STUDIES ON THE FORMATION OF FISH EGGS

IX. The Fate of the Yolk Vesicle in the Oocyte of the Flounder, *Liopsetta obscura*, during Vitellogenesis*

Kiichirô YAMAMOTO
Faculty of Fisheries, Hokkaido Univiversity

This study has been carried out in order to clarify the fate of the yolk vesicles in the oocyte of the flounder. on this subject the present author has already reported that the yolk vesicles of herring and smelt oocytes appear to give rise to the cortical alveoli by migrating towards the periphery of the ooplasm (Yamamoto, 1956 d, e). One noteworthy difference between the vesicles of the two kinds of fishes lies in the nature of polysaccharides occurring in the vesicles, that is, the vesicles of herring oocytes contain mucoids alone throughout vitellogenesis, while polysaccharides contained in the smelt vesicles are transformed from mucoids into mucoids associated with the acid mucopolysaccharides in the course of vitellogenesis. Previously, the writer reported on the origin of the yolk vesicle and the chemical nature of cortical alveoli in the present species (Yamamoto, 1956 b, c), but the relation between the two elements remained open at that time. Hence, after having investigated a large number of preparations the problem has been further attacked as described here.

Before proceeding further, the author would like to express his cordial gratitude to Professor Tohru Uchida, Director of the Akkeshi Marine Biological Station, under whose guidance this work has been performed. The writer is also indebted to Dr. Sajiro Makino, Professor of Hokkaido University, for kind advice.

MATERIAL AND METHOD

This study was carried out exclusively with the oocytes of the flounder, *Liopsetta obscrua*. The material was obtained in a similar way as that described in the former paper (Yamamoto, 1956a). For fixation, Bouin-Allen's, formol-alcohol and Regaud's solution were used. Sections, 10 micra in thickness, were made by the usual paraffin method.

The presence of polysaccharides in the oocyte was examined by means of the periodic acid Schiff reaction (P.A.S.) and Bauer's reaction (cf. Glick, 1949; Lison, 1953). For the differentiation of the P.A.S. -positive substances use was made of the saliva test for glycogen (cf. Ichikawa. 1953), the combined staining method for aminopolysaccharides (Monnè and Slautterback, 1950), the metachromatic staining with toluidine blue for sulfated mucopolysaccharides (cf. Glick, 1949) and Highman's method with methylene blue for the determination of the relative acidity of mucoproteins (cf. Ichikawa, 1953).

* Contributions from the Akkeshi Marine Biological Station, No. 86.

For details in the practice of these cytochemical techniques the former papers may be consulted (Yamamoto, 1956 b, d).

OBSERVATIONS

1. The fate of the yolk vesicle during vitellogenesis

As was described in a former paper (Yamamoto, 1956 b), the yolk vesicles of the flounder make their appearance as minute granules distributed in the periphery of the oocyte in the form of a thin layer. They gave a strong P. A. S. reaction (Fig. 1). Immediately after the formation of the vesicles, yolk globules of small size are seen in the vicinity of the vesicles, intermingled with each other. As the yolk globules exhibited a very faint P. A. S. reaction, it was easy, when the material was fixed well, to distinguish the globules from the vesicles. With the growth of oocytes, the yolk vesicles and the yolk globules continue to be formed centripetally until the ooplasm is filled with them (Figs. 2 and 3). During this period, the globules increase rapidly in size, but the vesicles slowly. At the primary yolk stage small vesicles of diverse forms are found located everywhere in the interstices between the enlarged globules (Fig. 4). In the oocytes of the secondary yolk stage, whilst the vesicles are located sparsely in the outer part and densely in the middle part, no yolk vesicles can be seen in the inner part of the ooplasm. They are spherical in form and gave a strong P. A. S. reaction. In well-fixed materials the yolk globules which are much larger than the vesicles always exhibited a faint P.A.S. reaction (Fig. 5). After careful observations on a large number of preparations, the writer thinks that this statement as to the globules is the correct interpretation, although he reported previously that the yolk globules of this stage located in the middle part of the ooplasm gave a strong P. A. S. reaction (Yamamoto, 1956b). Such an extraordinary response of the yolk globules to the polysaccharide test has been considered to be caused by the diffusion of the vesicle contents. Hereafter, the yolk vesicles, as was described in the previous paper (Yamamoto, 1956 b), gradually migrate towards the outer part of the ooplasm and finally are found arranged regularly in the periphery of the cortical cytoplasm, roughly forming a row (Figs. 6, 7 and 9). Consequently, it seems beyond doubt that the cortical alveoli of the flounder may also be derived from the yolk vesicles as in the case of the herring and the smelt.

2. On the chemical nature of polysaccharides occurring in the yolk vesicles

Polysaccharides contained in the yolk vesicles of the flounder remained unaltered in chemical nature throughout vitellogenesis. The polysaccharide substances reacted positively on the P. A. S. test and Bauer's reaction. Saliva was inactive with respect to the P. A. S.-positive substances. Moreover, the contents of the vesicles were not stained metachromatically either with toluidine blue or with methylene blue in acid solutions, regardless of whether or not the preparations were pre-treated with chromic acid. These

results fit in completely with those in the herring. Therefore, it seems most justifiable to form the same conclusion as that arrived at in the herring, i. e., the mucopolysaccharides in the vesicles may be regarded as neutral mucopolysaccharides (mucoids) and the vesicles contain the mucoids alone throughout vitellogenesis.

DISCUSSION

In contrast to herring and smelt oocytes, flounder oocytes have the vesicles much smaller than the globules. Although some confusing figures have resulted on account of this fact, there remains little doubt that the fate of the yolk vesicles of the flounder is quite similar to what happens in the cases of the herring and the smelt. The vesicles of the flounder exhibited always a strong P. A. S. reaction throughout vitellogenesis. On moving towards the periphery, they appear to give rise to the cortical alveoli which play an important role at fertilization. On the other hand, the yolk globules are accumulated in the cytoplasm, independently of the yolk vesicle. They continue to grow during the vitellogenesis and finally by their confluence become a yolk mass which is supplied as the nutritive material for later development. The globules gave always a very faint P. A. S. reaction. Therefore, the yolk vesicles differ from the yolk globules in their origin and fate in vitellogenesis and in the role in development.

In connection with this, some consideration should be given to the cortical granules of sea urchin eggs. As has already been pointed out by Kusa (1954), these granules have much in common with the cortical alveoli of fish eggs both in chemical nature and function. The granules are also composed of polysaccharide substance combined with sulfuric acid residues (Monnè and Hardè, 1950) and break down at fertilization (Moser, 1939; Motomura, 1941; Endo, 1952). According to Monnè and Hardè (1950), the prospective cortical granules are at first scattered throughout the whole cytoplasm and migrate into the cortex during maturation. As a similar feature has been demonstrated in the prospective alveoli of fish eggs, it seems very interesting to make clear the origin of the prospective cortical granules scattered throughout the cytoplasm.

SUMMARY

1. The yolk vesicles and the yolk globules in the flounder oocyte appear to be quite independent of each other. The former seem to give rise to the cortical alveoli embedded in the cortical cytoplasm, while the latter are accumulated in the extravesicular cytoplasm and grow into a continuous yolk mass enclosed by the cortical cytoplasm.

2. During vitellogenesis, the polysaccharide substances contained in the vesicles undergo no chemical change. They seem to be neutral mucopolysaccharides.

LITERATURE

Endo, Z. (1952). The role of the cortical granules in the formation of the fertilization membrane in eggs from Japanese sea urchins, 1. *Expl. Cell Res.* 3, 406-418.

Glick, D. (1949). *Techniques of histo- and cytochemistry.* 531p. London; Interscience Publishers.

Ichikawa, O. (1953). *Cytochemistry.* (in Japanese). 478p. Tokyo; Honda Shoten.

Kusa, M. (1954). The cortical alveoli of salmon egg. *Annot. Zool. Japon.* 27, 1- 6.

Lison, L. (1953). *Histochimie et cytochimie animales.* 2e Édit., 607p. Paris; Gauthier-Villars.

Monné, L. & Slautterback, D.B. (1950). Differential staining of various polysaccharides in sea urchin eggs. *Expl. Cell. Res.* 1, 477-491.

———— & Hardé (1950). On the cortical granules of the sea urchin egg. *Ark. f. Zool.* 1, 487-498.

Moser, F. (1939). Studies on a cortical layer response to stimulating agents in *Arbacia* egg, 1. *J. Exp. Zool.* 80, 423-446.

Motomura, I. (1941). Materials of the fertilization membrane in eggs of Echinoderms. *Sci. Rep. Tohoku Imp. Univ., Ser. IV.* 16, 345-363.

Yamamoto, K. (1956a). Studies on the formation of fish eggs, I. Annual cycle in the development of ovarian eggs in the flounder, *Liopsetta obscura. J. Fac. Sci. Hokkaido Univ. Ser.* VI, 12, 362-373.

———— (1956b). Ditto. III. Localization of polysaccharides in oocytes of *Liopsetta obscura. Ibid.* 12, 391-399.

———— (1956c). Ditto. IV. The chemical nature and the origin of the yolk vesicles in the oocyte of the flounder, *Liopsetta obscura, Jap. Jour. Zool.* 11, 567-577.

———— (1956d). Ditto. VII. The fate of the yolk vesicle in the oocyte of the herring, *Clupea pallasii,* during vitellogenesis. *Annot. Zool. Japon.* 29, 91-96.

———— (1956e). Ditto. VIII. The fate of the yolk vesicle in the oocyte of the smelt, *Hypomesus japonicus,* during vitellogenesis. *Embryologia,* 3, 131- 138.

PLATE I

Explanation of Figures

All figures are photomicrographs from sections of the flounder oocytes. Magnification of the figures is about 400 times.

Fig. 1. Early period of the yolk vesicle stage

Fig. 2. Middle period of the yolk vesicle stage

Fig. 3. Later period of the yolk vesicle stage

Fig. 4. Primary yolk stage

Fig. 5. Secondary yolk stage

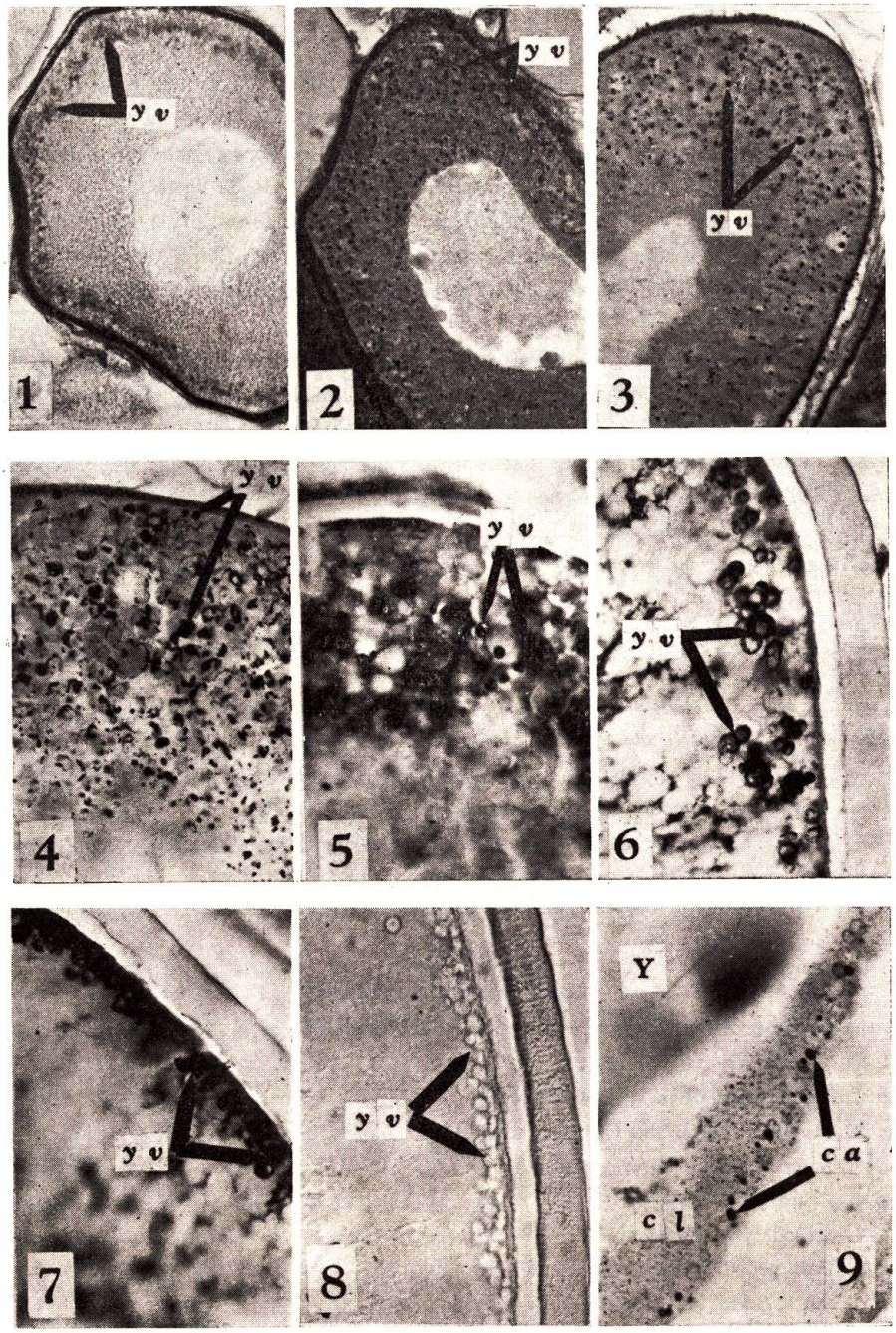
Fig. 6. Eary period of the tertiary yolk stage

Figs. 7 and 8. Later period of the tertiary yolk stage

Fig. 9. Maturation stage

All figures except 8 were made from Bouin-Allen and P. A. S. preparations treated with saliva prior to the P. A. S. test. Fig. 8 was taken from a Bouin-Allen and toluidine blue preparation.

c. a. Cortical alveoli, c. l. Cortical layer, Y. Yolk, y. v. Yolk vesicles



K. YAMAMOTO: Formation of Fish Eggs (IX)