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Ultrastructural Aspects of Yolk Absorption in the Vitelline Syncytium of the Embryonic Rockfish, *Sebastes schlegeli*

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Abstract The vitelline syncytium of the embryonic rockfish, *Sebastes schlegeli*, was examined by electron microscopy. The syncytium encloses the entire yolk mass in the yolk-sac, separating it from the embryonic body and the circulating fetal blood. Numerous small yolk droplets fused into coagulated masses were detected in the syncytial cytoplasm near the border with the yolk mass. Two structurally different regions were distinguished in the syncytium: one characterized by an extensive network of the smooth surfaced endoplasmic reticulum, numerous mitochondria and a large number of glycogen granules, and the other by compactly arranged cisternae of the rough surfaced endoplasmic reticulum and developed Golgi complexes. In some surface areas where the endothelial wall of blood vessels is incomplete and the fetal blood is in direct contact, the syncytium showed finely vacuolated cytoplasm forming an intricate structure between the cytoplasmic processes of the blood cells. These characteristic features of the vitelline syncytium are discussed in view of its functional significance in yolk absorption.

The yolk is the sole nutrient source for the developing embryos of oviparous fishes until they begin to feed. Biochemical evidence shows that yolk phosphoproteins and phospholipids decrease in accordance with the development of fish embryos (Yamagami, 1960a, b; Monroy et al., 1961). The whole yolk mass in a developing fish embryo after the closure of the blastopore is enclosed by the vitelline syncytium, or the periblast, a tissue specially differentiated for yolk absorption (Yamada, 1959a, b; Williams, 1967). The yolk, therefore, must be utilized by the fish embryo via the vitelline syncytium. The function of this tissue is probably to transform yolk substances into materials readily utilizable by the rapidly developing embryo.

Very little has been known of the ultrastructural and functional characteristics of the vitelline syncytium. To elucidate the functional significance of this tissue in yolk absorption, we examined the fine structure of the vitelline syncytium in intra-ovarian embryos of the rockfish, *Sebastes schlegeli*. This fish is an ovoviviparous species, but the embryos are considered to depend solely on the yolk for their nutrients because they do not have any specific organ for absorbing maternal substances such as the trophotania found in *Characodon eiseni* (Mendoza, 1972) and *Oligopus longhursti* (Wourms and Cohen, 1975).

Material and methods

Pregnant rockfish, *Sebastes schlegeli*, were captured from the coastal waters near Hakodate and reared in a culture tank with running sea water at Hokkaido Marine Cultivation Center at Shikabe. Intra-ovarian embryos were taken out by incision of their ovaries. The embryos were found ready to hatch twisted in the vitelline membrane, but some had stretched their bodies out of the membrane probably because of a mechanical stress at sampling.

The embryos were fixed with a mixture of 2% paraformaldehyde and 2.5% glutaraldehyde in 0.05% cacodylate buffer (pH 7.4) for 1.5 hours (Karnovsky, 1965), and then post-fixed with 1% osmium tetroxide in phosphate buffer for 1 hour. After being dehydrated through a series of graded alcohol concentrations, they were embedded in an Epon-812 resin mixture. Ultrathin sections were cut using a Porter-Blum ultramicrotome, stained doubly with uranyl acetate and lead citrate, and examined with a Hitachi HU-12 electron microscope. Some thick sections (0.5～1.5 μm in thickness) were stained with methylene blue

and azur II for light microscopic observations (Richardson et al., 1960).

Observations

The intra-ovarian embryos obtained by ovarian incision were identified externally to correspond to stage 28 (pre-hatching) in the development of *Fundulus* (Oppenheimer, 1937). An embryonic body of about 5 mm long was provided with a relatively large yolk-sac, about 1.2 mm in diameter, in the anterior half of the body (Fig. 1). The alimentary canal was twisted half way to the anus at the point where the liver and the pancreas lie to the right and left sides of the canal, respectively. The posterior end of the canal, or the rectum, was enlarged containing an opaque substance. The mouth had opened and the pectoral fins and other fin folds were well developed. Pigmentation was taking place in the peritoneal wall and at the top of the head. The heart was beating regularly but streaming of blood and blood cells was difficult to observe.

In section, yolk was observed as a large homogeneous mass, the whole surface of which was directly enveloped by a thin syncytial tissue, the vitelline syncytium (Fig. 2). Dorsally to the yolk-sac were the alimentary canal, the liver and the pancreas all of which lie on the syncytial wall intervened by a thin layer of cells and blood vessels. These and other blood vessels on the syncytium surface were bordered by thin endothelial walls. In some places, however, the endothelial walls were found to be still incomplete and the fetal blood was in direct contact with the syncytium. This was particularly evident in the area where the sinus venosus is contiguous to the syncytium at the anterior part of the yolk mass. One large oil globule (0.5 mm in diameter) occupied the lower central region of the yolk mass. The dorsal half of the oil globule was embedded in the yolk mass while its ventral half was enclosed by a thin layer of the vitelline syncytium lined externally by the ventral dermal wall of the yolk-sac.

The vitelline syncytium was about 0.09 mm in average thickness except the portions contiguous to the sinus venosus and the oil globule, where the maximum thickness measured 0.35 mm.

Electron microscopic observations of the vitelline syncytium revealed characteristic localization and distribution of some cytoplasmic organelles and inclusions. Numerous vesicular yolk droplets bounded by a single membrane, 0.2 μm to 1 μm in diameter, were distributed in the border region between the syncytium and the yolk mass (Fig. 3). They were mostly oval or round but sometimes complicated in shape. Further interior in the syncytium smaller yolk droplets were gathering in places to form coagulated masses (Fig. 4). Some of the coagulated droplets had lost the limiting membrane and their contents showed a decreased density. Some myelinated or vacuolated structures of various sizes and shapes were found frequently near the coagulated droplets (Fig. 4). Exceptionally large yolk droplets (3 μm$\sim$36 μm in diameter) were sometimes observed in the syncytium (Figs. 2, 5). Such large yolk droplets were more often detected in the ventral region near the oil globule.

Abundant mitochondria were distributed randomly in the interior of the syncytium (Fig. 6), but not in the border region facing the yolk mass where many vesicular yolk droplets were found (Fig. 3). The mitochondria were mostly oval or rod-shaped with an average length of 0.8 μm but some were elongated up to 8 μm in maximum length. They had numerous tubular cristae and the matrix of a low electron density with no internal granules. Both the inner and the outer membranes were undulated.

A compact network consisting of abundant tubules of smooth surfaced endoplasmic reticulum (SER) extended throughout the syncytium (Fig. 3). In the inner region, this network was particularly conspicuous in close association with mitochondria (Figs. 6, 7). On the other hand, compactly arranged cisternae of rough surfaced endoplasmic reticulum (RER) extended in portions across the syncytium forming a stratified structure (Fig. 8). The stratified cisternae of these extensive RER fields often contained a moderately dense granular substance. Many Golgi complexes consisting of numerous vesicles, some large round bodies and poorly developed Golgi

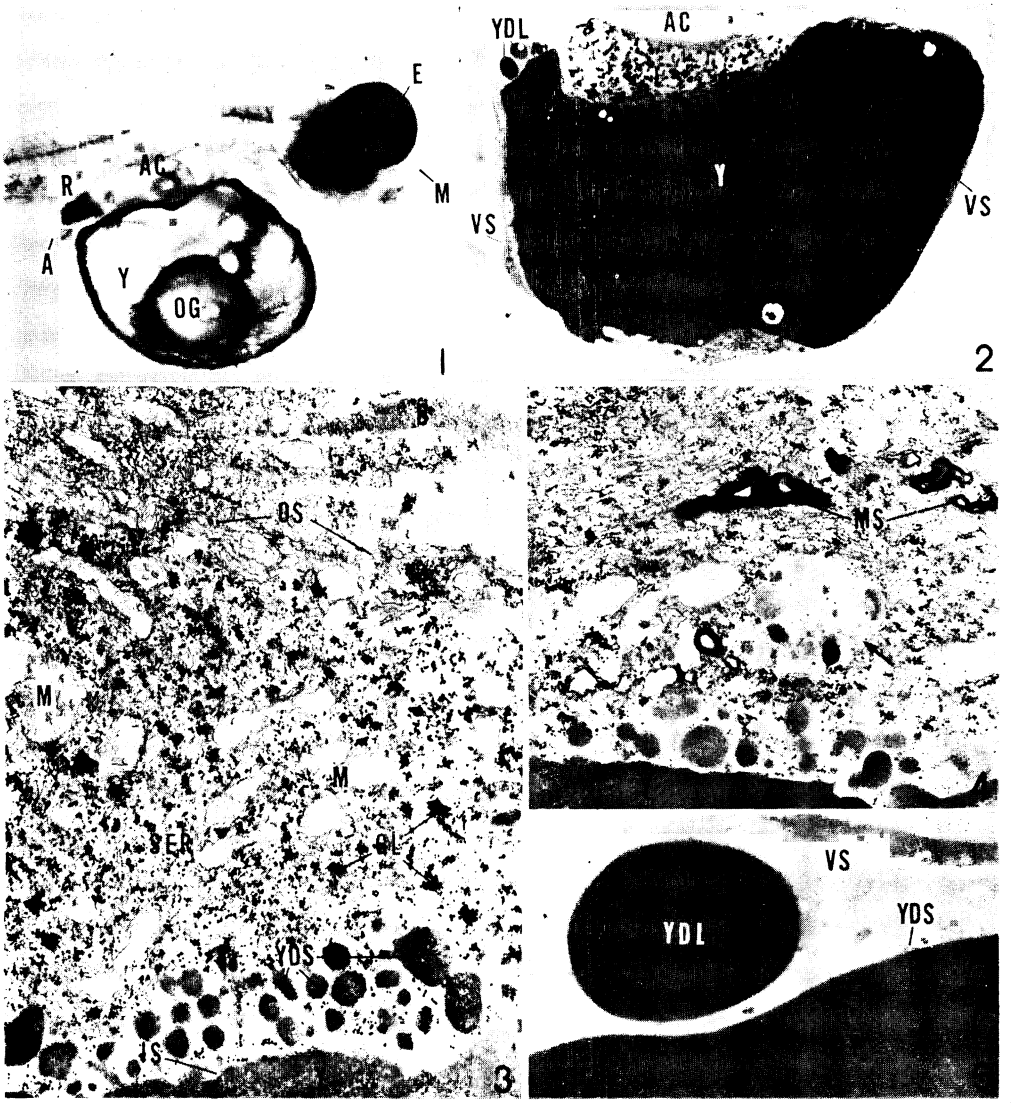

Fig. 1. Stretched intra-ovarian embryo of *Sebastes schlegeli*. The yolk-sac includes a large yolk mass (Y) and an oil globule (OG). A, anus; AC, alimentary canal; E, eye; M, mouth; R, rectum. ×24.

Fig. 2. A sagittal section of the yolk-sac showing the large smooth yolk mass (Y) enveloped by a thin layer of vitelline syncytium (VS). Several large yolk droplets (YDL) are seen in the syncytium. AC, alimentary canal; L, liver. Epon section stained with methylene blue and azur II. ×80.

Fig. 3. Vitelline syncytium extending over the yolk mass (Y) with the border region containing numerous small yolk droplets (YDS). The outer surface of the syncytium (OS) is contiguous to the endothelium of a blood vessel in which fetal blood (B) is contained. Abundant mitochondria (M) and glycogen granules (GL) are distributed in the syncytium. IS, inner surface of the syncytium; SER, smooth surfaced endoplasmic reticulum. Stained with uranyl acetate and lead citrate. ×11000.

Fig. 4. A mass of coagulated small yolk droplets (arrow) adjacent to the border region where a number of yolk droplets are segregating from the yolk mass (Y). Some of the coagulated droplets of a decreased density are losing their limiting membrane. MS, myelinated structures. Stained with uranyl acetate and lead citrate. ×11000.

Fig. 5. One large (YDL) and many small (YDS) yolk droplets segregated from the yolk mass (Y) in the vitelline syncytium (VS). Thick Epon section stained with methylene blue and azur II. ×1000.

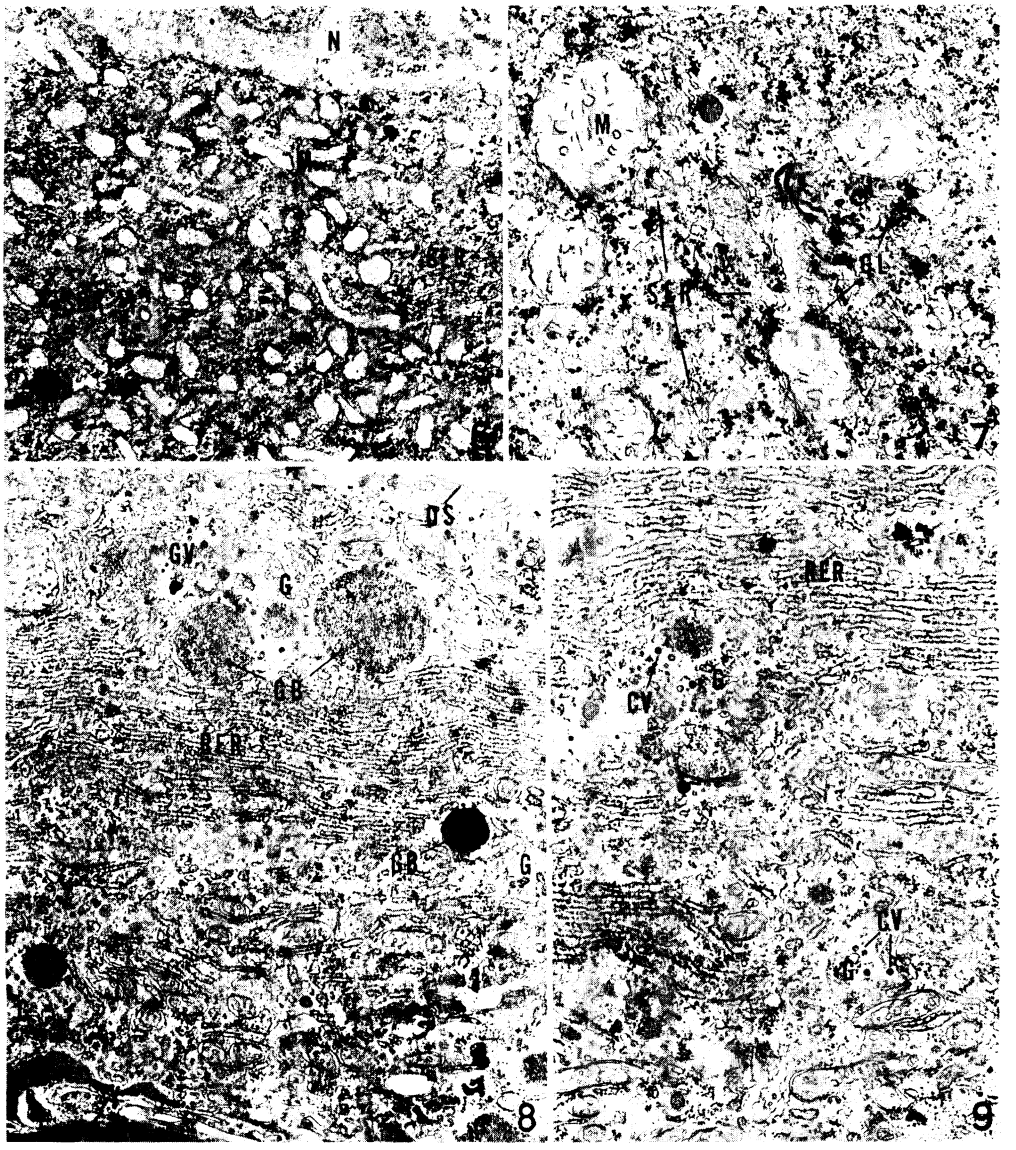

Fig. 6. Numerous mitochondria (M) of various shapes and smooth surfaced endoplasmic reticulum (SER) extended in the syncytium. N, nucleus of the syncytium. Stained with uranyl acetate and lead citrate. ×6000.

Fig. 7. Developed smooth surfaced endoplasmic reticulum (SER) in close association with mitochondria (M). GL, glycogen granules. Stained with uranyl acetate and lead citrate. ×18000.

Fig. 8. Stratified cisternae of rough surfaced endoplasmic reticulum (RER) in close association with Golgi complexes (G) consisting mainly of vesicles (GV) and large bodies (GB). Two types of large bodies are distinguished as to the density. OS, outer surface of the syncytium; Y, yolk mass. Stained with uranyl acetate and lead citrate. ×7800.

Fig. 9. Golgi complexes (G) accompanied with coated vesicles (CV) in the RER region. RER, rough surfaced endoplasmic reticulum. Stained with uranyl acetate and lead citrate. ×12000.

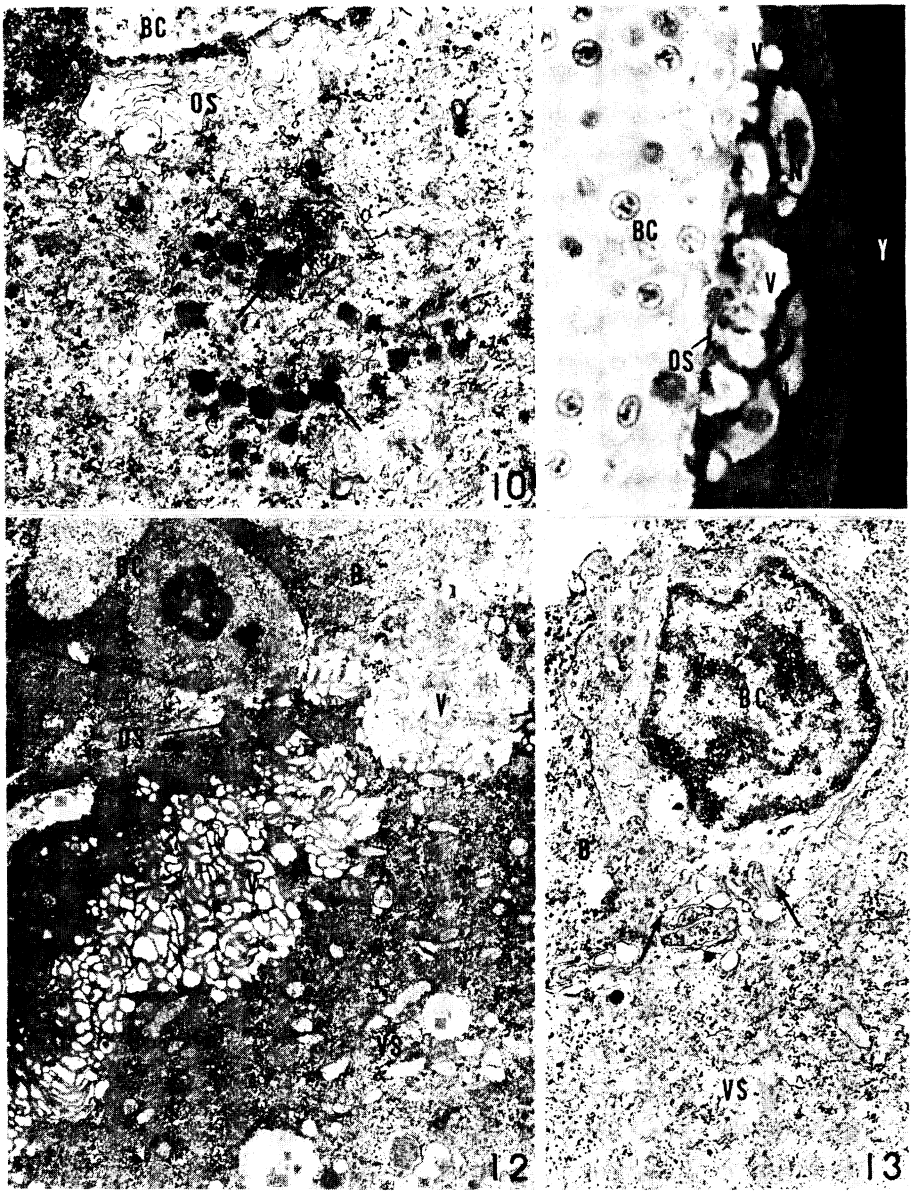

Fig. 10. Vesicles containing a dense granular substance (arrows) in the surface area of the syncytium. A similar substance is noted in the fetal blood (B). BC, blood cell; OS, outer surface of the syncytium. Stained with uranyl acetate and lead citrate. ×13500.

Fig. 11. Vacuolated structures (V) in the outer surface of the syncytium (OS) facing the sinus venosus. Blood cells (BC) are in direct contact with the syncytium where large nuclei (N) are accumulated around the vacuoles. Y, yolk mass. Thick Epon section stained with methylene blue and azur II. ×1000.

Fig. 12. Fine cytoplasmic network found in the vacuolated structures in the area shown in Fig. 11. B, fetal blood; BC, blood cells; OS, outer surface of the syncytium; V, vacuolated structure; VS, vitelline syncytium. Stained with uranyl acetate and lead citrate. ×5000.

Fig. 13. Intricate cytoplasmic interdigitations (arrows) between a blood cell (BC) and the syncytium (VS). B, fetal blood. Stained with uranyl acetate and lead citrate. ×7800.

cisternae were observed among the stratified cisternae of the RER. Some of the vesicles were enwrapped with a rough surfaced membrane, taking the appearance of coated vesicles (Fig. 9). The large round bodies were observed to be of two types: one filled with a dense homogeneous material and the other with a moderately dense granular substance (Fig. 8).

Abundant glycogen granules were distributed throughout the syncytium except in the RER fields. These granules were particularly conspicuous in the inner region where they frequently aggregated to form separate clumps (Fig. 3).

The fetal blood contained a dense granular material (Figs. 10, 12). The syncytium surface contiguous to the fetal blood showed for the most part a complicated configuration with intricate cytoplasmic projections. Just inside this area were noted some vesicles which contained a dense granular material similar to that found in the fetal blood (Fig. 10). The portion facing the sinus venosus where the endothelial wall was incomplete showed a characteristic feature. By light microscopy, vacuolization of the cytoplasm was observed to occur in this portion. The vacuolization seemed to proceed as if the cytoplasm was eroded or disintegrated from the outer surface where many blood cells were accumulated (Fig. 11). Large nuclei of the syncytium were often found around the vacuolated area. Ultrastructure of this area revealed numerous vacuoles surrounded by a fine network of the syncytial cytoplasm (Fig. 12). Some of the blood cells accumulated in this area often extended intricate cytoplasmic processes into the syncytial cytoplasm (Fig. 13).

Discussion

Histological and histotopographical aspects of the vitelline syncytium in *Sebastes schlegeli* were basically similar to those in the pond smelt, *Hypomesus olidus*, and salmonids, *Oncorhynchus keta* and *Salmo irideus* (Yamada, 1959a, b). Electron microscopic observations of the present study, on the other hand, disclosed characteristic localization and distribution of cell organelles in the syncytium and inclusions which seemed to be involved in active absorption of yolk. Two different regions were distinguished in the syncytium based on the fine structure; one is characterized by the presence of a compact network of SER, numerous mitochondria and a large number of glycogen granules, and the other by developed stratified cisternae of RER, Golgi complexes with abundant vesicles and some large bodies.

Compact distribution of SER in association with mitochondria has been known to occur in cells of some mammalian tissues, such as liver, intestine, testis and adrenal cortex, in which active carbohydrate or lipid metabolism is taking place with participation of these cell organelles (Yamada, 1965; Orrenius and Ericsson, 1966; Niedelen, 1967; Cardell et al., 1967; Friend and Brassil, 1970). Another instance of developed SER is found in chloride secreting cells in which the SER is involved in the active transport of inorganic ions (Philpott, 1967). Biochemical investigations have confirmed that the SER and mitochondria both participate in carbohydrate or lipid metabolism (Stein and Shapiro, 1958; Senior and Isselbacher, 1960; Hulsmans, 1961; Wilgram and Kennedy, 1963; Stetten and Taft, 1964). Furthermore, the co-existence of mitochondria and SER-derived microsomes was suggested to be essential for phospholipid synthesis in vitro (McMurray and Dawson, 1969). On the other hand, the function of RER is the synthesis of proteinous substances and that of Golgi complexes is the addition of carbohydrates to the proteins (Jamieson and Palade, 1968a, b; Ashley and Peters, 1969; Zagury et al., 1970; Case, 1978).

Therefore, the region of the vitelline syncytium characterized by extensive SER, developed mitochondria and a large number of glycogen granules is probably responsible for carbohydrate or lipid metabolism or both. Active glycolysis, synthesis of fatty acids and the regulation of these metabolic pathways are considered to be major events in this region. The region characterized by RER and developed Golgi complexes associated with numerous vesicles must be involved in the synthesis and transport of proteinous substances needed for embryonic development.

Yolk substances in the chick embryo are

absorbed by phagocytotic activity of the endoderm cells which are in immediate contact with the yolk (Bellairs and New, 1962; Bellairs, 1963; Williams, 1967). In the fish embryo, however, yolk substances are absorbed by phagocytotic activity of the vitelline syncytium. The absorbed yolk is probably transformed into lower molecular substances by the activity of lysosomal enzymes in the syncytium. A part of the degraded substances may be directly utilized by the embryo and others may serve as materials for the synthesis of high molecular substances, which may be transported into the fetal blood by the membrane system of SER and Golgi complexes.

Yamada (1959b) observed in salmonid fry that large nuclei of the vitelline syncytium migrate towards the vitelline veins and appear to liberate their Feulgen-positive contents into vitelline veins. Large nuclei of the syncytium in embryos of the muskellunge, *Esox masquinongy*, and the zebrafish, *Brachydanio rerio*, contain 4 to 20 folds of DNA in comparison with somatic cell nuclei of the same embryo (Bachop and Schwartz, 1974). These facts and the characteristic feature of the syncytium in the portion facing the sinus venosus, where an intimate relationship between the syncytium and fetal blood cells was observed, are of particular interest in considering that the function of the syncytium might be more than simply the transport of yolk substances into the blood. Elucidation of the relationship between the fetal blood cells and the vitelline syncytium awaits further observations.

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卵黄吸収時におけるクロソイ胎仔の卵黄多核質の微細構造

清水幹博・山田寿郎

出産間近のクロソイ *Sebastes schlegeli* 胎仔の卵黄多核質を電顕で観察し，卵黄吸収時における卵黄多核質の機能を考察した．

クロソイ胎仔の卵黄は一つの大きな均質塊で油球を含み，腹部中央に位置する卵黄嚢内に存在する．卵黄塊はその全表面を卵黄多核質によって直接おおわれ，多核質の外表面には血管が分布している．多核質と卵黄塊との境界域には卵黄塊から分離した多くの卵黄小顆粒が認められ，卵黄多核質は微細構造的に二つの部域に区分される．一つは滑面小胞体が広く密に分布し，糸粒体およびグリコーゲン顆粒に富む部域であり，他の一つは粗面小胞体およびゴルジ装置がよく発達している部域である．前者は脂質および糖質代謝に関与し，後者は蛋白質代謝に関与していると考えられる．また，卵黄多核質の外表面が血管と接する所では，細胞膜が入り組んで複雑な構造を呈している．特に静脈洞と接する部位では，血管内皮は未発達で血液および血球が直接多核質に接し，特異な空胞様構造が認められた．

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