Notes on the Mechanism of Monospermic Fertilization in Fish Eggs[1)2)]

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The fact that fertilization in sea urchin eggs proceeds monospermic, has been emphasized since the time of Loeb and Lillie and its mechanism has been often discussed by many authors. As in sea urchin eggs, fertilization in fish eggs is also monospermic except for that in eggs of the Elasmobranchii. In contrast with that in sea urchin eggs, particular attention has scarcely been paid to the mechanism of monospermic fertilization in fish eggs. This is probably due to the general belief that the fish egg is enclosed with a compact membrane which admits no spermatozoa to penetrate excepting at only one point where a canal for the penetration of the sperm, a micropyle, is opened, and the micropyle is just as large as the head of a spermatozoon. In addition, it has also been commonly held that with formation of the perivitelline space the micropyle becomes narrow to refuse the admission of a second spermatozoon once the egg is fertilized (Ziegler '02).

These beliefs may be nearly truth, but afford even now some scope for discussion, accordingly should be reinvestigated because there have been no careful observations and experiments concerning them. In the present paper, the author therefore should like to present some considerations on these problems referring to the data obtained from experiments mainly on eggs of the Pacific herring, *Clupea pallasii*, and of the dog salmon, *Oncorhynchus keta*.

I

Differing from the case of the so-called marine invertebrate ova, in the case of fish eggs the part through which sperm penetrates into the egg is obviously restricted to only one definite point, a micropyle which is a small opening found in the compact egg membrane (chorion). The first subject to be considered here, accordingly, is naturally the construction of the micropyle.

Though the micropyle of fish eggs is in general funnel-shaped with small inner opening, there are two types, a simple and a complicated one. The former is a fine indentation formed merely by the depression of the chorion without any remarkable change in its thickness and a canal is found in the center of this indentation

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as shown by His in the salmon egg. The latter is also a fine indentation, but in this case the margin of the inner end of the canal protrudes inwards being thickened. The micropyle of the dog salmon egg belongs to the simple type and that of the herring egg to the complicated one (Kanoh '49).

As to the micropylar canal itself, it is, however, closely similar in each case having small size capable of allowing passage of only one spermatozoon of the same species. To describe it in detail, the outer opening of the canal of the salmon egg is about 9μ and its inner one about 3 μ in diameter, and these of the herring egg about 3 μ and 1.5 μ in diameter respectively. On the other hand, the head of a spermatozoon of the salmon measures about 3 μ and that of the herring about 2 μ in diameter. Therefore, even if more than one spermatozoa can enter the canal at the same time, only one of them ought to be in principle permitted to pass through the canal and to reach the egg cortex.

In fact, observations on the fresh herring egg have revealed as a rule that when the egg is inseminated, several spermatozoa reach the vicinity of the micropyle to begin an active sliding movement and enter the canal one by one, in file. Yet it is only the first one that can, passing through the canal, reach the egg cortex, even if the second one follows just behind the first (Yanagimachi and Kanoh '53). The same is also observed in the salmon egg (Kanoh, unpublished). It is however to be noted here that in the case of the herring and also of the salmon egg, supernumerary spermatozoa which have entered the canal in file are observed to be suddenly extruded from the canal sooner or later with formation of the perivitelline space following the penetration of the first spermatozoon into the egg cortex.

The block to polyspermy is supposed accordingly to be not only dependent upon the morphological status of the size of the micropylar canal in the chorion, but also upon the change occurring in the egg proper after fertilization, though it is certainly as a rule only a single spermatozoon that can pass through the canal.

II

Regarding the changes occurring in the egg cortex, it is well known that alveoli embedded in the cortex begin to break down when the egg is activated and in consequence perivitelline space is formed colloidosmotically (Yamamoto, T. '44, Kanoh '50, '53). Thus, the sudden extrusion of supernumerary spermatozoa in the canal after the penetration of the first spermatozoon into the egg cortex may be due to the pushing out of the substance eliminated from the egg cortex at the time of activation induced by the first spermatozoon.

In conncetion with these events, attention must be given to the relationship between the structure of the micropyle and the perivitelline pressure, of which the latter is evidently demonstrated with ease to be exerting considerable high power by the bursting out of the perivitelline substance when the chorion is torn with a needle.

As the micropyle is funnel-shaped and thus the chorion of this part is depressed inwards, it may happen therefore that when the perivitelline pressure begins to develop, the depth of such micropylar depression is in some extent lost owing to the pushing action of this inner pressure, consequently the canal becomes narrow.

On the other hand, since the chorion of the salmon egg swells in fresh water (Aoki '39), narrowing of the micropylar canal must be accelerated by such swelling of the chorion in the case of the salmon egg. In the case of the herring egg, on the contrary, swelling of the chorion is not conspicuous in sea water, but it should be noted here that the micropylar structure of the herring egg is complicated as noted above. That is : protruding thickened margin of the inner end of the canal may be the structure providing more facilities for narrowing of the opening. Further, as it shows different staining reaction from other parts of the chorion (Kanoh '49), it may be conceivable, differing from other parts, to have a delicate nature liable to narrowing, with special physico-chemical reaction to sea water or probably to substances released from the egg proper at the time of egg activation. At any rate, it is certainly in fact that once the perivitelline space begins to appear, spermatozoa, as a rule, can not pass entirely through the canal though they can enter it repeatedly.

Thereupon, it may be concluded that the block against supernumerary spermatozoa in fish eggs has been not only established primarily in the morphological status of the micropyle itself but also it may be increased secondarily by changes in the micropyle occurring after the penetration of one spermatozoon, in other words, after the activation of the egg, in addition to the changes in the egg proper.

Such being the case, the micropyle may be of great importance in the problems concerned ; attempts have therefore been made to determine whether or not the sperm might penetrate into the naked egg deprived of its chorion.

As to the methods of removing the chorion, mechanical and chemical ones are devised. In the unfertilized herring egg, it has been possible frequently to remove the chorion mechanically without injuring the egg cortex nor inducing egg activation by gentle treatment with fine scissors and needle. When the unfertilized naked egg thus obtained is inseminated with fresh milt, cleavage begins sometimes to proceed apparently in polyspermic fashion. In the salmon egg, however, no results have been obtained for such mechanical operation has proved unsuccessful. On the other hand, chemical removing of the chorion has been achieved by use of proteolytic enzymes in cases of both herring and salmon egg. That is to say, healthy unfertilized naked egg can be obtained in the case of herring by treatment of trypsin and in that of salmon by pancreatin (Yamamoto, T. S. '57, Kanoh & Yamamoto '57). Experiments of insemination performed on such naked eggs thus obtained have yielded a high percentage of cases, both herring and salmon eggs, showing polyspermic cleavage.

On the contrary, when once a normally fertilized or an artificially activated

egg is deprived of its chorion after formation of the perivitelline space, it is impermeable for spermatozoa, in other words, it shows no reaction to any superimposed insemination. Similar attempts have also been made by Kagan ('35) in the case of *Fundulus* eggs after formation of the perivitelline space, but the results were always negative. Furthermore, it has happened on rare occasions in the case of herring eggs that even after formation of the perivitelline space spermatozoa can enter through the inner end of the micropylar canal into the perivitelline space, but no penetration of such spermatozoa into the egg cortex takes place (Yanagimachi & Kanoh '53).

The findings noted above seem to indicate therefore the high possibility that in fish eggs protective change against penetration of supernumerary spermatozoa is supposed to occur after fertilization or activation as also in sea urchin eggs. Yet this change spreads over the entire surface of the egg proper not so rapidly as in the case of sea urchin eggs at the moment of fertilization by the first spermatozoon, though it can not be absolutely denied that artificial removing of the chorion, particularly in the case of unfertilized eggs, by such mechanical or chemical treatments as described above, might have impaired the egg cortex in some invisible way.

Sections of the herring eggs fixed just after insemination have often revealed that additional to the first spermatozoon one or two spermatozoa which are regarded to have nothing to do with further development have penetrated into the egg cortex just beneath the micropyle. But, no figures of degenerating sperm heads have been found in the sections (Kanoh '53). Accordingly, such extra spermatozoa having penetrated in the egg cortex may be eliminated shortly from the cortex into the perivitelline space with the progress of the changes in the cortex induced by the first spermatozoon.

Thus the change in the egg cortex to inhibit polyspermy may occur first at the penetration point of the spermatozoon, viz., at the point just beneath the micropyle and spread from this point over the entire surface of the egg cortex.

III

General conclusions concerning the mechanism of monospermic fertilization in fish eggs, thereupon, may be drawn from the considerations mentioned above as follows: supernumerary spermatozoa are restricted in their penetration into the egg, first, mechanically by the primary morphological status of the micropyle and then prevented to made penetration also mechanically by the pushing action of the substance eliminated from the egg cortex at the time of activation and by secondary changes in the micropyle resulting from physico-chemical reactions of this part, and physiologically by the protective change occurring in the egg cortex itself after the penetration of the first spermatozoon. Thus in these respects, the micropylar system is naturally of importance, but most essential are the changes in the egg proper, particularly those occurring at the point just beneath the

micropyle, which must be the subject of future research.

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