development of the Dog Salmon Egg without Breakdown of Cortical Alveoli¹⁾

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(With 11 Text-figures and 1 Table)

As a rule the fish egg, awaiting insemination, possesses cortical alveoli. When the insemination is performed in isotonic Ringer's solution, the entry of the spermatozoon induces the breakdown of cortical alveoli and the formation of perivitelline space in the egg (T. Yamamoto, 1961). On the other hand, it is known that the salmonid egg inseminated in isotonic Ringer's solution does not show any morphological change above mentioned, though it receives a single effective spermatozoon. The egg is activated and shows the breakdown of cortical alveoli when it is immersed in tap water. In the analysis of the mechanism of egg activation, several authors found that the salmonid egg is able to initiate the development without breakdown of cortical alveoli (Kano, 1952; Kusa, 1953; Devillers *et al.*, 1953; T.S. Yamamoto, 1962, 1964, 1966). For example, the immersion of the salmon egg in isotonic CaCl_2 solution induces bipolar differentiation without breakdown of cortical alveoli. Very little information is available on the developmental fate of the egg whose cortical alveoli remained unbroken. In order to analyse the significance of the breakdown of cortical alveoli in the early development of the salmon egg, the development of the egg treated with isotonic CaCl_2 solution in various ways was investigated in the present study.

Material and Methods: Materials used were the dog salmon, *Oncorhynchus keta*, obtained from the Chitose Branch, Hokkaido Salmon Hatchery. After several washing with isotonic Ringer's solution²⁾, the eggs were inseminated in the same solution. As has been reported already, the salmon egg does not show any morphological change of activation though it receives a single effective spermatozoon by this procedure (*cf.* Kusa, 1950; T.S. Yamamoto, 1966). The inseminated eggs thus obtained were separated into six groups of 100 or 200 eggs each. They were immersed in the following solutions and the development was observed:

1) Contribution No. 783 from the Zoological Institute, Faculty of Science, Hokkaido University, Sapporo, Japan.

2) Composition of isotonic Ringer's solution: M/6.5 NaCl 100 parts + M/6.5 KCl 2.8 parts + M/10 CaCl_2 3.4 parts (pH 7.2)

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- Group 1. Isotonic CaCl_2 solution
 Group 2. Isotonic CaCl_2 solution (12 hours)→Tap water
 Group 3. Tap water (5 minutes)→Isotonic CaCl_2 solution
 Group 4. Tap water (5 minutes)→Isotonic CaCl_2 solution (12 hours)→Tap water
 Group 5. Tap water (5 minutes)→Isotonic Ringer's solution
 Group 6. Tap water (control)

These egg groups were placed in the petri-dishes (11.5 cm in diameter and 4.5 cm in depth) containing test solutions, which were gently renewed twice every day. The temperature was maintained at 10.0–10.5°C. When the eggs died, they turned opaque in tap water or CaCl_2 solution. In Ringer's solution, the cytolysis of dead eggs was appreciated through the translucent egg membrane. These dead eggs were discarded every day.

The eggs were fixed in Bouin's fluid. After the removal of the egg membrane from the fixed egg in 70 % alcohol, the blastoderm was observed under the dissecting microscope. For cytological observation, the blastoderm was embedded in paraffin by the ordinary method. Sections of 10 μ thick were stained with Delafield's hematoxylin.

Results

The first experiment was carried out in order to know the developing capacity of the eggs subjected to various treatments with different salt solutions. The eggs were fixed 12 days after the beginning of the experiment and their blastoderm was examined. At the end of experiment, the control eggs (Group 6) reached the stage of about 5 mm embryo which showed differentiation of brain and optic cup (Mahon and Hoar, 1956). The number of eggs forming a distinct embryonic shield in each group is shown in Table 1. In Groups 1, 2, 3, and 5, the blastoderm was indistinct and its degeneration was clearly recognized. It was further noticed that, during the experiment, a number of dead eggs appeared in Groups 1, 2, and 5.

Table 1. Developing capacity of the salmon egg treated with isotonic CaCl_2 solution. Eggs were fixed 12 days after the beginning of the experiment and the blastoderm was examined

Group	1	2	3	4	5	6
Number of eggs used	200	200	200	200	200	200
Number of eggs formed embryo	0	0	0	196	0	186
Percentage of survival	0	0	0	98	0	93

The determination of the exact time of the appearance of dead eggs in each group serves for the analysis of the cause of the death. For this purpose, dead eggs were counted and removed every day from each experimental group. The

result is summarized in Fig. 1. As seen in this figure, dead eggs first appeared at the third and fifth days in Groups 1 and 2 respectively. With the lapse of time, the number of dead eggs increased rapidly in these groups. In Group 3, several dead eggs were observed at the ninth day. The mortality of the eggs in Group 4 was very low and was nearly the same as that of the control (Group 6). Dead eggs in Group 5 first appeared at the second day. The number of dead eggs gradually increased and, up to the end of the experiment, about half of the eggs underwent cytolysis. When the mortality of Groups 1 and 2 was compared with that of Group 5, it was apparent that the increase of the number of dead eggs was more rapid in the former than in the latter. This fact suggests that there are

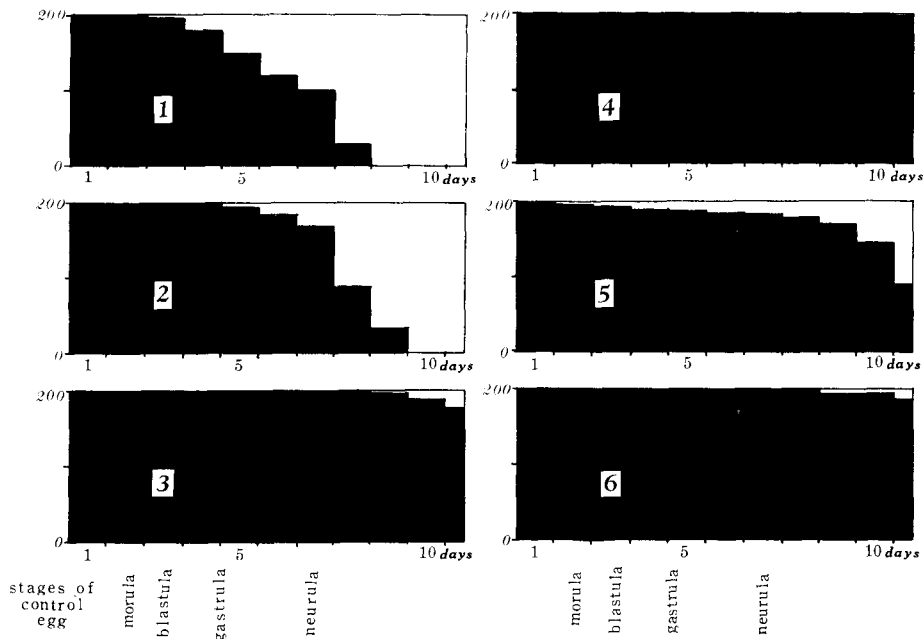
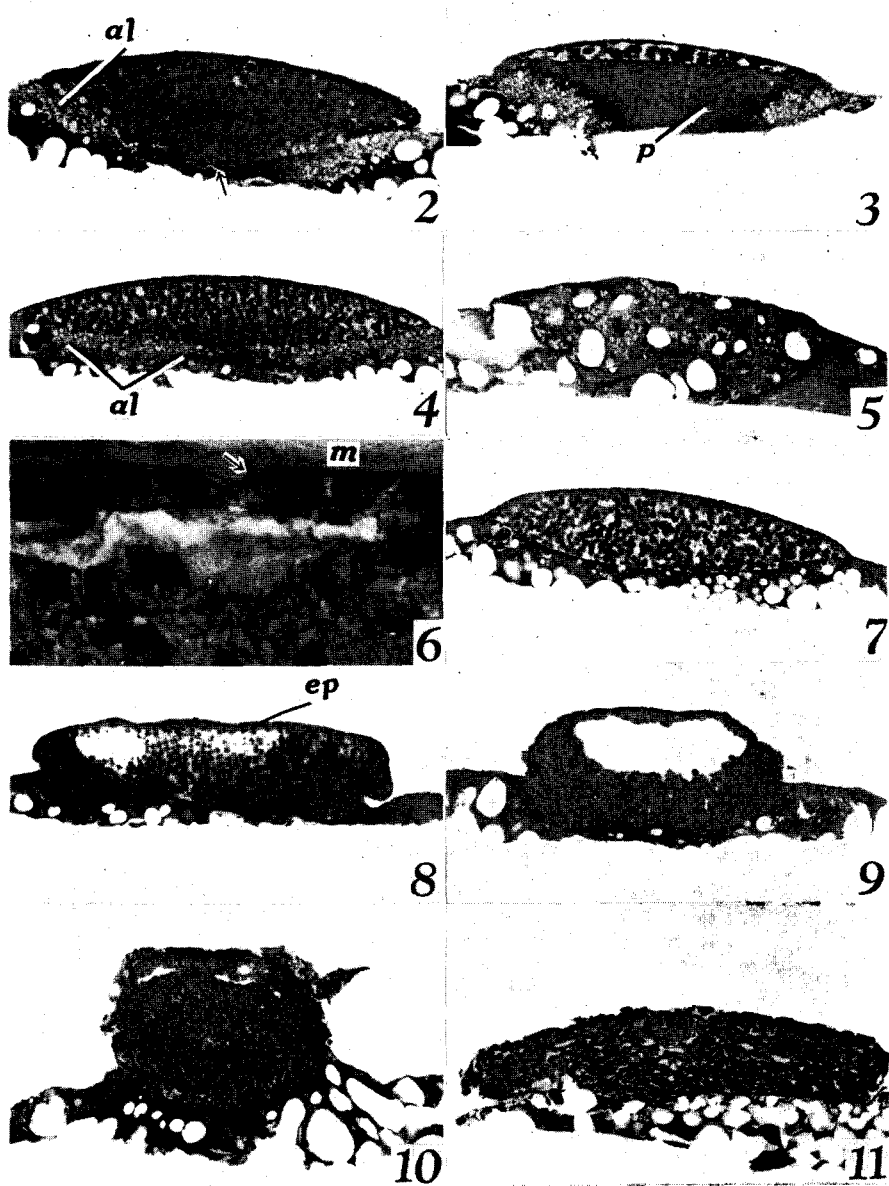


Fig. 1. Developing capacity of the salmon eggs of Groups 1-6. Black columns indicate the number of eggs surviving at the corresponding day. Abscissa; days after the beginning of the experiment. Ordinate; number of eggs.

different mechanisms causing the death of eggs between these groups. Probably the rapid increase of the number of dead eggs in Groups 1 and 2 may indicate that some pathological changes of the protoplasm occur simultaneously in most eggs.

In order to know what pathological changes occur in these eggs, the structure of blastoderm was observed in sections provided from the eggs of Groups 1-5.

Group 1: When the inseminated eggs were immersed in isotonic CaCl_2 solution, they stuck on the bottom of petri-dish with a remarkable increase in the transparency of the egg membrane. These facts indicate that, at the time of immersion, rapid physico-chemical changes occur in the egg membrane. As for the egg nucleus, anaphase movements were initiated within 40 minutes after immersion. By 70 minutes, the second polar body was formed. The haploid egg pronucleus moved toward the central part of blastodisc and united with the sperm pronucleus at 2–2.5 hours after immersion. Eggs fixed at 4.5 hours after immersion had definite cleavage spindle in the blastodisc. Although these karyological changes indicated the occurrence of activation in the egg, the breakdown of cortical alveoli did not take place and perivitelline space was hardly visible between the plasma surface and the egg membrane. With the lapse of time, the blastodisc became distinct at animal pole of the egg. The cortical alveoli remaining unbroken were found in the margin of the blastodisc. In spite of the presence of cortical alveoli, the cleavage of the egg normally proceeded. The cleavage rate was nearly the same as that of the control egg (Group 6), and the egg reached morula stage at about 24 hours after immersion. As the development proceeded, the cortical alveoli which had been formerly observed in the margin of the blastodisc came to be found in the portion underneath the blastodisc. In some cases a few cortical alveoli were found in the space among the blastomeres. At late morula stage (1.5–2 days old), the first pathological change in the arrangement of blastomeres was recognized in the blastodisc of a small number of eggs. In this case, the lower central portion of the blastodisc was crowded with blastomeres and there was no space between them (Fig. 2). With the lapse of time, the blastomeres occupying this portion were fused with each other and formed giant syncytial cells. Afterwards, they united with syncytial periblast. These eggs therefore appeared to have a considerable large periblast (Fig. 3). As these figures were not found in sections provided from the eggs developed for more than 4 days, these pathological eggs may die in the early period of the experiment (*cf.* Fig. 1). Most eggs however proceeded normally with their development and at the third day reached blastula stage. Except for the presence of cortical alveoli in the portion underneath the blastoderm, the internal structure of these eggs was similar to that of the control eggs (Fig. 4). By 4 days after the beginning of the experiment, the eggs showed an indication of epiboly. Owing to the lack of perivitelline space, the surface of the blastoderm was pressed toward the inner surface of the egg membrane at this time and the contact of both surface became intimate. Because of this reason, the blastoderm was hardly separated from the egg membrane when the removal of the membrane of the fixed egg was made in 70% alcohol. With these changes, the cells composing the blastoderm showed a pathological change. The blastoderm cells gradually lost their contour and at the sixth day the degeneration of the blastoderm was clearly recognized (Fig. 5). Probably these eggs would turn opaque within a few days.



Figs. 2-11. Sections of the blastoderms of the developing salmon eggs. Fig. 2, 2 day-old egg of Group 1. The cortical alveoli (al) remaining unbroken are found in the margin of the blastodisc. An arrow indicates crowding of the blastomeres. $\times 70$; Fig. 3, 2.5 day-old egg of Group 1. This egg has very large periblast (p) and is apparently pathological. $\times 70$;

Group 2: When the eggs were transferred into tap water, they were at 4 cell stage. As has already been reported by Kusa (1953), the cortical alveoli accumulated in the margin of blastodisc did not break down even after the transfer of the egg into tap water. Thus the perivitelline space was not found in these eggs. The cleavage pattern and the blastula formation were normal and the egg showed an indication of epiboly at 3-4 days after the beginning of the experiment. As in the case of Group 1, the surface of blastoderm at this stage was in close contact with the inner surface of the egg membrane. At the fourth day, the disintegration of the blastoderm cells was recognized in the portion immediately beneath the inner surface of the egg membrane (Fig. 6). The degeneration of the blastoderm was evident in all eggs 5 days old. These eggs may turn opaque in tap water within a few days.

Groups 3: As has been observed by K. Yamamoto (1951) and Kanoh (1956), the eggs were stimulated by first 5 minutes' immersion in tap water and showed the breakdown of cortical alveoli in isotonic CaCl_2 solution. Therefore the perivitelline space was visible in these eggs. The cleavage proceeded normally and the egg reached early blastula stage at the third day after the beginning of the experiment (Fig. 7). First pathological indication was observed at mid-blastula stage. As has been described by several authors (Saito, 1950; Mahon and Hoar, 1956), the blastocoel or the segmentation cavity was not distinct in the egg of this fish. At blastula stage of the normally developing egg, the blastoderm was consisted of one cell-layered wall, epiblast, and round or pear-shaped cells, the latter being loosely suspended within the lumen enclosed by the former. In the experimental eggs, however, most of the inner loose cells gathered closely on the periblast of the egg. Furthermore, some of the inner loose cells had contact with the cellular wall of the blastoderm, epiblast (Fig. 8). Thus there appeared a large cavity in the central part of the blastoderm (Fig. 9). The proliferation of the blastoderm cells continued for several days which caused the collapse of the central cavity of the blastoderm (Fig. 10). The indication of epiboly could not be detected in these eggs. At the sixth or seventh day after the beginning of the experiment, the nucleus and cytoplasm of the blastoderm cells were homogeneously stained with hematoxylin. These eggs underwent degeneration within a few days. The most important character of the development in this group was therefore that

Fig. 4, 3 day-old egg of Group 1. The cortical alveoli (al) remaining unbroken are found underneath the blastoderm. Except for the presence of the cortical alveoli, the structure of the blastoderm seems to be normal. $\times 70$: Fig. 5, 6 day-old egg of Group 1 showing the disintegration of the blastoderm. $\times 70$: Fig. 6, 4.5 day-old egg of Group 2. The blastoderm cells suffer mechanical damage (arrow) in the portion just beneath the egg membrane (m). $\times 480$: Fig. 7, 3 day-old egg of Group 3. It seems to be at normal blastula stage. $\times 70$: Figs. 8 & 9, 3.5 day-old eggs of Group 3 showing the formation of the cavity in the central part of the blastoderm. ep, epiblast. $\times 70$: Fig. 10, 4.5 day-old egg of Group 3. The central cavity of the blastoderm is collapsed by the proliferation of the blastoderm cells. No indication of epiboly is detected. $\times 70$: Fig. 11, 4.5 day-old egg of Group 5. Regular arrangement of cellular epiblast is not observed. $\times 70$.

the pathological change occurs at blastula stage and that the egg shows no indication of epiboly.

Group 4: The development of the eggs of this group was, as expected from the result shown in Table 1, the same with that of the control eggs (Group 6), and no pathological indication of the development was detected.

Group 5: In order to ascertain whether the abnormality of the development observed in the eggs of Group 3 was caused by the osmotic pressure of the environmental solution or not, the eggs pretreated with tap water for 5 minutes were immersed in isotonic Ringer's solution. The perivitelline space was formed between the plasma surface and the membrane in these eggs. During the cleavage, the arrangement of the blastomeres was normal at early stages but became irregular in late stages. Furthermore it should be noticed here that the cleavage of these eggs clearly retarded in Ringer's solution. When the eggs of the control group reached morula stage, the eggs of this group were at 16 cell stage. With the lapse of time, the irregular arrangement of the blastomeres became evident. The blastoderm at blastula stage did not show the regular cell arrangement of epiblast (Fig. 11). At the fifth day after the beginning of the experiment, the blastoderm cells showed vacuolization and at the eighth day the degeneration of the blastoderm was clearly detected.

Discussion

The main purpose of this investigation is to know whether the salmon egg initiated the development without breakdown of cortical alveoli can complete the embryonic development or not. The results obtained from the eggs of Groups 1 and 2 show that the egg is able to perform epiboly to some extent without breakdown of cortical alveoli. Since the eggs of Group 3 showed a pathological change at mid-blastula stage, the development of the eggs of Group 1 may be influenced by the osmotic pressure or the ionic effect of the unusual external solution. The eggs of Group 4 showed the normal development during the entire period of the experiment. These eggs had been treated for 12 hours with isotonic CaCl_2 solution. Probably this fact suggests that, except for the breakdown of cortical alveoli, the immersion in isotonic CaCl_2 solution for less than 12 hours exerts little or no effect on the development of the salmon egg. Therefore it may be reasonable to consider that the results obtained from the eggs of Group 2 indicate the mere influence of the presence of cortical alveoli on the development. Up to early gastrula stage, the development of the eggs of Group 2 proceeded normally. Owing to the lack of the perivitelline space, the formation of which is resulted from the breakdown of cortical alveoli (T. Yamamoto, 1961), the surface of the blastoderm was intimately contacted with the inner surface of the egg membrane. It has been observed in the development of a number of animals that the cells formed by the division of the egg do not show the cell growth in the cleavage stages but begin to increase in their size during gastrulation. This results in the increase of the

volume of the embryo in late developmental stages. These facts may hold true in the development of the salmon egg. Probably the blastoderm of the developing salmon egg may increase its volume during gastrulation. Since the egg is closely enclosed with the rigid membrane, the growth of blastoderm results in the increase of the pressure inflicted on the surface of the blastoderm. This increase of pressure may cause the mechanical damage of the cells composing the surface of blastoderm which leads the egg to its death. The above consideration is supported by the fact that the first pathological change of the blastoderm cells in the eggs of Group 2 is found in the portion immediately beneath the egg membrane.

The egg of Group 1 showed an indication of epiboly. However the egg of Group 3, which was allowed to develop in the same solution with the egg of Group 1 after breakdown of cortical alveoli, raised a pathological change at blastula stage. If these results are compared with those obtained from the egg of Group 1, another possibility is considered as to the cause of the death of eggs developed without breakdown of cortical alveoli. That is, the molecular configuration of the protoplasm of the developing egg is different according to whether the alveolar breakdown is induced or not. The difference might be reflected on the developmental capacity of the egg.

The cleavage of the eggs pretreated with tap water was abnormal in isotonic Ringer's solution (Group 5). This may not be due to the high osmotic pressure of the environmental solution, because the development of the eggs pretreated with tap water was normal up to early blastula stage in isotonic CaCl_2 solution. Preliminary experiments showed that the salmon eggs develop normally in M/3 sucrose solution. According to Domurat (1964) who immersed the trout egg in paraffin oil at 2 cell stage, the development is normal up to blastula stage. In order to explain these results, the relation between the egg activation and the environmental solution should be mentioned. The salmon egg does not show any visible change in isotonic Ringer's solution. It is activated and initiates the development when immersed in hypotonic salt solution or non-electrolyte solution (without regarding its osmotic pressure) (Kano, 1950). If the egg is treated with isotonic CaCl_2 solution, it extrudes the second polar body and shows bipolar differentiation. The breakdown of cortical alveoli, however, does not occur in these eggs (Kano, 1952; Kusa, 1953). Probably the environmental solution directly exerts its action on the plasma membrane of the salmon egg (T.S. Yamamoto, 1966). Accordingly, the modes of action of the above mentioned solutions on the plasma membrane are different from each other. Therefore, the different results of the development observed between the eggs of Groups 3 and 5 may be explained as a result of the different reactivity of the plasma membrane of the developing egg to each environmental solution.

Recently Iwamatsu (1965) treated the medaka egg immediately after fertilization with acetone and inhibited the breakdown of cortical alveoli. In this case, the separation of the blastomeres is noticed during the cleavage. With the aids of the electron microscope, Iwamatsu (1965) found an outer covering layer, "surface

gel layer", on the blastodisc of the normally developing medaka egg. In developing acetone-treated eggs whose cortical alveoli remained unbroken, however, he failed to demonstrate such a layer on the surface of blastodisc. Since the surface gel layer seems to combine closely the blastomeres, he supposed that the separation of blastomeres observed in the acetone-treated egg is due to the absence of this layer. In the present study, the separation of the blastomeres was not detected during the cleavage of the eggs whose cortical alveoli remained unbroken. By treating the unfertilized herring egg with high temperature, Kanoh and Yanagimachi (1956) found that the eggs initiate the development without breakdown of cortical alveoli after insemination. According to them, the cleavage proceeds normally in these eggs and no separation of blastomeres is detected. Therefore a question arises as to whether the blastoderm of the eggs of Groups 1 and 2 is covered with surface gel layer or not. According to the description of Trinkaus (1951) on the surface gel layer of the *Fundulus* egg, it is the outer layer or membrane of the egg itself in fertilized and unfertilized eggs, in oviducal eggs, and in developing eggs of all stages. At blastula stage, the gel layer constitutes the outer layer of the cellular epiblast, to which all epiblast cells are attached. In sections of the blastoderm, the epiblast cells are rounded only on their inner surfaces but their outer surfaces are flattened, conforming to the smooth contour of the outer blastoderm surface. The outer surfaces of these cells are in contact with and bounded together by a common membrane, the surface gel layer. Although the author failed to actually observe the gel layer in both experimental and control salmon eggs, the cellular arrangement of epiblast in the blastoderm of the eggs of Groups 1, 2, and 6 was quite similar to that of the *Fundulus* egg (Trinkaus, 1951). This may suggest the presence of the surface gel layer in the developing salmon egg whose cortical alveoli remained unbroken. Furthermore, the eggs of Groups 1 and 2 showed an indication of epiboly. Since the surface gel layer plays an important role during the process of gastrulation (Devillers, 1950), this fact may also indicate the presence of the gel layer in the above mentioned salmon egg. However there is another possibility that, in the egg developing without breakdown of cortical alveoli, the blastomeres are closely combined with each other by unknown substance of cell surface other than the gel layer. According to Osanai (1960), the blastomeres of the sea urchin egg are combined with each other by two kinds of substance, i.e. hyaline layer substance and something other than the hyaline layer on the cell surface. It is not clear in the present study why the blastomeres of the salmon egg developing without breakdown of cortical alveoli do not separate during the cleavage.

Summary

1. The eggs of the dog salmon, *Oncorhynchus keta*, inseminated in isotonic Ringer's solution were treated with various salt solutions and their development was observed.

2. When the eggs were immersed in isotonic CaCl_2 solution, they initiated the development without breakdown of cortical alveoli. In some eggs, the cell contour of the blastomeres became indistinct at the late morula stage and showed an indication of degeneration. Most of the eggs however reached blastula stage and initiated epiboly. During the gastrulation, the surface of the blastoderm was pressed toward the inner surface of the egg membrane, by which the blastoderm cells suffered a mechanical damage. All eggs died at gastrula stage.

3. If the eggs were transferred into tap water after 12 hours' immersion in isotonic CaCl_2 solution, they developed normally without breakdown of cortical alveoli and reached blastula stage. Mechanical damage of the blastoderm cells was also observed in this case, which may cause the death of the egg.

4. The eggs immersed in isotonic CaCl_2 solution after 5 minutes' pretreatment with tap water showed the breakdown of cortical alveoli. The eggs developed normally and reached early blastula stage. At mid-blastula stage, the eggs formed a large cavity in the central part of the blastoderm. No indication of epiboly was detected within 6 days.

5. The cleavage rate of the eggs pretreated with tap water for 5 minutes was retarded in isotonic Ringer's solution, and the arrangement of the blastomeres became irregular in late stages. The blastoderm cells showed vacuolization at the fifth day and showed an indication of degeneration.

6. Discussion was given on the relation between the breakdown of cortical alveoli at egg activation and the faculty of the developing egg to combine closely the blastomeres.

The author is grateful to Professor Atsuhiko Ichikawa, Professor Yasuhiko Kanoh and Dr. Chiaki Katagiri for their valuable advice and to Mr. Yoshinari Yanagita and Mr. Yuki-hisa Hirao for technical assistance. Thanks are also due to the staff of Hokkaido Salmon Hatchery for their allowing the collection of the materials used in this study.

References

- Devillers, Ch. 1950. Action des solutions hypertoniques sur le développement de l'oeuf de truite. C.R. Acad. Sci. **230**: 471-472.
- Devillers, Ch., A. Thomopoulos, et J. Colas 1953. Differentiation bipolaire et formation de l'espace périvitellin dans l'oeuf de *Salmo irideus*. Bull. Soc. Zool. France **78**: 462-470.
- Domurat, J. 1964. Embryonic development of the rainbow trout, *Salmo irideus* Gibb., and the brown trout, *Salmo trutta* L., in a non-aqueous medium. Zool. Pol. **14**: 125-151.
- Iwamatsu, T. 1965. Effect of acetone on the cortical changes at fertilization of the egg of the medaka, *Oryzias latipes*. Embryologia **9**: 1-12.
- Kanoh, Y. 1950. Ueber Wasseraufnahme und Aktivierung der Lachseier. I. Annot. Zool. Japon. **24**: 13-21.
- Kanoh, Y. 1952. Ueber die Beziehung zwischen dem Zerfallen der Kortikalalveoli und der Entwicklung bei Fischeiern (in Japanese with German Résumé). Jap. J. Ichthyol. **2**: 99-103.

- 1956. Ueber Wasseraufnahme und Aktivierung der Lachseier. IV. Vorläufiges Experiment in Betreff des Calciumproblems. Jour. Fac. Sci. Hokkaido Univ., Ser. VI, Zool. 12: 259-263.
- Kanoh, Y. and R. Yanagimachi 1956. Ueber den japanischen Hering (*Clupea pallasii* C. et V.). III. Der Beginn der Entwicklung ohne Zerfallen der Kortikalalveoli. Jour. Fac. Sci. Hokkaido Univ., Ser. VI, Zool. 12: 264-272.
- Kusa, M. 1950. Physiological analysis of fertilization in the egg of the salmon, *Oncorhynchus keta*. I. Why are the eggs not fertilized in isotonic Ringer's solution? Annot. Zool. Japan. 24: 22-28.
- 1953. Significance of cortical change in the initiation of development of the salmon egg (Physiological analysis of fertilization in the egg of the salmon, *Oncorhynchus keta* II). Annot. Zool. Japon. 26: 73-77.
- Mahon, E.F. and W.S. Hoar 1956. The early development of the chum salmon, *Oncorhynchus keta* (Walbaum). J. Morph. 98: 1-48.
- Osanai, K. 1960. Development of the sea urchin egg with the inhibited breakdown of the cortical granules. Sci. Rep. Tôhoku Univ., 4th Ser., Biol. 26: 77-87.
- Saito, S. 1950. The embryological study of fishes. I. General observation on the early development of the dog-salmon, *Oncorhynchus keta* Walbaum. Jour. Fac. Agr. Hokkaido Univ. 48: 267-289.
- Trinkaus, J.P. 1951. A study of mechanism of epiboly in the egg of *Fundulus heteroclitus*. Jour. Exp. Zool. 118: 269-319.
- Yamamoto, K. 1951. Activation of the egg of the dog salmon by water and the associated phenomena. Jour. Fac. Sci. Hokkaido Univ., Ser. VI, Zool. 10: 303-318.
- Yamamoto, T. 1961. Physiology of fertilization in fish eggs. Intern. Rev. Cytol. 12: 361-405.
- Yamamoto, T.S. 1962. Activation of dog salmon egg by heavy metal ions. Jour. Fac. Sci. Hokkaido Univ., Ser. VI, Zool. 15: 148-156.
- 1964. Effects of acidulated Ringer's solution on the egg of dog salmon, *Oncorhynchus keta*. Jour. Fac. Sci. Hokkaido Univ., Ser. VI, Zool. 15: 426-438.
- 1966. Independence between sperm-entry and activation in the dog salmon egg (in Japanese with English Résumé). Zool. Mag., Tokyo 75: 297-302.