ON THE VITELLINE SYNCYTIUM AND THE ABSORPTION OF THE YOLK IN THE FRY OF TWO SALMONIDS

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Introduction

In his previous paper, the writer reported the structure of the yolk-sac in the pond smelt fry with special reference to the vitelline syncytium which is considered to play some important role in the yolk absorption. As is already described by Cordier (1941), the yolk in the yolk-sac of rainbow trout is enclosed by a vitelline syncytium which he considered as a dialyzing membrane to filter the yolk.

The structure of the yolk-sac in dog salmon and rainbow trout, both treated in this study, does not differ essentially from that of pond smelt. However, on the surface of the vitelline syncytium, there are many vitelline veins in which blood containing a vast number of blood corpuscles is circulating. This is in striking contrast to the structure of the yolk-sac in pond smelt. In the latter species, the fetal blood has no blood corpuscles and flows inside of the yolk-sac wall around the whole yolk mass. In the present study, besides observations on the general transition of the yolk mass in the yolk-sac, attention has been paid especially to the relation between the blood cells and the syncytial nuclei by tracing the behaviour of the nuclei in histological preparations, as blood cells were unobservable in the study of the pond smelt.

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Materials and Method

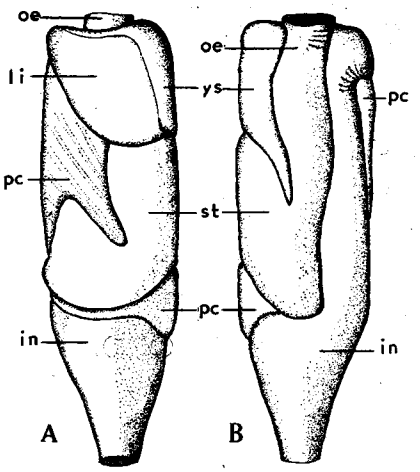
The histologically examined fry ranged respectively from 2.6 to 4.0 cm in the case of dog salmon (*Oncorhynchus keta*) and 1.6 to 3.8 cm in rainbow trout (*Salmo irideus*). The age of these materials in days after hatching was mostly uncertain excepting some of the rainbow trout fry.

The method of preparing the microscopic preparations is the same as described in the previous paper.

Observations

Transition of the yolk mass in outline

Each of the young fry examined is either provided with or is without a yolk-sac in the belly, but for a considerable period the fry which have lost their yolk-sac, in external appearance, maintain more or less some remnant yolk mass or only vitelline syncytium



Textfig. 1. Schematic drawings of ventral and dorsal views of the digestive tract and its accessory organs in the rainbow trout, 3.1 cm in body-length. ×5
A. Ventral view
B. Dorsal view
in: intestine; li: liver; oe: oesophagus; pc: pyloric caeca; ys: yolk and vitelline syncytium

embedded in their ventral cavity.

For instance, when the viscera are taken out of the ventral cavity of the rainbow trout fry, 3.1 cm in body-length, their dorsal and ventral views are as shown in Textfig. 1. The alimentary canal turns twice being twisted to form the stomach. Under the anterior part of the stomach the triangular liver closely adheres. One can easily recognize also a tapering organ adherent to the left side of the stomach in the dorsal view. This is still existing yolk which is absent in larger fishes. In section, the base of this tapering yolk mass which is adjacent to the liver consists almost entirely of vitelline syncytium and a little yolk, while the slender arm is composed entirely of syncytium (Fig. 1). Fine vitelline veins pass all over the whole surface.

It is impossible to make whole serial sections of the fry which retains still a large amount of yolk, but the periphery of the yolk mass, especially at its anterior part, is converted into vitelline syncytium which a worker has less difficulty in sectioning. Accordingly, the cutting was made from anterior to posterior of the body as far as the point where it came almost impossible.

The anteriormost section through the yolk mass in the two-day-old fry of the rainbow trout is shown in Fig. 2. It passes also through the sinus venosus and the posterior part of the auricle. A hollow space can be seen at the middle of the yolk mass. That may be the site which was originally filled with some fatty substance as is shown in the yolk-sac of the pond smelt fry. The peripheral portion of the yolk mass as well as the circumferance of the above mentioned hollow space is transformed into vitelline syncytium in which many vitelline veins are distributed. This is essentially the same over the whole surface of the yolk mass (Fig. 3). But the thickness of the syncytial wall is the largest in its anterior portion where the area is rich in vitelline veins and nuclei.

Later on, the yolk comes to occupy only the left side of the body, although it is not clear whether it occured because the yolk absorption proceeded more rapidly in the right side than in the left, or because the displacement of the yolk took place in later stages (Fig. 4). As this figure is a frontal view of the section, passing through the cuvierian duct, the yolk mass is seen at the left in the figure. A hollow space is at the top of the mass.

With the decrease of the amount of yolk mainly in the right side of the body, the

site comes to be occupied by the growing liver. After all, the rise and fall of the bulk of these two masses leads to the situation as is already shown in Textfig. 1. Most of the vitelline veins distributed over the whole surface of yolk join into one to open into the sinus venosus, but some of them enter into the liver directly. It seems, therefore, that the vitelline veins which remain when the yolk has been absorbed become the radicles of the hepatic or portal veins according to the outgrowth of the liver to the yolk area.

Detailed observation on the vitelline syncytium

As yolk of eggs of dog salmon or rainbow trout take the form of the so-called massed yolk which differs from that of pond smelt eggs, sections of the yolk show a continuous smooth mass. In the peripheral part which is contact with the vitelline syncytium, however, the yolk mass contains many spherical yolk drops (Fig. 5). As they also exsist in the inner part of the syncytium, the boundary between yolk and syncytium is quite indistinct. The vitelline veins running through the syncytium often lack their endothelial wall, where exposed blood corpuscles are directly in contact with the syncytium.

The nuclei scattered in the syncytium are extremely varied in shape, measuring in diameter about 20 to 30μ on the average (Fig. 6). They contain two or more nucleolus-like bodies which are similar both in size and shape to the nuclei of erythrocytes. The bodies are stainable with haematoxylin in blue, with azan in red, and are positive to the Feulgen reaction, showing that they consist exclusively of chromatin.

The nuclei migrate toward the vicinity of the vitelline veins, and after their arrival at the goal they liberate their contents into veins (Figs. 5, 7 and 8). As for the liberation of the nuclear contents, three different ways may be distinguished. One of them is the direct contact of the nuclei with the exposed blood corpuscles at the portion where no endothelium is visible (Fig. 5). The second, encountered rather abundantly in the cases, is that there is a slight discrepancy between the positions of the liberating nuclei and those of the veins (Fig. 7). And the third is the liberation of the nuclear contents at just outside of the endothelial wall (Fig. 8). In the latter two cases, the nuclei seem likely to be secreting cells, while in the former case, their behaviour calls to our mind an amoeboid movement. The meaning of this difference in the behaviour of the nuclei cannot be explained.

The phenomenon of the liberation of the nuclear contents comes to be seen in anypart of the syncytium which is in contact with the vitelline veins, according to the advance in the yolk absorption. In looking over Figs. 9, 10 and 11, it may be well interpreted that the extension of the vitelline veins in the syncytium takes place after the degeneration of the nuclei has been completed. In Fig. 9, it is shown that the liberation of the nuclear contents into a vitelline vein is being performed by many nuclei standing on the wall of the vein. After the liberation has been completed, the sites are subsequently vacuolized (Fig. 10). Then, these vacuoles are penetrated by a number of blood corpuscles and become filled with them (Fig. 11). In this way, the vitelline veins extend their influence all over

the vitelline syncytium which is being increasingly invaded by the veins.

On the multiplication of the vitelline nuclei, no typical amitotic figures were observed in the present study, likewise as in the case of pond smelt.

Discussion

The vitelline syncytium should be derived from subgerminal periblast which appears as early as in late cleavage stage in dog salmon (Saito, 1950). Recently, studies on the structure of the periblast and its nuclei in the developing salmon eggs were carried out by Nishida (1958a,b). His description on the structure of the periblast and the size and shape of its nuclei are quite in accord with the structure of the vitelline syncytium and size and shape of the vitelline nuclei which was observed in the present study. Therefore, it is obvious that the vitelline syncytium in the yolk-sac of the hatched fry is descended from the periblast of the early embyonic stage, and further that the essential nature of the periblast is invariable throughout the whole course of yolk absorption, excepting the fact that the vitelline syncytium is provided with many veins while the periblast is not yet. As even before the blood vessels develop and the blood begins to circulate, the yolk was being absorbed by the embryo through the interposed periblast, the role which the periblast or vitelline syncytium plays in the yolk absorption may have not any direct relationship with the presence of the blood vessels. However, the distribution of the blood vessels over the yolk mass seems to be necessary to accelerate the yolk absorption according to the increasing consumption of yolk by the rapidly growing embryo. Therefore, after the vitelline veins have developed, the best objective to observe in order to understand the function of the vitelline nuclei play in the yolk absorption is supposed to be their behaviour in the vicinity of the veins.

Observation clearly showed that the nuclei migrate towards the vitelline veins and liberate their contents into them. But it is difficult to say whether the migration of the nuclei is really autonomous or that a movement of the ground substance of the syncytium which may also be absorbed through the veins causes the nuclei to migrate. It must be noted here, however, that the syncytium is continuously transferred into blood at the portion where blood vessels lack their endothelium, but the nuclei are not always concentrated at that portion.

The contents of the nuclei under liberation react to the Feulgen test, suggesting that the nuclei play a role to synthesize DNA and to provide the embryo with it. In the case of pond smelt, the liberation of nuclear contents was not observed but the nuclei tended to adhere to the outer surface of the syncytium on which the colourless blood was circulating. This is considered, therefore, to be equivalent to the liberation phenomenon in the present study on salmon and trout.

In general vertebrate embryogenesis, most erythrocytes are said to arise from blood islands in yolk region. Some investigators insist that yolk spheres give rise to newly formed

cells which, then, turn into erythrocytes (Lepeschinskaja, 1936; Chishima, 1956). In this connection, it is very interesting that the vitelline nuclei contain some chromatin nucleoli which are similar in size to the nuclei of erythrocytes. But it can scarcely be said that these nucleoli change into nuclei of erythrocytes. Rather, they seem to be destroyed and to disappear when the vitelline nuclei liberate their inner substance. Consequently, the sites of the nuclei turn into vacuoles which come to be occupied by a number of infiltrated blood cells from the vein. After all, the contents of the nuclei are replaced by several in number erythrocytes, but it should not be considered that the nuclei themselves change into or produce erythrocytes.

The yolk is transformed into vitelline syncytium which consists of nuclear and cytoplasmic substances, and it supplies a demand of a developing embryo or a fry. The vitelline syncytium is probably not a mere dialyzing membrane as is considered by Cordier (1941). Furthermore, the yolk, including the vitelline syncytium in a broad sense, is not merely a mass of inert nutrient substance but it should be called an 'active substance' in which some important biochemical syntheses are taking place.

The vitelline nuclei are comsumed one after another in the course of yolk absorption, neverthless, the syncytium in any stage retains a vast number of nuclei. How do these nuclei arise ? According to the accepted opinion, the vitelline nuclei are believed to multiply in amitosis. But this opinion seems to be insufficient to explain the phenomenon described here, as there was almost no figure in the stage of amitotic division. In considering Lepeschinskaja's insistence that yolk spheres form new cells, an elaborate study on this subject is required.

Summary

The process of yolk absorption in the hatched fry of dog salmon and rainbow trout was traced mainly by means of histological observations of the yolk-sac. The yolk is enclosed by vitelline syncytium in which are distributed many vitelline veins which are contributing to absorb the yolk. The veins often lack their endothelium, and the syncytium is continuous to the blood at that portion. The syncytium has several large vacuoles which are considered to be filled with some fatty substance.

After the disappearance of the yolk-sac, the yolk mass remains for a considerable period in the abdominal cavity of the fry, and it comes finally to consist practically of syncytium only. The vitelline nuclei migrate towards the veins into which they liberate their centents. Three happenings can be distinguished based on the behaviour of the nuclei; the direct destruction of the nuclei at the endothelium-less portion, the liberation of nuclear substance at a slightly distant portion from a vein, and the liberation at the outside of the endothlium. After the nuclear contents have been liberated, the sites are consequently vacuolized. Then, the vacuoles are filled with blood cells. In such a way, the vitelline syncytium is increasingly invaded by the veins.

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EXPLANATION OF PLATES

All figures except Fig. 8 are sections fixed in Bouin and stained with haematoxylin. The length of the fish is shown in body-length.

PLATE I

Fig. 1. Cross section through the region of liver and vitelline syncytium in the drawings in Text-fig. 1. Rainbow trout, 3.1 cm. ×40 li: liver; st: stomach; vs: vitelline syncytium

Fig. 2. Sinus venosus and anterior part of yolk mass in the central cavity of the rainbow trout fry, two days after hatching, 1.6 cm. ×64 au: auricle; sv: sinus venosus; y: yolk

Fig. 3. A part of yolk-sac wall and underlying vitelline syncytium taken from the lateral side of yolk-sac of the rainbow trout fry, two days after hatching, 1.6 cm. ×260 ep: epithelium; vs: vitelline syncytium; y: yolk

Fig. 4. Yolk mass situated at the left body side (right in the figure). At the top of the mass is a hollow space. Dog salmon, 2.7 cm. ×26 cd: cuvierian duct; y: yolk

Fig. 5. Peripheral yolk and vitelline syncytium in which three amoeboid nuclei are visible. Rainbow trout, two days after hatching, 1.6 cm. ×260 n: nucleus

Fig. 6. Various shaped and sized nuclei in the developed vitelline syncytium. Rainbow trout, forty days after hatching, 2.5 cm. ×260

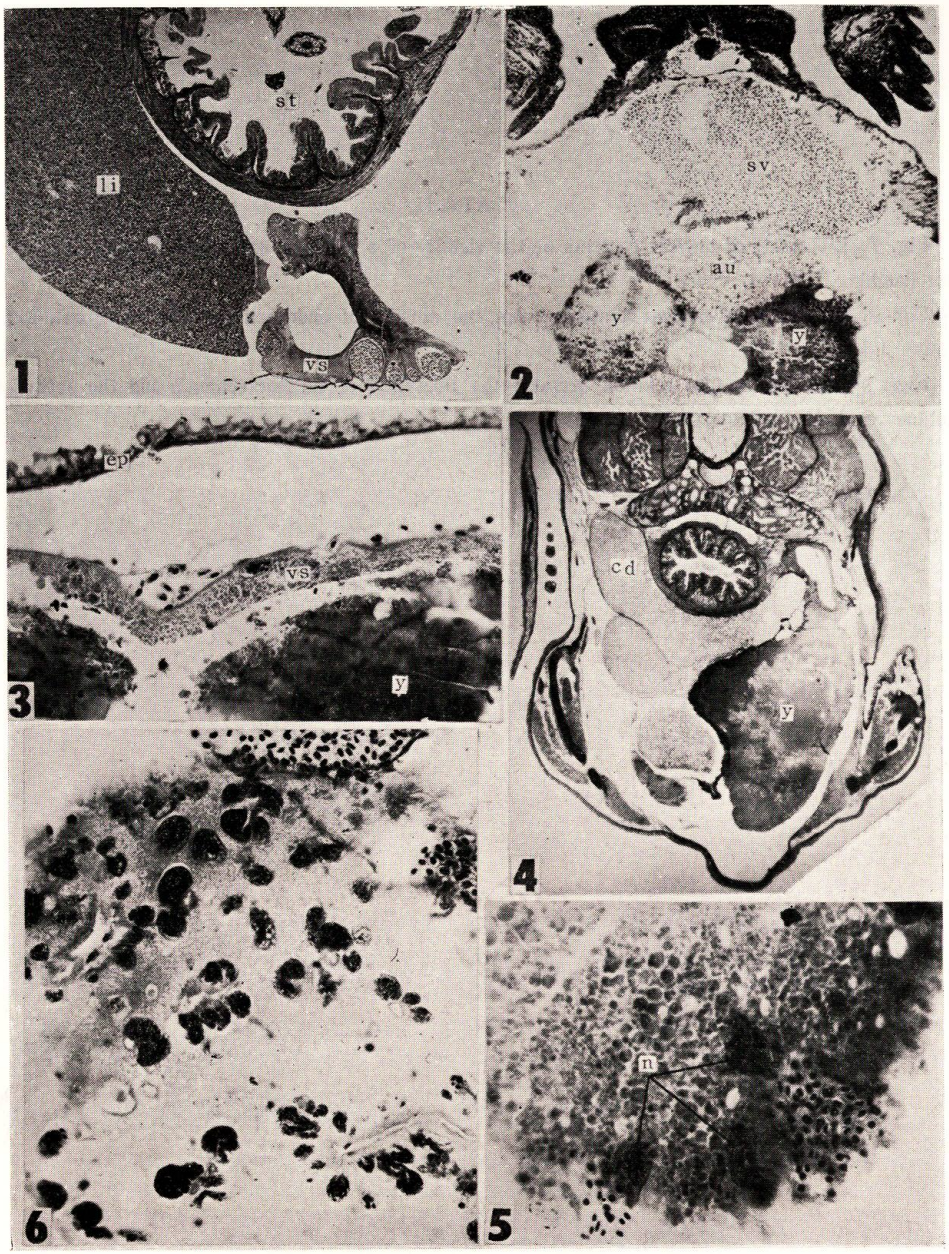

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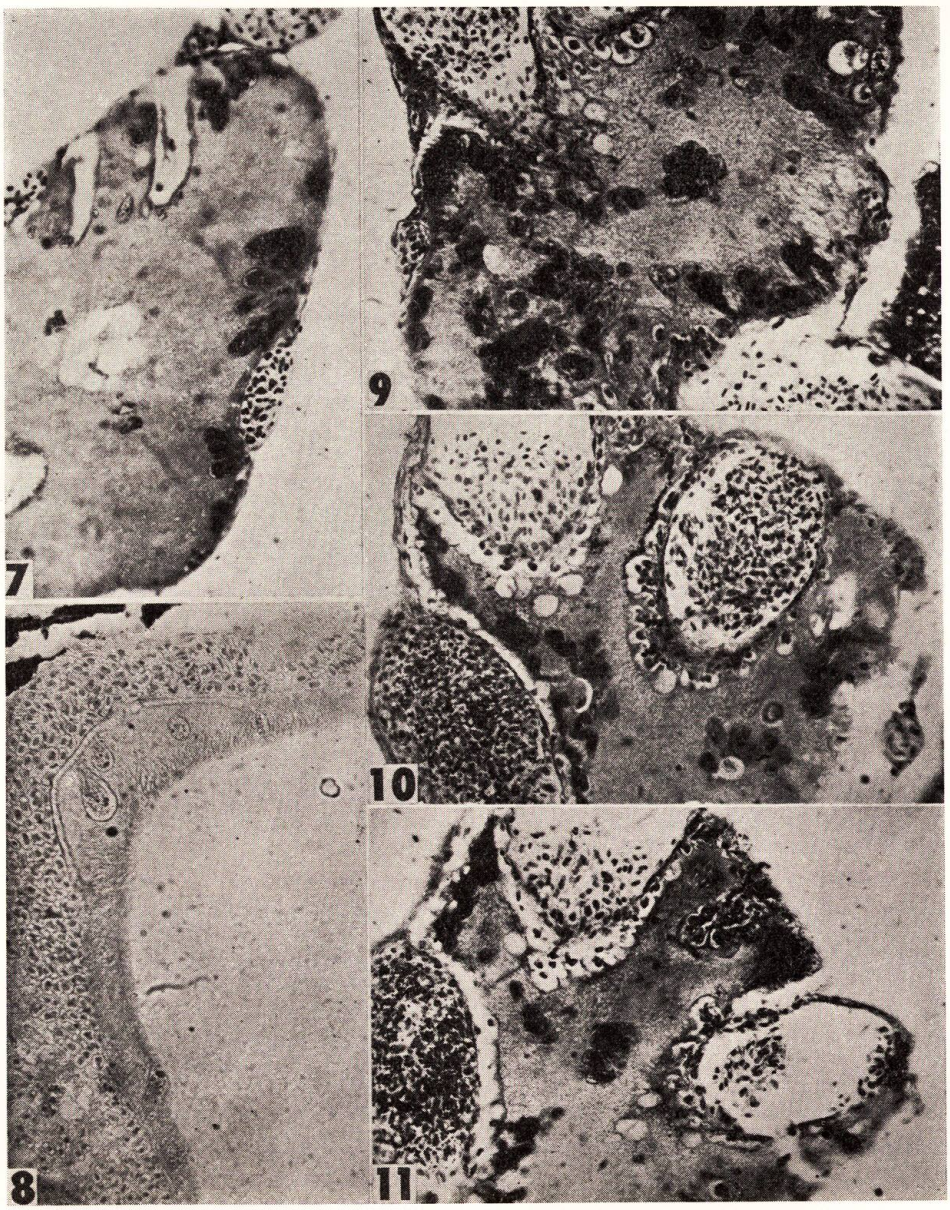
PLATE II

Fig. 7. Liberation of nuclear contents at the vicinity of a vitelline vein. Rainbow trout, forty days after hatching, 2.5 cm. ×260

Fig. 8. Liberation of nuclear contents from the outside of endothelium. Rainbow trout, 2.3 cm. ×260 Feulgen

Figs. 9–11. Showing the relation between the liberation of nuclear contents and the extension of vitelline veins. Dog salmon, 4.0 cm. ×260



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