THE STRUCTURE OF THE YOLK-SAC AND THE ABSORPTION OF THE YOLK IN THE POND SMELT FRY

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INTRODUCTION

Most of the teleost fry just after hatched out have a large yolk-sac in the belly, and live continuing the differentiation and the growth of the body structure without taking any foods until yolk in the yolk-sac has been consumed. Hitherto, on the utilization of yolk in stages of embryonic development, it has generally been accepted that the yolk is taken into the embryonic cells as an inactive nutrient material or a mere energy source of the developing embryo, and is consequently transformed into cytoplasm. Recently, Bellairs (1958), who studied the conversion of intra-cellular yolk drops into cytoplasm in chick embryo, discovered the appearance of mitochodria within the yolk drops and suggested that the yolk is not inert. There is available only a little imformation about the absorption of yolk in fish fry, viz., that the yolk drops are dissolved out from the yolk-sac and are transferred to every part of body, but detailed study of this process has not been advanced.

In the present study, the strucutre of the yolk-sac was principally observed to find out the manner of the absorption of yolk in the pond smelt fry. The yolk in the yolk-sac is not intracellular as shown by Bellairs but is extra-cellular; the results described here seem to show that the yolk, also, in this case, is not only a mass of inactive substance.

The writer is greatly indebted to Prof. S. Saito for his kind criticism and for the revision of this paper. Hearty thanks are due to Mr. K. Hamada of our laboratory for his helpful advices. To Mr. Z. Kofuji and Mr. S. Tanaka, the writer expresses his sincere gratitudes for their kind aid in collection of materials used.

MATERIAL AND METHOD

The eggs and young fry of the pond smelt (*Hypomesus olidus*) used in this study were taken in Lake Ōnuma and the old waterway of the Ishikari River (Ishikari-Furukawa). The developing eggs, artificially fertilized by members of the Fisheries Association of those actual places, were brought into laboratory and kept in water regulated within the temperature range of 7°C to 10°C. The eggs were hatched out in about three weeks after insemination. While the hatched fry were fixed daily up to the ninth day after hatching, some of the living fry were observed under low magnification. All fishes had died within ten days after hatching.

The agents employed in fixation were Bouin's, Carnoy's, Gilson's, Regaud's fluids and neutral formalin. Paraffin or carbowax sections were made from 6μ to 10μ in thickness. For histological preparations, the sections were stained with Delafield's haematoxylin or

Heidenhain's azan. Besides above, histochemical tests were also made such as the PAS reaction for polysaccharides, the Feulgen for DNA, Sudan III or Sudan black B for lipids and Romieu's reaction for cholesterol. The procedures for these reactions followed Lison (1953).

OBSERVATIONS

The general features and internal structure of the hatched fry

The just hatched fry has an appearance of a slender transparent needle-like form; its total length is about 5 mm. The oval shaped yolk-sac, about 0.6 mm in length, is embedded in the front region of the belly (Figs. 1 and 2). The original fin is a continuous thin membrane around the caudal end from dorsal to ventral, beginning at the ear and ending at the posterior portion of the heart. At the base of the ventral membranous fin, chromatophores are arranged in a row. The alimentary canal is a straight duct rather uniform in size, above both sides of which are the pronephric ducts (Fig. 3). Beneath the notochord is the dorsal aorta but the venous system is yet indistinct. Along the right side of the gut, posterior from the middle of the yolk-sac, there lies the pancreas (Fig. 8), while the liver is at the left just behind the yolk-sac (Fig. 13). Between the two organs, a duct opens just under the gut at the dorsal side of the yolk-sac (Fig. 8). This duct running through the liver to enter the posterior part of the gut will be mentioned later in detail. The air-bladder has not yet developed. The heart consists of two cell layers, endocardium and myocardium, lying in the pericardiac cavity (Fig. 2). A constriction divides it into two chambers, ventricle and auricle, the latter being situated rather more dorsalward than the former, so the heart takes somewhat S-shaped form. Forward of the heart is the ventral aorta which connects through the gill to the dorsal aorta running below the notochord to the caudal end. Backward of the heart, the auricle communicates with a wide funnel-like cavity which opens to the anterior part of the yolk-sac; there the sinus venosus or cuvierian duct is yet undifferentiated. The fetal blood is colourless and has no blood corpuscles.

The structure of the yolk-sac

The upper half of the oval shaped yolk-sac is embedded in the belly in the region of about 1.2 mm from the head end; its lower half is covered with the skin which overspreads the whole body surface. In this wall of yolk-sac, no blood vessels are observed either in sections or in external view. The contents of the yolk-sac is not a simple mass of yolk, but it consists of an anterior oil globule, the outermost wall of syncytium and the inner yolk mass. Detail of each costituent is given as follows.

The oil globule: The glistening oil globule always occupies the most anterior part of yolk-sac; the diameter of the globule is less than 0.2 mm (Figs. 1 and 2). The front two-thirds of the surface of this sphere is covered with a cytoplasmic wall of syncytium while the remaining one-third is in contact with the yolk (Figs. 4 and 9). As such large oil globule cannot be seen in the egg which is in the early developmental stages, it must be

assumed that the small oil droplets scattered among the yolk drops have been gathered and fused gradually into one during the later stages. When a formalin-fixed fry is put into water, the oil globule becomes gradually larger because of the inflow of some fine granules, probably oil droplets, from the yolk region. Finally, the syncytial wall ruptures then the contents flows out. Occasionally, when the rupture occurred at the most anterior part of the wall, the inner oil flowed into the funnel-like cavity in front of the yolk-sac. It passes through the heart, the gill region and reaches up to the dorsal aorta. Furthermore, observing a living fry under microscope, it can be found that the syncytial wall facing toward the funnel-like cavity vibrates with the pulsation of the heart. These facts prove a direct relationship between the heart and the yolk-sac.

The oil which flowed out from the body is soluble in benzine, acetone and alcohol.

The vitelline syncytium: Under the dermal wall of yolk-sac, the oil globule and the reserved yolk mass are enclosed in a syncytial tissue, in which a number of large nuclei are spotted (Fig. 2). This vitelline syncytium may have been derived from the periblast which is generally seen under the germ-disc in the early stages of fish embryogenesis. The anterior syncytial wall, inside of which is only the oil globule but no yolk, is thicker than the other portion which is in contact with the yolk mass (Figs. 3, 4 and 5). The nuclei in the anterior portion are round and numerous; they measure about 11μ in diameter (Fig. 3), twice as much of those in the liver cells. On the contrary, those in the other portion are more or less depressed and prolonged in shape according to the thinning of the ground substance, and the number is not so great (Fig. 5). Therefore, it can be said that most of the nuclei are concentrated in the anterior portion. Among the nuclei, no typical phases of nuclear division either in mitosis or in amitosis can be observed, though there are a few amitotic figures which do not take a typical gourd-shaped form but only show a considerably slender form without a marked constriction. But it is uncertain that these are true amitotic figures. Normally, one nucleolus besides chromatin reticulum is visible in each nucleus, but sometimes there are two or more in a large one. The nucleoli are stainable in red with azan and are Feulgen-positive, showing that they are not true nucleoli.

The ground substance of the syncytium takes an appearance of a fine granular structure stainable with light-green. It corresponds to the cytoplasm of somatic cells. In the interior of the syncytium, which is in contact with the yolk, there are various sized PAS-positive bodies (Fig. 6). Some of the large ones among them are similar to the nuclei in outline as well as in size (Fig. 10), but are negative to the Feulgen reaction.

In the contact surface of the syncytium with the yolk, especially in the portion just behind the oil globule, phases of transformation from yolk to syncytium can be observed. The process will be described below.

The yolk: The yolk of the pond smelt egg is a so-called non-massed yolk. In section, there are a plenty of yolk drops of various sizes. Observing the sections, fixed in Bouin or

Gilson and stained with haematoxylin, one sees that the yolk drops in the yolk-sac may be roughly divided into two; one group comprises the nearly spherical vacuolated drops showing some bubbly structure stained intensely, and the other the amorphous smooth drops stained faintly (Figs. 5 and 8). The former has a tendency to be located in the central region of the yolk-sac, contrary to the latter in the peripheral. In the periphery of the yolk-sac, where especially the yolk comes in contact with the oil globule, the smooth yolk drops melt in a liquid state, in which some fibrous structure appears (Fig. 9). Between this liquid yolk and the outer syncytial wall, there is often a portion where no clear boundary can be found. The above-stated difference between the two kinds of yolk drops is quite indistinct in the sections fixed with Carnoy or Regaud. The honey-combed yolk drops containing many vacuoles give negative PAS reaction, while the smooth drops as well as liquid yolk react faintly. Moreover, the PAS-positive granules are present in the inner part of the syncytium as described already (Fig. 6). The chemical content of the vacuoles within the vacuolated yolk drops is judged to be cholesterol or its derivatives from the positive result of the formalin-fixed frozen sections to which Romieu's reaction for cholesterol was applied.

The other body structures related to the yolk-sac

The funnel-like cavity in front of the yolk-sac is filled with fluid which is stained faintly with haematoxylin. In sagittal section, a crescent-shaped space can be seen in contact with the front wall of the sycytium resulting from the coagulation of this fluid by fixation (Fig. 7). The fluid shows positive reaction with PAS; it is clearly shown in the PAS-applied sections to fill the heart, the other blood vessels and also the interstitial spaces of various tissues, as well as the funnel-like cavity (Fig. 11). Accordingly, the fluid may be the fetal blood which is devoid of any blood corpuscles. Moreover, it can be said that the circulatory system in the larval stage of the pond smelt is still an opened one. Besides with the PAS, the fluid is stainable with Sudan black B in the Regaud-fixed carbowax sections (Fig. 12).

A duct opens at the posterior portion of the yolk-sac, between the gut and the upper surface of the vitelline syncytium. In the cross section, as shown in Fig. 8, the opening of the upper half of this duct is a thin cellular membrane, while the lower half is in common with the wall of the vitelline syncytium. Tracing the duct to the caudalwards, one finds that it expands at the portion behind the yolk-sac, behind which it gets gradually narrower as it passes through the liver close to the pancreas (Fig. 13). Finally, the duct ends in being united with the gut together with the other duct running out from the pancreas. In general morphogenesis, the liver and pancreas are formed from a blind sac projected from alimentary canal. Accordingly, the duct may be called here the "hepatic duct", assuming its appearance at the site mentioned as an indication of a growing stage of the liver and the pancreas. It is difficult to consider, therefore, that it would play a part in the digestion of yolk, though the anterior end of this duct has the intimate relation with the yolk in position.

Further change of the yolk-sac

From the external view, the yolk-sac almost disappears in the fry aged five days after hatching. But internally, it takes about nine days to exhaust the yolk, including the syncytium, completely.

The first visible change occurring in the yolk-sac is the gradual decrease in amount of the vacuolated yolk drops. Then, the relative amount of the smooth yolk drops increases, and at last, the inner part of the yolk-sac becomes filled with only the smooth drops or the liquid yolk (Fig. 14). The peripheral syncytium thickens according to the diminishing of the yolk. The nuclei increase in number remarkably (Fig. 15). The spherical nuclei are transformed into oval or more irregular shapes, and have a tendency to arrange themselves radially in the outer edge. A chromatin network appears in each of the multiplied nuclei and it becomes so conspicuous, that it is difficult to identify them as nuclei at a glance, but they give clearly the Feulgen-positive reaction (Fig. 16).

The oil globule decreases in size and at last disappears when the yolk drops are completely transformed into syncytium or liquid yolk. The hepatic duct of which its anterior end opens at the dorsal surface of vitelline syncytium becomes enlarged and comes to occupy the yolk-sac region (Fig. 17). The bulge of the duct takes an appearance of a blind sac neighboring to the remaining syncytium which is under absorption (Fig. 18). In this manner, the whole yolk as well as the syncytium is consumed entirely, leaving there only a large space which is the anterior bulge of the hepatic duct (Fig. 19). Blood corpuscles are not yet recognizable even in this stage.

DISCUSSION

John (1932), who has studied the origin of erythrocytes in herring, recognized that the posterior part of the heart communicated to the funnel-like cavity in front of the yolk-sac, which was filled with colourless blood in which no blood corpuscles were observed. That finding is quite in accord with the observation made in the present study on the pond smelt fry. Moreover, the facts that there is no vitelline vein on the surface of the yolk-sac, that the anterior wall of the syncytium holding the oil globule vibrates with the heart beats, and that a part of the oil globule flows into the heart through the funnel-like cavity, all suggest that the yolk in the yolk-sac is absorbed at its anterior part into the blood in some way.

The circulatory system remains to be called an opened one in the young fry in which only the main arteries have been differentiated. The venous blood which returns from the caudal end may flow back to the heart passing around the outer surface of the vitelline syncytium and through the anterior cavity of the yolk-sac. In other words, it can be regarded that the whole yolk mass which is enclosed by the syncytial wall is contained in a large vein.

In the foregoing account, the yolk is not descrided as dissolving out of the yolk-sac in the form as it is. Cordier (1941) considered in his experiment on injection of acid dyes in

trout yolk-sac that the vitelline syncytium plays a role as a dialyzing membrane through which the yolk is utilized by the embryo. His concept that the syncytium has a role to filter the yolk seems to be quite possible, but it must be noted here that the syncytium is originally formed from the conversion of yolk. The fact that the phase of transformation from yolk to syncytium is always observable seems to show the necessity of the conversion of yolk into such cytoplasmic substance in the process of the yolk absorption.

The matured egg of the pond smelt is supposed to be composed of peripheral yolk vesicles and inner yolk globules, according to the study of ovogenesis in smelt, *Hypomesus japonicus* (K. Yamamoto, 1956 and 1958). As the yolk vesicles are liberated in the perivitelline space when the egg is fertilized, there remain the yolk globules only. Now, in the yolk-sac, two kinds of yolk drops can be distinguished; these, of course, should be derived from a single kind of yolk, viz., from yolk globules. In considering the quantitative change of the two kinds of yolk, it can be assumed that the absorption of yolk may take place through the change as follows: the yolk globules — the vacuolated yolk drops — the smooth yolk drops — the liquid yolk — the vitelline syncytium. With regard to the PAS reaction, as the conversion of yolk progresses the reaction tends to increase in intensity. It is a proof to indicate that the synthesis or the liberation of polysaccharides may take place in these yolk drops.

It has long been considered possible that the periblast is concerned in the absorption of yolk in developing fish eggs. T. S. Yamamoto (1957) estimated the role of the periblast in herring, as it plays a part in the absorption of yolk after gastrulation from the observation that at the stage it comes to contain more polysaccharides, SH, and lipids than the other part. In the pond smelt fry, the vitelline syncytium contains many PAS-positive bodies, and it holds at the anterior a large oil globule instead of a weak staining for lipids of the syncytium by Sudan black B.

The periblastic nuclei have been believed to originate in excess spermatozoa in polyspermy or in the dropping down of one of the daughter nuclei when a mitotic division occurred in marginal blastomeres, and to multiply in amitosis. Lepeschinskaja (1936) reported in chick the new formation of cells from yolk without nuclear division and insisted that the cells develop into erythrocytes. A similar opinion was expressed by Chishima (1956) in frog embryo. If the opinion of those workers is correct, the newly formed cells which were indicated in thir opinions, contradictory to usual biological common view, may correspond to the periblastic nuclei in fish embryogenesis. Nishida (1958 a,b) stated regarding his histochemical observation of the periblast in salmon, that he found there some nucleus-like bodies which seem to contain nucleotides, throwing some doubt upon the accepted theory on the multiplication of periblastic nuclei.

So far as the present study is concerned, it is difficult to make any statement about the question on the origin of nuclei in the vitelline syncytium. However, it must be empha-

sized that even the amitotic figures are scarcely recognizable, of course no mitotic, in spite of the marked increase in the number of nuclei which accompany with the diminishing of yolk. Moreover, there can be seen, also in this case, the Feulgen-negative nucleus-like bodies in the syncytium.

Most of these nuclei are concentrated in the anterior end of the yolk-sac; in considering the close connection of the appearance of nuclei with the absorption of yolk, it is reasonable to conceive that the absorption takes place mainly in this portion.

According to the general review by Bracht (1950), eggs can be distinguished into two types on the basis of the pattern of the synthesis of nucleic acids: the one contains a considerable quantity of RNA which may be transformed into DNA during development, and the other synthesizes both the nucleic acids simultaneously. Brachet also says that, with some exceptions, the oligolecithal eggs with total cleavage belong to the former type, while the large ones with partial cleavage and provided with a vitelline syncytium do to the latter. In this connection, the vitelline syncytium appears to have much importance in the synthesis of nucleic acids. The marked increase in number of nuclei in the process of the yolk absorption may be possibly concerned with this matter. In another study on the yolk-sac of rainbow trout, the present writer recognized obvious phenomenon that the contents of the syncytial nuclei are liberated in vitelline veins (Yamada, 1959). In the pond smelt, therefore, it is not unreasonable to consider that the peripheral situation of the multiplied nuclei which are probably under the degenerating phase as shown in Fig. 18 may have close connection with that phenomenon.

The oil droplets lying among yolk granules begin to gather and fuse into one during development to form a large oil globule. As these oil droplets are known as glycerides (K. Yamamoto, 1958), the large globule in the yolk-sac must also be made up of neutral fats. The invariable situation of the oil globule in the yolk-sac suggests its some playing role in the absorption of yolk as a source of fatty acids.

The hepatic duct enlarges in keeping with the progress of the yolk absorption and finally it comes to occupy the region where the yolk has been embedded, as if it had pushed the yolk forewards. The ultimate fate of this duct remains unknown as the more advanced stages could not be observed. It is difficult to consider, at least, that the duct takes any part in the absorption of yolk.

SUMMARY

Observations were made of the structure of the yolk-sac and the associated internal body structures in the pond smelt fry (*Hypomesus olidus*). In the yolk-sac, the yolk mass and a large oil globule are enclosed by a syncytial tissue in which a number of large nuclei are scattered. In the larval stage, the circulatory system of this species is still to be called an opened one. The fetal blood is colourless and has no blood corpuscles. It can be

considered that the contents of the yolk-sac is embedded as a whole in a large vein, viz., the yolk-sac, in which the fetal blood is circulating. The yolk does not dissolve out from the yolk-sac in the form in which it exsists. It is transformed into the cytoplasmic syncytium through changes in form such as, in turn, vacuolated yolk drops, smooth yolk drops and liquid yolk. In this yolk and the cytoplasmic syncytium, the synthesis of polysaccharides and nucleic acids may be taking place. The chemical nature of the oil globule is probably that of neutral fats, while that of the vacuoles within the vacuolated yolk drops may be cholesterol. As the diminishing of the yolk-sac proceeds, the wall of syncytium thickens in which the nuclei remarkably increase in number though the typical figures of the nuclear division can never be observed, and there are often Feulgen-negative nucleus-like bodies in the inner part of the syncytium. The hepatic duct enlarges forwards and comes to occupy the yolk region, leaving only a wide cavity which is the anterior bulge of the duct, when the whole yolk as well as the syncytium has been completely absorbed.

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EXPLANATION OF PLATES

PLATE I

All figures (1–6) represent external view or sections of just hatched fry.

Fig. 1. External view of the anterior half of the body in the living state. ×40

Fig. 2. Sagittal section through the heart and the yolk-sac. Bouin-haematoxylin. ×80 fc: funnel-like cavity; ht: heart; l: liver; og: oil globule; vs: vitelline syncytium; y: yolk

Fig. 3. Cross section through the anterior wall of the vitelline syncytium. Bouin-haematoxylin. ×250 ch: notochord; da: dorsal aorta; g: gut; pn: pronephric duct; vs: vitelline syncytium

Fig. 4. Cross section through the middle part of the oil globule. Bouin-haematoxylin. ×250 og; oil globule; vs: vitelline syncytium

Fig. 5. Cross section through the middle part of the yolk-sac. Bouin-haematoxylin. ×250 vs: vitelline syncytium; ys: smooth yolk drop; yv: vacuolated yolk drop

Fig. 6. Cross section through anterior part of the yolk-sac showing the PAS-positive bodies. Bouin-PAS. ×250 n: nucleus; pb: PAS-positive body

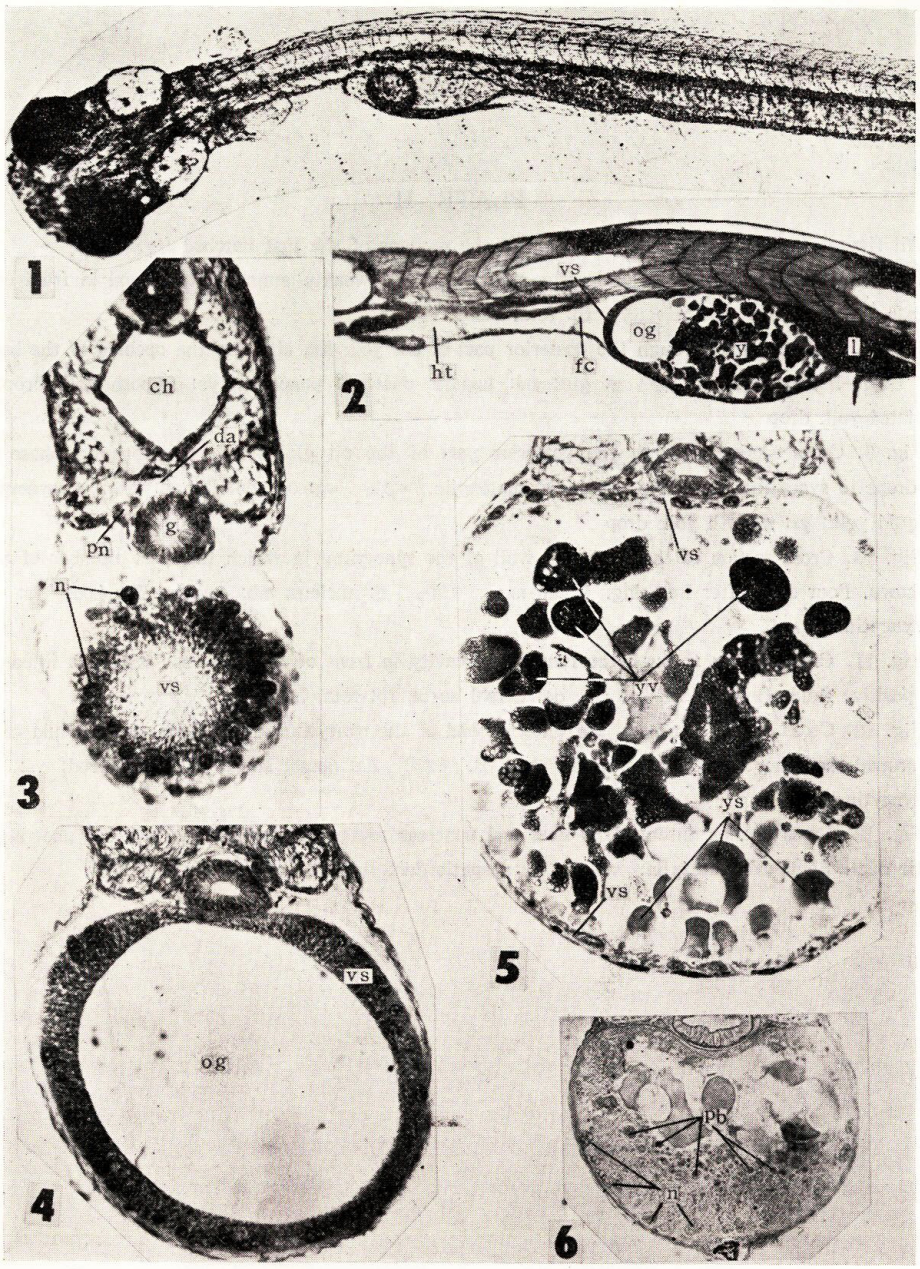

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PLATE II

All figures (7-13) except Fig. 10 represent the sections of the just hatched fry.

Fig. 7. Sagittal section same as in Fig. 2 showing the weakly stained fetal blood in front of the yolk-sac. Bouin-haematoxylin. ×65 fb: fetal blood

Fig. 8. Cross section through the posterior part of the yolk-sac showing the opening of the hepatic duct. Bouin-haematoxylin ×250 g: gut; hd: hepatic duct; p: pancreas; ys: smooth yolk drop; yv: vacuolated yolk drop

Fig. 9. Cross section through the posterior part of the oil globule. A transforming phase from yolk drops to syncytium is visible. Bouin-haematoxylin. ×250 og: oil globule; vs: vitelline syncytium; yl: liquid yolk; ys: smooth yolk drop

Fig. 10. Cross section of the thickened wall of the syncytium in which the vast number of nuclei are shown. Four days after hatching. Bouin-azan. ×800 n: nucleus; nb: nucleus-like body; vs: vitelline syncytium

Fig. 11. Cross section through the funnel-like cavity in front of the yolk-sac which is filled with PAS-positive fluid. Bouin-PAS. ×250 da: dorsal aorta; fb: fetal blood

Fig. 12. Cross section through the anterior end of the syncytium. The black stained fluid can be seen around the syncytium. Regaud-Sudan black B. ×250 da: dorsal aorta; fb: fetal blood; vs: vitelline syncytium

Fig. 13. Cross section through the liver and pancreas region just behind the yolk-sac showing the hepatic duct. Bouin-haematoxylin. ×250 hd: hepatic duct; l: liver; p: pancreas

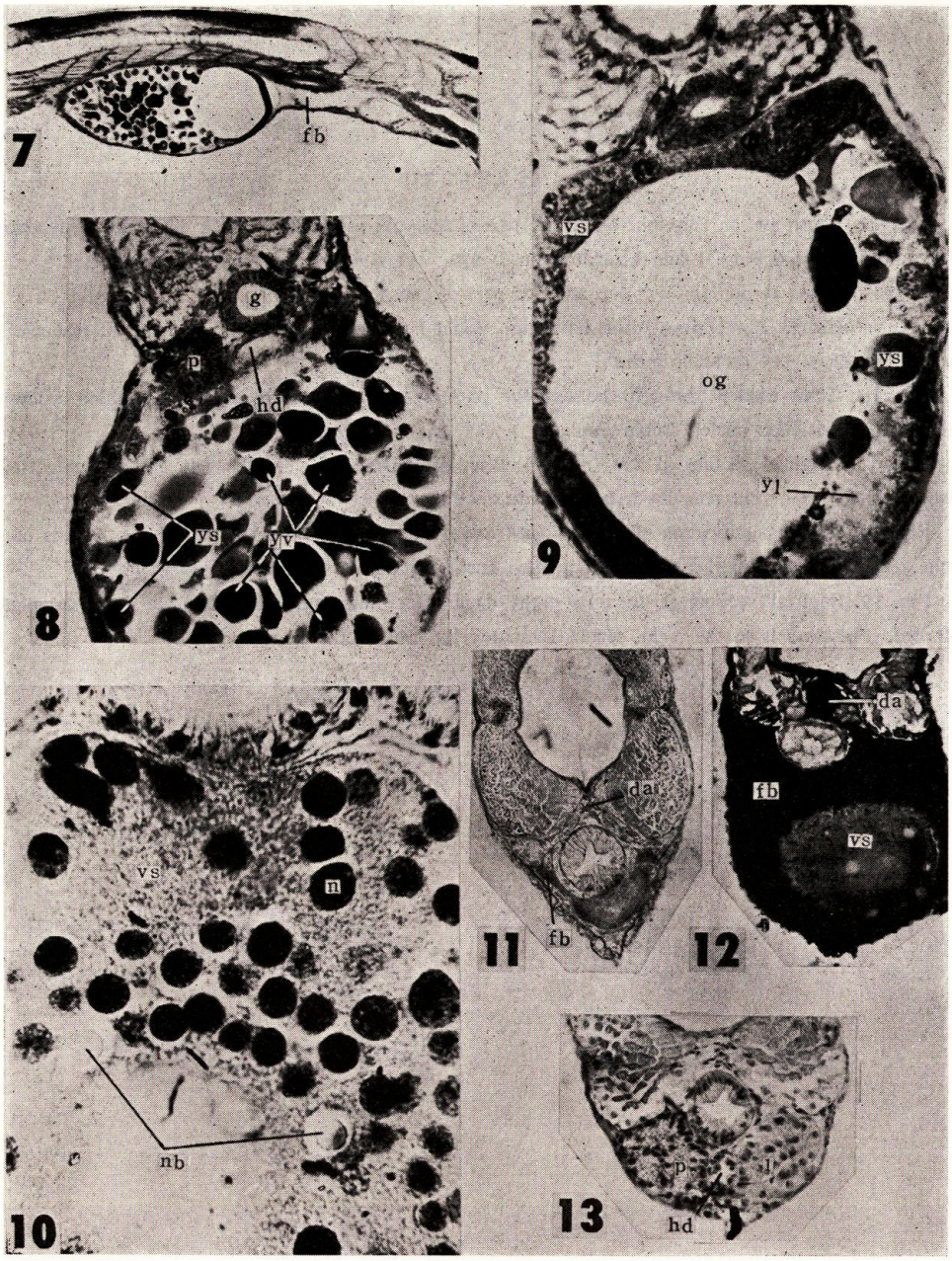

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PLATE III

Fig. 14. Cross section through the middle part of the yolk-sac in which yolk drops are disappearing. Three days after hatching. Bouin-haematoxylin. ×320 vs: vitelline syncytium; yl: liquid yolk

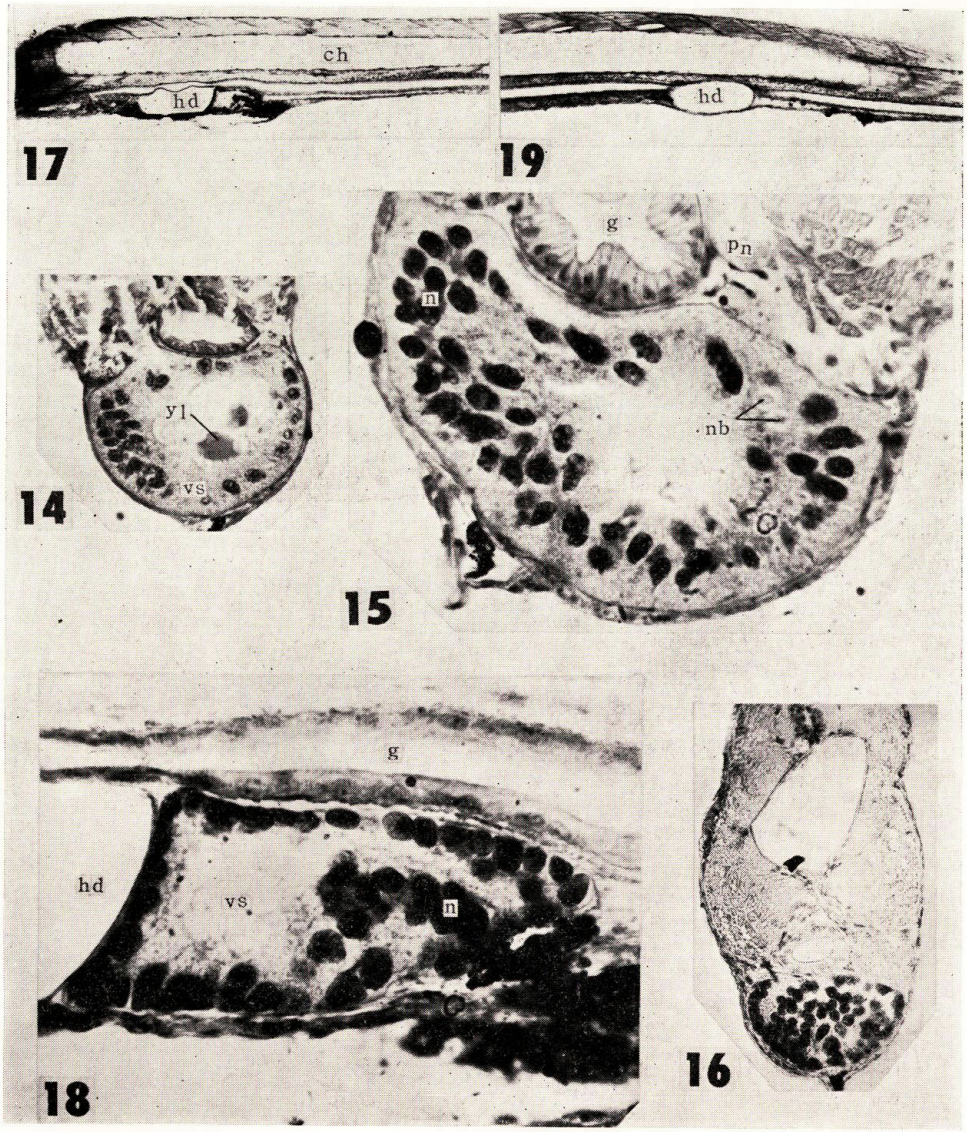
Fig. 15. Cross section through the anterior part of the yolk-sac. The nuclei are irregular in shape and are vacuolized. Three days after hatching. Bouin-haematoxylin. ×640 g: gut; n: nucleus; nb: nucleus-like body; pn: pronephric duct

Fig. 16. Cross section through the anterior part of the yolk-sac showing the vitelline syncytium filled with multiplied nuclei. Bouin-Feulgen. ×250

Fig. 17. Sagittal section of the fry five days after hatching. The head is to the right. Bouin-haematoxylin. ×65 ch: notochord; hd: hepatic duct

Fig. 18. High magnification of the yolk-sac region in Fig. 17. Note the peripheral position of the nuclei. Bouin-haematoxylin. ×640 hd: hepatic duct; n: nucleus; vs: vitelline syncytium

Fig. 19. Sagittal section of the fry eight days after hatching. The yolk has been completely absorbed. The head is to the right. Bouin-haematoxylin. ×65 hd: hepatic duct



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