HOKKAIDO UNIVERSITY

Title	STUDIES ON THE FORMATION OF FISH EGGS： X II. On the Non-massed Yolk in the Egg of the Herring, Clupea pallasii
Author(s)	YAMAMOTO, Kiichiro
Citation	北海道大學水産學部研究彙報, 8(4), 270-277
Issue Date	1958-02
Doc URL	http://hdl.handle.net/2115/23013
Type	bulletin (article)
File Information	8(4)_P270-277.pdf

Instructions for use

Hokkaido University Collection of Scholarly and Academic Papers : HUSCAP

STUDIES ON THE FORMATION OF FISH EGGS

XII. On the Non-massed Yolk in the Egg of the Herring, *Clupea pallasii*

Kiichiro YAMAMOTO
Faculty of Fisheries, Hokkaido University

According to the form of the yolk, one can classify fish eggs into two different types, massed-yolk eggs and non-massed-yolk ones. Of these two types, the former has already been studied in the previous work, resulting in the conclusion that a continuous mass of yolk in the flounder is derived from yolk globules alone and the lipids in the globules consist mainly of phospholipids (K.Yamamoto 1957).

As for the formation of non-massed yolk, on the other hand, there can be found several valuable papers, such as those of Lams (1903) on *Osmerus*, of Konopacka (1935) on *Cyprinus* and *Gobio*, and of Osanai (1956) on *Lefua*; for the chemical nature of non-massed yolk there are the papers of Konopacka (1935) and Mas (1952). In spite of these works, there still remain many controversial points requiring elucidation. The present writer has studied some of those points, using the oocyte of the herring as the material, and obtained some findings worthy to be noted here.

Before proceeding further, the writer would like to express here his cordial thanks to Professor Tohru Uchida, Director of the Akkeshi Marine Biological Station, under whose guidance the main part of this work has been performed. The writer is also indebted to Professor Sajiro Makino, Faculty of Science, and Professor Hisanao Igarashi, Faculty of Fisheries, for their valuable advices, and also to staff members of the Akkeshi Marine Biological Station for their kindly aid extended in collecting the material.

MATERIAL AND METHOD

The oocytes of herring, *Clupea pallasii*, were obtained in a similar way to that described in the former paper (K. Yamamoto 1955a).

The lipids occurring in the yolk globules were demonstrated by Ciaccio's test and the modified Sudan black B and alcohol method. Moreover, the chemical nature of lipids was investigated by Baker's test for phospholipids, Schultze's test for cholesterol and Smith's Nile blue staining for the differentiation of phospholipids from glycerides.

Details concerning the practice of these cytochemical techniques may be learned by consulting the previous paper (K.Yamamoto 1957).

RESULTS

1. Formation of non-massed yolk globules

In herring oocytes, yolk vesicles are formed as primary vitelline particles. At the beginning of their formation, the vesicles are minute and spherical, and found scattered

in the ooplasm. They gave always a negative Ciaccio's reaction and positive P. A. S. reaction (K. Yamamoto 1955*a*). The vesicles then increase rapidly in size and number, and are found arranged in the periphery of the ooplasm, roughly forming a row. At the same time, very minute granules showing a strong Ciaccio's reaction are seen in the extravesicular cytoplasm. Being flocked together near the periphery of the ooplasm, the granules form a narrow ring situated outside the vesicle layer (Fig. 1). As the oocyte grows further, the formation of the vesicles proceeds by and by, to occupy a large part of the ooplasm. During this period, the Ciaccio-positive granules also increase in size and number, though not markedly (Fig. 2). At the next stage, the Ciaccio-positive granules grow rapidly in size and develop into so-called yolk globules. The yolk globules showing a strong and homogeneous Ciaccio's reaction appear at first in the peripheral cytoplasm and then invade the interstices between the yolk vesicles, and finally they fill the inner cytoplasm surrounding the nucleus. In the meanwhile, the vesicles show no marked changes, and still remain sudanophobic (Figs. 3 and 4). Then, the globules continue to grow in size and number; this activity appears to be more vigorous in the inner part of the ooplasm than in the outer part. Consequently, the vesicles seem to be shifted towards the periphery of the ooplasm, where they are found forming a thick layer as shown in figures 5 and 6a. The shifted vesicles are still much larger in size than the globules.

At the stage when the germinal vesicle nears its migration, the globules attain conspicuously large size and are now no less in dimension than the vesicles. Therefore, in Ciaccio preparations it becomes difficult to demonstrate under low magnification the presence of the vesicles situated in the peripheral ooplasm (Fig. 7). During the pre-maturation and maturation stages, the fusion of the yolk globules seems to occur. The globules grow much in size but decrease in number. Now the globules are much larger in size than the vesicles. It is noteworthy that the affinity of the globule for Sudan III or Sudan IV decreases clearly and the fatty droplets, which were stained with Sudan III and dissolved out with fat solvents, make appearance in the ooplasm during this period. In Ciaccio preparations the globules and follicle layer gave a positive reaction, while the cytoplasm, yolk vesicles and egg membrane were always negative (Fig. 8). Even in ripe eggs, there are found many yolk globules of large size, but no yolk in a continuous mass.

2. The chemical nature of lipids present in the yolk globules

As already mentioned above, the yolk globules of herring oocytes were strongly Ciaccio-reactive throughout vitellogenesis. To investigate the chemical nature of lipids present in the globules, the oocytes in the secondary yolk stage were subjected to several cytochemical tests following Lison's table for lipid analysis (1953). The results obtained are as follows: The modified Sudan black B and alcohol method clearly demon-

strated the presence of lipids in the globules, being stained deeply in blue black (Fig. 6B). Unstained formalin-fixed frozen sections were almost colourless and proved to contain no discernible carotinoids. Schultze's test for cholesterol was also negative. By the application of Baker's test for phospholipids, the globules were deeply stained blue black. In contrast to the globules of the flounder, however, the yolk globules of the herring oocytes were stained blue black even after being treated with pyridine, although some reduction of coloration could be detected under the treatment (Figs. 9a and 9b). The globules were stained blue by Smith's Nile blue staining for the differentiation of blue-stained acid lipoid from red-stained neutral fat (Fig. 10).

From the data above, it is evident that the globules of the herring oocytes contain no appreciable quantities of glycerides and carotinoids and cholesterol as well as those of the flounder, but noteworthy it is that the yolk globules of the herring oocytes showed a positive reaction for both the standard acid haematein test and pyridine extract test. Cain (1947) reported a similar fact that the epidermal cells and plasmosomes of *Glassiphonia* give a positive reaction for both the standard acid haematein test and pyridine extraction test. From the detailed examination with Baker's test, he concluded that a substance which reacts positive for both the acid haematein test and pyridine test cannot be regarded as a phospholipid, and that only a blue-black coloration given by the acid haematein test but not by the pyridine extraction test indicates phospholipids. Therefore, the results obtained in the present study are apt to lead to the conclusion that the main part of the lipids demonstrated in the globules of the herring must be some kinds of conjugated lipids other than phospholipids. However, it seems necessary to undertake further detailed examinations before this conclusion may be fully accepted, because the specificity of the Baker's test depends on the relatively greater affinity of phospholipids among conjugated lipids for the mordant (Cain, 1947), whereas the globules are certainly composed of complicated lipid-protein complexes. Consequently, the resistance of the lipids in the globule against the fat solvents depends not only on the affinity of lipids for the mordant, but also on the quantitative difference of the constitutional components and the combining state of lipid-protein components. In this regard, together with taking into consideration biochemical results showing that fish eggs contain a large quantity of phospholipids but a meagre amount of other conjugated lipids (Young and Phinney 1951, Igarashi *et al.* 1955, 1956a, b), it seems justifiable to surmise that a weak reduction of coloration of pyridine extracted preparations does not necessary indicate the presence of a small amount of phospholipids, or rather the resistance of the lipids against pyridine extraction.

DISCUSSION

Concerning the formation of non-massed yolk of fish eggs, Lams reported detailed observations on *Osmerus eperlanus* as early as 1903. In *Osmerus*, fatty globules

appear as primary vitelline element. After the fatty globules have been formed into two layers, inner and outer, the yolk globules begin to be accumulated in the cytoplasm between the egg membrane and the fatty globules. The formation of the yolk globules then proceeds towards the interstices between the fatty globules of the outer layer, and finally all ooplasm between the fatty globules comes to be filled with the yolk globules. Lams' findings on *Osmerus* agree with the writer's findings on *Clupea* in so far as the formation of yolk globules is concerned, that is, the globules appear at first in the periphery of the coplasm and are formed centripetally. But there can be found a marked difference between the two species. In *Osmerus*, fatty globules composed of two layers have already been accumulated in the inner and outer regions of the ooplasm before the yolk globules begin to be formed, while in *Clupea* only yolk vesicles have been found prior to the formation of yolk globules and the appearance of fatty droplets is recognized in far more advanced stages. However, as Lams did not make sure of the fatty nature of the globules by reliable techniques, it seems necessary to confirm whether the fatty globules are really fatty in nature or whether they correspond to the yolk vesicles composed mainly of mucopolysaccharides. Furthermore, the findings on the closely related species such as *Hypomesus japonicus* (K. Yamamoto 1955b) show that only yolk vesicles have been found in the outer regions of the cytoplasm before the formation of yolk globules begins to start.

Another striking report concerning the formation of non-massed yolk is that of Konopacka (1935) on *Cyprinus* and *Gobio*. Her findings fit in pretty well with those of the present writer. In these species as well as the present material, yolk vesicles which were designated by her as "gouttes claires" are firstly formed in the ooplasm of a peripheral region and then accumulated inwardly. Soon after, the granules of small size make appearance in the peripheral cytoplasm and then in the interstices between the vesicles. Along with the proceeding of vitellogenesis, these granules increase in size and number, and grow into yolk globules designated as "plaquete vitelline". During the later phase of vitellogenesis, one noteworthy difference between her materials and the herring can be recognizable. In *Cyprinus* and *Gobio* the vesicles situated in the inner part of the ooplasm, exclusive of the peripheral region, break down and the material contained in the vesicles play a supplementary part in the formation of the yolk globules; in *Clupea* the vesicles of the inner ooplasm do not break down and are only shifted towards the periphery of the oocyte as mentioned above. It is difficult to determine with certainty whether all vesicles present in the inner part of the ooplasm are shifted intact towards the periphery of the oocyte or not, because of the difficulty in preparing good sections from the advanced stages of fish eggs. But it seems improbable that most vesicles situated in the inner part break down and participate in the formation of the yolk globules. If that were true, the cortical layer could not be

embedded thoroughly with cortical alveoli as is really seen in ripe eggs, because the surface of the oocyte increases enormously with the growth of the oocytes, while the formation of the vesicles comes to an end in earlier stage and the number of the vesicles remains unaltered thereafter as confirmed by Konopacka (1935), K. Yamamoto (1956), and Osanai (1956). On the basis of the above considerations, therefore, it is most reasonable to consider that the yolk vesicles scarcely participate in the formation of the non-massed yolk, but give rise to the cortical alveoli by shifting towards the periphery of the ooplasm as already asserted by the present writer (1956) and Osanai (1956).

The presence of lipids in the non-massed yolk of fish eggs has already been demonstrated by Konopacka (1935) working on *Gobio* and *Cyprinus*, by Mas (1952) on *Perca*. On the other hand, the presence of lipids in the globules has also been established in *Oryzias* (T. S. Yamamoto 1955) and *Liopsetta* (K. Yamamoto 1957) whose globules give rise to a continuous mass of yolk. Therefore, the findings obtained in the present study offer further evidence for the conclusion that the yolk globules of fishes, regardless of whether or not they give rise to a continuous mass of yolk, contain a large quantity of lipids. However, the ratio between lipids and proteins in yolk globules is not always similar in all species and in all yolks at different stages. In *Gobio* and *Cyprinus* the yolk globules at the beginning of formation are composed mainly of lipids and the lipid components of the globules then come to combine with protein substances as yolk formation proceeds (Konopacka 1935); *Liopsetta* globules, judging from the result of Sudan staining tests, become rich in lipids with the growth of oocytes (K. Yamamoto 1957). On the other hand, the globules of the herring oocytes contain much lipids from the beginning of formation and the lipid-protein ratio in the globules appear to remain almost unchanged until the time when fat droplets are formed.

As to the quality of lipids in the globules, Konopacka (1935), from the result of Smith-Dietrich test, asserted that the lipids in the yolk globules of *Gobio* and *Cyprinus* are phospholipids. A similar conclusion was obtained by the present writer, using the Baker's test and Smith's Nile blue staining test, that the globule lipids in the flounder must be phospholipids composed mainly of lecithin, but the substance seems to be changed in nature during vitellogenesis. On the other hand, the yolk globules of herring oocytes showed a different reaction in response to the Baker's test giving only a weak reduction of coloration for the pyridine extraction test. But this difference in reaction is considered to depend partly upon the difference of the nature of lipid itself, but rather more upon the quantitative difference of constitutional components and the combining state of lipid-protein components in the globules. Taking into consideration the results of biochemical analysis, therefore, it seems probable that the globule lipids of fish eggs consist mainly of phospholipids.

SUMMARY

1. The formation of yolk globules in the herring oocytes commences in the periphery of the ooplasm and proceeds inwardly until a greater part of the ooplasm is filled with them. At the last stage of vitellogenesis, the globules become large in size by their fusion, but make no continuous mass of yolk. The yolk vesicles appear to play no role in the formation of the yolk globules.

2. The presence of lipid is established in the yolk globules of all stages by cytochemical techniques. The globules in the oocytes of the secondary yolk stage showed a weak reduction in response to the Baker's pyridine extraction test, but they are supposed to be composed mainly of phospholipids.

LITERATURE

Cain, J.A. (1947). An examination of Baker's acid haematein test for phospholipines. *Quart. J. Micro. Sci.* 88, 457–478.

Igarashi, H., Zama, K. & Katada, M. (1955). Studies on the phosphatide of aquatic animals. 1. On the egg lecithins of Shark. (in Japanese with English summary). *J. Agr. Chem. Soc. Jap.* 29, 454–457.

———, ——— & ——— (1956a). Ditto. VIII. On the egg lecithins of pollacks (*Theragra chalcogramma*). (in Japanese with English summary). *Ibid.* 30, 566–568.

———, ——— & ——— (1956b). Ditto. IX. Cephaline, neutral fat and unsaponifiable material of the eggs of pollack (*Theragra chalcogramma*). (in Japanese with English summary). *Ibid.* 30, 568–572.

Konopacka, B. (1935). Recherches histochimiques sur le dèvèloppement des poissons. 1. La vitellogenèse chez le goujon et la carpe. *Bull. Acad. Polonaise Sci. et Let. B*, 163–182.

Lams, H. (1903). Contribution a l'etude de la genèse du vitellus dans l'ovule de teleosteens. *Arch. d'Anat. Micro.* 6, 633–653.

Lison, L. (1935). *Histochimie et cytochimie animales.* 2 edit. 607 p. Paris, Gauthier-Villars.

Mas, F. (1952). Contribution a l'histologie de l'ovogènèse chez un teleostéen, *Perca fluviatilis L. Bull. Soc. France* 77, 414–425.

Osanai, K. (1956). On the ovarian eggs of the loach, *Lefua echigonia*, with special reference to the formation of the cortical alveoli. *Sci. Rep. Tohoku Univ. Ser.* IV, 12, 181–188.

Yamamoto, K. (1955a). Studies on the formation of fish eggs. V. The chemical nature and the origin of the yolk vesicle in the oocytes of the herring, *Clupea pallasii*. *Annot. Zool. Japon.* 28, 158–162.

——— (1955b). Ditto. VI. The chemical nature and the origin of the yolk vesicle in the oocyte of the smelt, *Hypomesus japonicus*. *Ibid.* 28, 233–237.

——— (1956). Ditto. VII. The fate of the yolk vesicle in the oocytes of the herring, *Clupea pallasii*, during vitellogenesis. *Ibid.* 29, 91–96.

——— (1957). Ditto. XI. The formation of a continuous mass of yolk and the chemical nature of lipids contained in it in the oocyte of the flounder, *Liopsetta obscura*. *J. Fac. Sci. Hokkaido Univ. Ser.* VI. 13, 346–353.

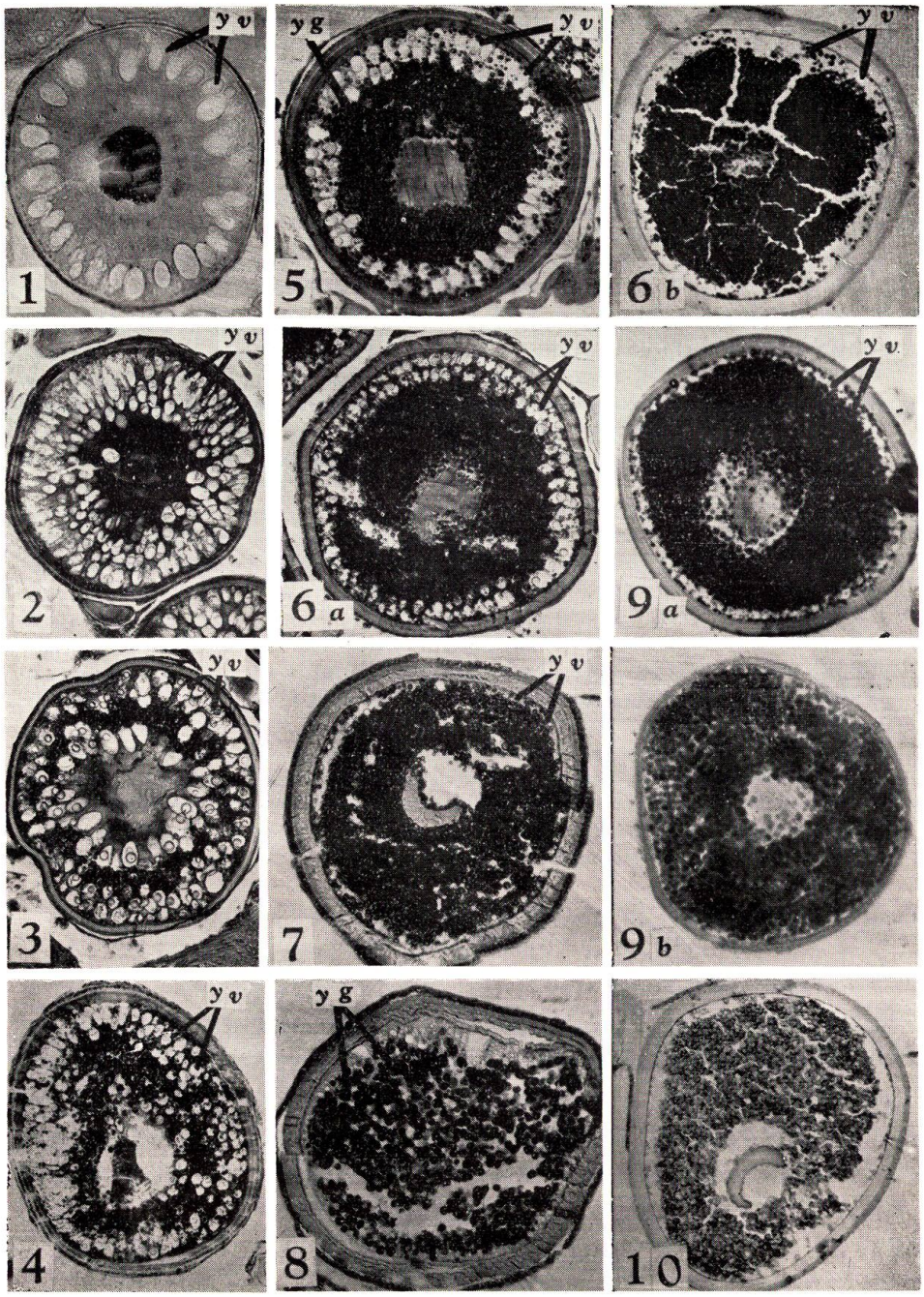
Yamamoto, T. S. (1955). Morphological and cytochemical studies on the oogenesis of the fresh-water fish, Medaka (*Oryzias latipes*). (in Japanese with English summary). *Jap. Jour. Ichthyol.* 4, 170–181.

Young, E.G. & Phinney, J. T. (1951). On the fraction of the proteins of egg yolk. *J. Biol. Chem.* 193, 73–80.

Explantion of Plate

All figures are photomicrographs from sections of herring eggs.

Figs. 1. and 2. Yolk vesicle stage. Regaud and Sudan IV preparations.
Figs. 3. and 4. Primary yolk stage. Preparations as above.
Fig. 5. Secondary yolk stage. Regaud and Sudan III preparation.
Fig. 6a. Same stage as above. Regaud and Sudan IV preparation.
Fig. 6b. Same stage as above. Modified Sudan black B and alcohol preparation.
Fig. 7. Tertiary yolk stage. Regaud and Sudan IV preparation.
Fig. 8. Maturation stage. Preparation as above.
Fig. 9a. Secondary yolk stage. Baker's acid heamatein preparation.
Fig. 9b. Same stage as above. Baker preparation with pyridine treatment.
Fig. 10. Same stage as above. Smith's Nile blue staining preparation.
y.v. yolk vesicle, y.g. yolk globule.



K. YAMAMOTO: Formation of Fish Eggs (Ⅻ)