Some Morphological and Physiological Aspects of the Eggs of Teleostean Fishes[1)]

By

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I

Since T. Yamamoto devised an excellent method for the observation of fertilization in the eggs of fresh-water fish in 1939, works on fish eggs have played an important part in recent advances in the fields of embryology and cell physiology. He found that the ripe unfertilized eggs of Medaka (*Oryzias latipes*) can be kept in isotonic Ringer's solution without any changes and that the development proceeds normally after insemination in this solution. Since the time of T. Yamamoto's paper extensive studies have been made on the physiology of fertilization or activation in fish eggs.

The advantages in the use of fish eggs are not far to seek : they are readily available in large numbers and are physiologically uniform; the large size of an egg is favorable for handling of the egg; they develop readily under laboratory conditions.

The present works, which were performed from 1952 to 1956, were undertaken in order to gain information on the physiology of fertilization or activation in the eggs of the teleostean fishes, Medaka (*Oryzias latipes*), Pacific herring (*Clupea pallasii*) and Dog salmon (*Oncorhynchus keta*).

II

As has long been well known, the egg of teleostean fishes is clothed in tough membrane. In many cases the egg membrane consists of more than two layers. The outermost one is called the adhesive layer in some fish eggs for the adhesion of the egg is mainly due to the presence of this layer. This is found typically in the eggs of herring (Kanoh '49). In the eggs of Medaka the adhesive layer is absent on the surface of the egg membrane but a filamentous structure of the vegetative pole is of service to the attachment of eggs.

The chemical nature of the egg membrane was studied histochemically (T. S. Yamamoto '55a, '57a) and enzymologically. Polysaccharides and protein are dominant constituents of the egg membrane and probably disulfide bridges are also contained, at least in the case of herring eggs. The presence of protein was ascertained by the enzymological experiment. The egg membranes of Medaka and herring are easily digested with proteolytic enzymes such as trypsin and pancreatin. In the case of salmon, the membrane of unactivated eggs is dissolved only when double treatment with acidulated Ringer's solution and pancreatin

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is performed (Kanoh and Yamamoto '57). Differing from the case of Medaka and herring the membrane of the activated salmon egg is, however, not dissolved with the double treatment applied on the unactivated eggs (T. S. Yamamoto'57b). This fact indicates, therefore, that the nature of the egg membrane of the salmon had been changed at the time of activation.

The entry of the spermatozoon into the egg is carried out only through the micropyle at the animal pole of the egg, because the spermatozoa can not pass through the egg membrane. Thus the micropyle has an important role in fertilization. The formation of the micropyle has been observed as reported in the study of oogenesis in Medaka (T.S. Yamamoto '55a). As has been described in some teleostean fishes by Eigenmann ('90), a particular cell penetrates into the micropylar canal. This particular cell called "micropylar cell" is different in size and in affinity to some dyes from the other follicular cells. It is found on the surface of young oocyte. As the formation of the egg membrane does not occur at the site of the micropylar cell, a pit through the egg membrane appears in this part of the egg surface. It was found in the study of ovulation in the eggs of salmon and herring that the micropylar cell degenerated *in situ* prior to ovulation (T.S. Yamamoto '55b).

The nucleus of ripe unfertilized eggs of fishes shows the metaphase spindle of the second maturation division lying near the periphery of the animal pole. In the preceding stages the nucleus of the egg shows germinal vesicle and is located almostly in the center of the oocyte. As the transition of the nucleus is commenced toward the animal pole, the chromosomes appear in the germinal vesicle. On the other hand, yolk granules which had been fully formed in the cytoplasm of oocyte begin to fuse from vegetative pole at the same time. Thus the time of the commencement of the nuclear transition coincides with that of the fusion of yolk granules. At the time of ovulation the nucleus of the salmon or herring eggs shows metaphase spindle of the second maturation division, in other words, the nucleus of these eggs attains the stage of matured eggs even in the ovary (T.S. Yamamoto '55b). This finding does not coincide with that in amphibian eggs in which the nucleus of the eggs just ovulated shows germinal vesicle.

The first visible change at fertilization is a wave-like breakdown of the cortical alveoli which are embedded in the cortical cytoplasm of ripe unfertilized egg (cf. Kanoh '52a). Since the release of colloidal substance from the cortical alveoli is the cause of the formation of the perivitelline space (T. Yamamoto '56), the chemical nature and the origin of the cortical alveoli have recently attracted attention. It has been ascertained histochemically by many authors that the main components of the cortical alveoli are polysaccharides (Kusa '54, Aketa '54, T.S. Yamamoto '55a, etc.). According to K. Yamamoto ('56) fish eggs are divided into two groups by the nature of polysaccharide substance in the cortical alveoli. Examples of the first group are herring and flounder eggs; their cortical alveoli contain mucoids alone. In the second group are the eggs of smelt, salmon and

Medaka; their cortical alveoli contain mucoids associated with acid mucopolysaccharides. However, the cortical alveoli of fish eggs may not contain polysaccharides alone. In fact the cortical alveoli of herring eggs show a distinct positive reaction to Millon's test for protein and also show the presence of disulfide bridges as a result of the application of the revised method of ferric-ferricyanide technique (T.S. Yamamoto '57a). Concerning the origin of the cortical alveoli the present author investigated in Medaka (T.S. Yamamoto '55a) and concluded that the cortical alveoli originate from the "vesicles" which appear in the cytoplasm of the young oocyte and correspond to the "proteineous yolk vesicles" of Guthrie ('28) or "gouttes claires" of Konopacka ('35, '37). The same conclusion had been reached by K. Yamamoto ('56) in some teleostean fishes.

III

The fertilization in fish eggs is monospermic. What kind of mechanism blocks polyspermy? Concerning this problem, two possibilities have been considered. The one is that the block of polyspermy may be due to instantaneous changes of plasma surface occurring at the time of entry of the effective spermatozoon. This is held true in the case of sea urchin eggs. The other possibility is that the instantaneous changes may occur at the micropylar canal of egg membrane, in other words, the mechanism blocking the polyspermy occurs in the egg membrane. As an experimental approach to decide this problem, the fertilization in denuded egg, of which the membrane had been removed by double treatment with acidulated Ringer's solution and pancreatin, was observed. The result indicated apparently that the fertilization was polyspermic in these eggs (Kanoh and Yomamoto '57). Thus, the latter possibility may be considered to be of primary importance in the case of fish eggs, though the question remains still unsolved because the plasma surface of the denuded egg might have been somewhat damaged with this treatment. On these problems further discussion has been given in greater detail by Kanoh ('57).

Differing from the eggs of herring and Medaka, the inseminated eggs of salmon remain unactivated in isotonic Ringer's, NaCl and KCl solutions (Kanoh '50). The activation of the eggs of salmon is induced when the eggs are immersed in hypotonic solution. Cleavages of the fertilized eggs are also considerably inhibited in the isotonic Ringer's solution (K. Yamamoto '51). Thus the isotonic Ringer's solution inhibits both the activation and the cleavage in the salmon eggs. In the eggs immersed in isotonic $CaCl_2$ or $MgCl_2$ solution, however, the formation of distinct blastodisc happens without breakdown of the cortical alveoli and female pronucleus locates in the center of the blastodisc (Kanoh '52b, Kusa '53). Furthermore, the fertilized eggs immersed in this solution begin to cleave without treatment with hypotonic solution. Thus the isotonic $CaCl_2$ or $MgCl_2$ solutions do not inhibit the formation of blastodisc nor the cleavage of the egg. In this connection, the present author has recently investigated the effects of $ZnCl_2$ or

$ZnSO_4$, known as very toxic salts, on the eggs of salmon. It was found that when the eggs are immersed in isotonic Ringer's solution containing Zn-salt, they show no indication of cytolysis and ooplasm of the eggs begins to form the blastodisc at animal pole without breakdown of cortical alveoli and in some eggs, cleavings of blastodisc without nuclear divisions are observed to be caused. Thus the toxic action of Zn ions is not found in this case, probably because that toxic action may be arrested antagonistically by the presence of Na and K ions (cf. Loeb and Gies '02). When the eggs inseminated in isotonic Ringer's solution are transferred into Zn-Ringer's solution, cleavings of blastodisc proceed with nuclear divisions but the blastomeres thus formed show irregular arrangement. Therefore, it is clear that Zn-Ringer's solution does not inhibit but induces both the formation of blastodisc and its cleavings in the salmon egg. As mentioned already, the effects of Ca or Mg ions are found by the use of isotonic solutions which do not contain Na and K ions, in other words, by the use of solutions of the single salt (Kanoh '52b, Kusa '53). The present author performed further experiments using Ca- or Mg-Ringer's solutions having the same concentrations of Zn-Ringer's solutions but there occurred no visible change in the eggs. Thus it is concluded that the effect of Zn ions is more remarkable than that of Ca or Mg ions.

These results seem to contribute to knowledge concerning the effect of bivalent cations, but further investigation is needed to clarify the mechanism of the formation of blastodisc by these ions.

IV

In this paper, the formation and structure of the fish eggs were described briefly and the effects of Zn ions on the intact eggs of salmon were discussed in comparison with those of Na, K, Ca and Mg ions.

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