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Accumulation of Lipid Particles in Xanthophores During the Larval Development of the Goldfish, *Carassius auratus*

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Abstract The ultrastructure of the xanthophores of larval goldfish at various developmental stages was examined, with special reference to the appearance and accumulation of lipid particles in the xanthophores. In xanthophores of the hatching larva, numerous pterinosomes were present but no lipid particles were observed. The lipid particles, homogeneously electron-dense and 60~80 mμ in diameter, appeared in the neighborhood of the nuclei of the xanthophores in larvae 5 days after hatching. They formed conglomerates of varying sizes. The conglomerates of lipid particles increased in size along with the development of the larvae, and a considerable number of lipid particles gradually become distributed freely into the dendritic portion of the xanthophores. The mechanism of accumulation of these lipid particles in the xanthophores is discussed in relation to those of other cell types on which earlier reports have been made.

Five different kinds of chromatophores, i.e. melanophores, xanthophores, erythrophores, leucophores and iridophores, are responsible for the coloration of fishes (Hama and Kajishima, 1967; Takeuchi, 1976). Xanthophores and erythrophores contain pteridines and carotenoids as a source of their coloration (Bagnara, 1966; Hama and Kajishima, 1967). Pteridines are carried in the pterinosomes, the cytoplasmic granules named by Hama et al. (1993) and Matsumoto (1965). However, many different cytoplasmic organelles have been considered as the location for carotenoid pigments (Matsumoto and Obika, 1968; Hawkes, 1974; Castrucci, 1975; Takeuchi, 1975; Winchester et al., 1976). In the xanthophores of the adult goldfish, we have demonstrated that numerous electron-dense particles of lipid nature, 60–80 mμ in diameter, filled the cytoplasm after fixation of the tissue with Dalton's chrome-osmium solution, and carotenoid pigments were thought to be dissolved in these small particles (Takeuchi and Kajishima, 1972).

In the hatching larva of the goldfish, the xanthophores contain only yellow-colored pteridines; these pteridines disappear during the course of development of the larvae, and lipids and carotenoid pigments gradually accumulate in the xanthophores (Hama and Fukuda, 1964). These facts led us to examine the ultrastructure of the xanthophores in larval goldfish at various developmental stages, with special reference to the appearance and accumulation of the said lipid particles in the xanthophore.

Materials and Methods

Larvae of three developmental stages, stage 25, 26+ and 27+ of a xanthic goldfish, *Carassius auratus* Linnaeus, in Kajishima (1960), were used as materials. The larvae were disected into head and trunk-tail parts in a chilled 2% glutaraldehyde solution (cacodylate-buffered at pH 7.2), and the head parts were fixed with the same solution for one hour at 4°C. These specimens were post-fixed with Dalton's chrome-osmium solution (Dalton, 1955), pH 7.2, for one hour at 4°C. Dehydration was carried out with a series of ice-cold ethanol baths, and embedding into epoxy resin was according to Coulter (1967). Ultra-thin sections of the dorsal skin of the head were made using a Porter-Blum MT-2 ultramicrotome with a glass knife, double-stained with a routine method using uranyl acetate and lead nitrate, and examined with a JOEL JEM 100B electron microscope at 80 kV.

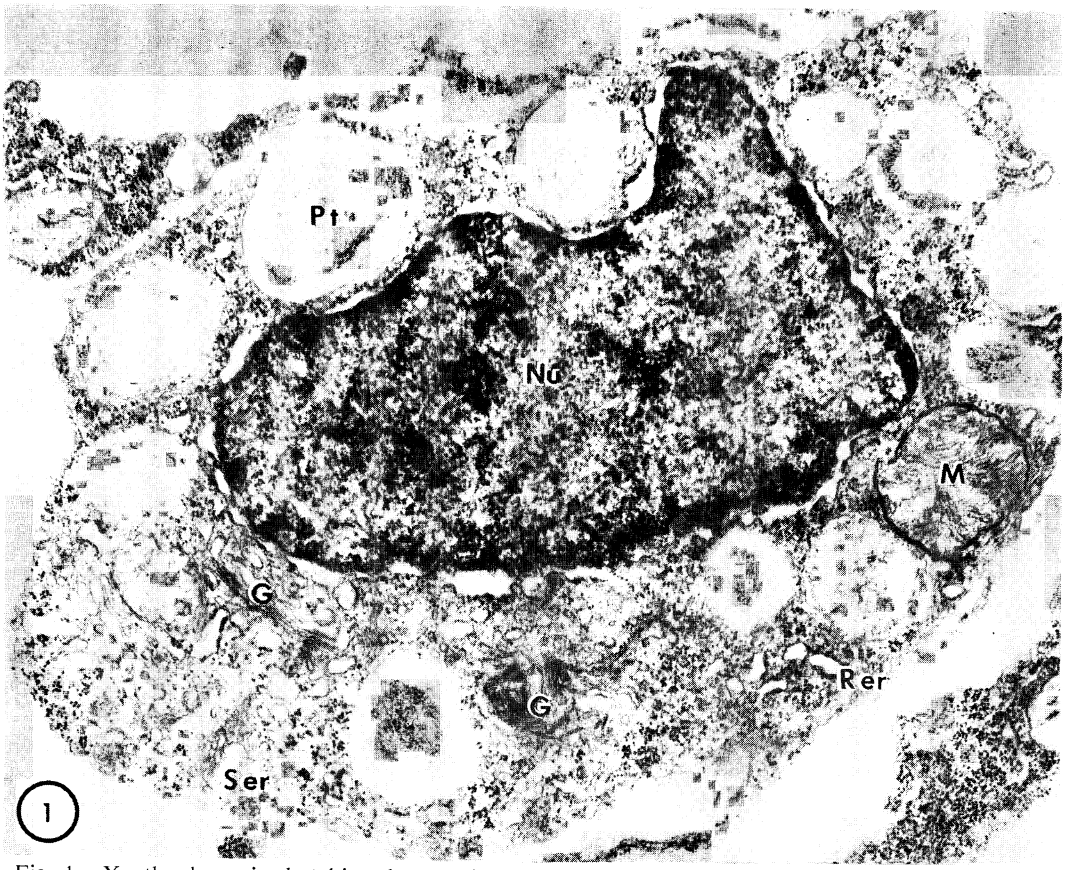


Fig. 1. Xanthophore in hatching larva of a goldfish. Numerous pterinosomes (Pt) are present in the cytoplasm. G, Golgi apparatus; M, mitochondria; Nu, nucleus; Rer, rough-surfaced endoplasmic reticulum; Ser, smooth-surfaced endoplasmic reticulum. ×26,000.

Results

Hatching larva: stage 25 in Kajishima (1960). In the larva at the hatching stage, the xanthophores were located adjacent to the collagen fibers underlying the basal lamina. The nucleus of the xanthophore was large and irregular in form (Fig. 1). In the cytoplasm, numerous pterinosomes, oval in shape and 0.8～1.0 μm in diameter, were present. The inner parts of the pterinosomes were filled with numerous fibrous and particulate materials (Fig. 1). One or two Golgi apparatus were usually observed in the neighborhood of the nucleus. Rough-surfaced and smooth-surfaced endoplasmic reticula, and large mitochondria having well-developed cristae, were also present, but no lipid-like particles were observed (Fig. 1). At the plasma membrane, no micropinocytotic vesicles were formed (Fig. 1).

Five days after hatching: stage 26+. In the cytoplasm neighboring the nucleus, lipid particles which were homogeneously electron-dense in their core and about 60～80 mμ in diameter, were present as conglomerates of varying sizes (Figs. 2, 3). The delineating membrane of the particle was indiscernible (Figs. 5, 6). Both rough-surfaced and smooth-surfaced endoplasmic reticula were intermingled with these lipid particles, the inner cores of which were usually electron-translucent (Figs. 3, 4). Several Golgi apparatus were observed either at the periphery or in the central region of these conglomerates of lipid particles (Figs. 3～5), but in occasional cases they were found apart from the conglomerates (Fig. 2). The cisternae of Golgi lamellae

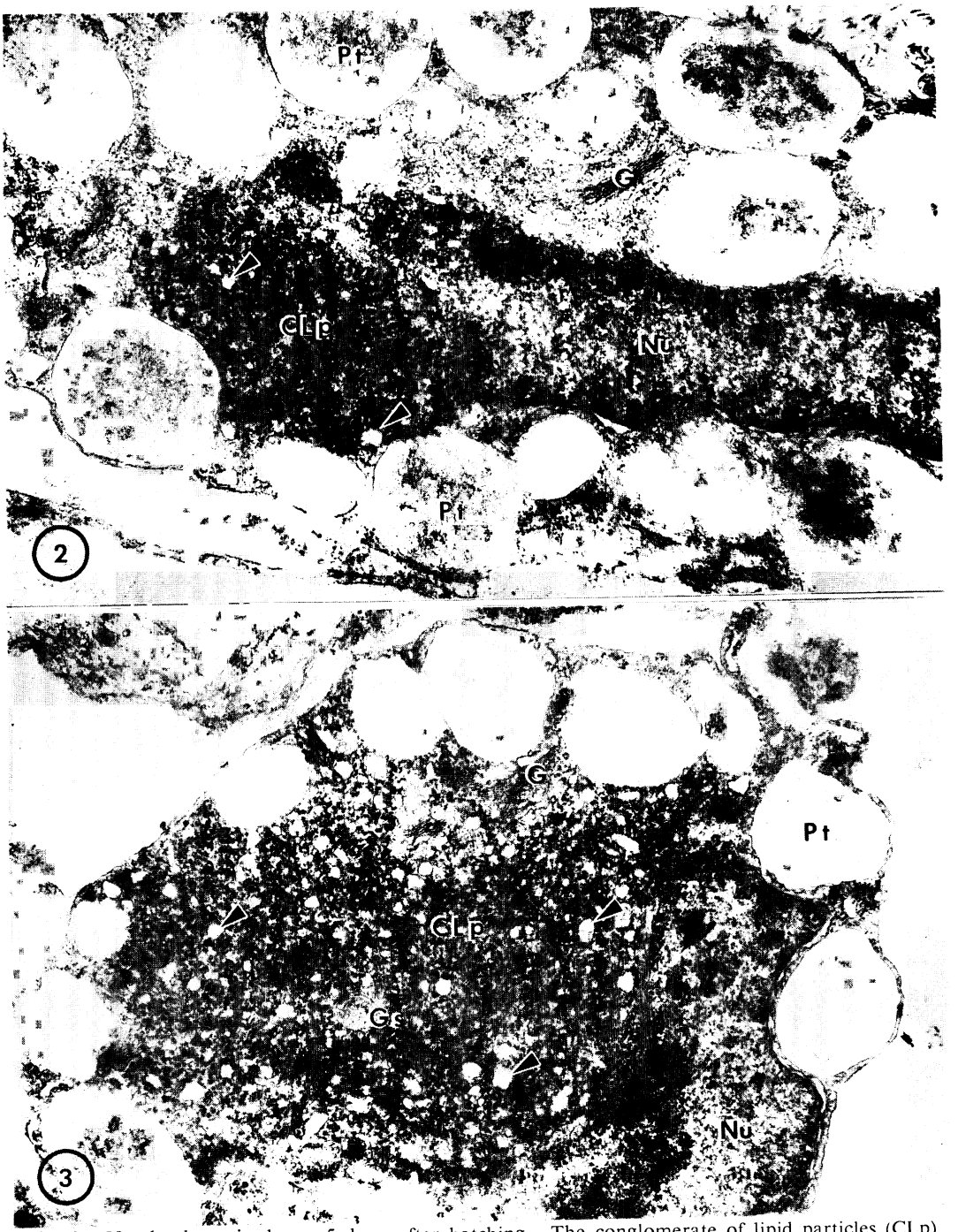


Fig. 2. Xanthophore in larva 5 days after hatching. The conglomerate of lipid particles (CLp) is observed in the neighborhood of the nucleus (Nu). Endoplasmic reticula are intermingled with lipid particles in this conglomerate (arrow-heads). Golgi apparatus (G) is present apart from the conglomerate. Pt, pterinosome. ×21,000.

Fig. 3. Xanthophore in the same material in as Fig. 2. Golgi apparatus (G) is at the periphery of the conglomerate of lipid particles (CLp). The granular structure (Gs) carrying fine particulate materials in its core is found in the conglomerate. Arrow-heads indicate the endoplasmic reticula in the conglomerate. Nu, nucleus; Pt, pterinosome. ×14,000.

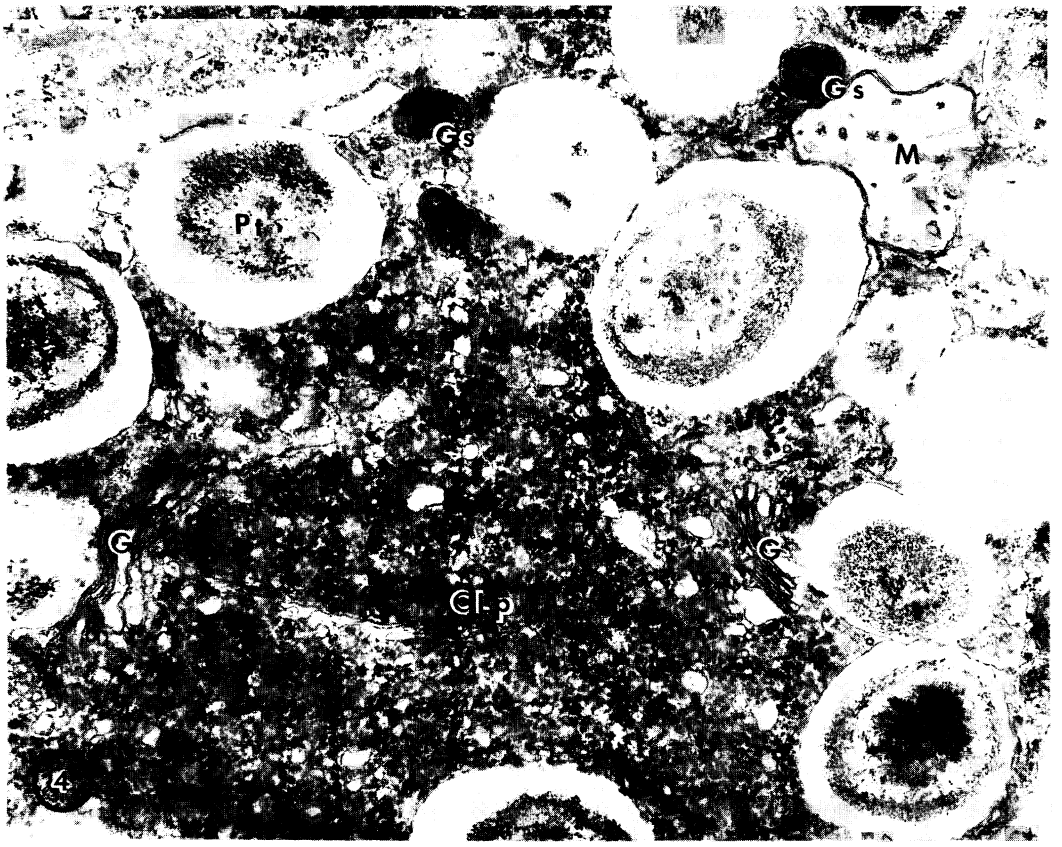


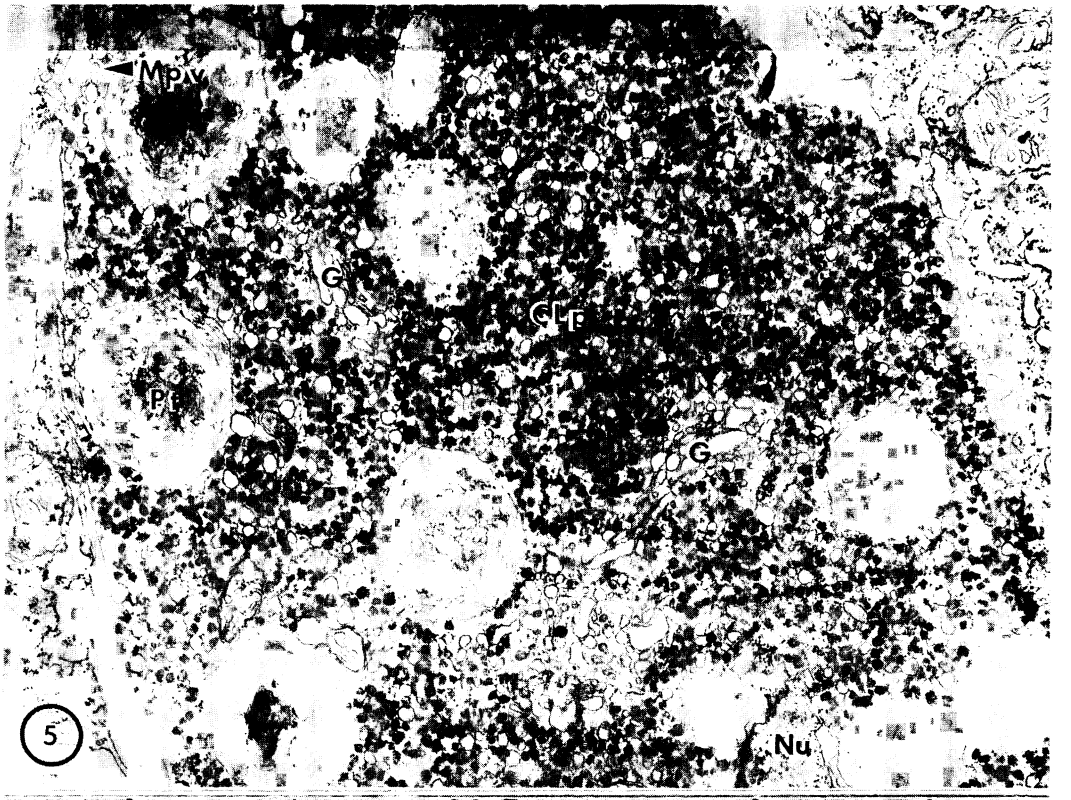
Fig. 4. Xanthophore in larva 5 days after hatching. Pterinosomes (Pt) increase in size as compared with those in the hatching larva. The granular structures (Gs) and Golgi apparatus (G) are found at the periphery of the conglomerate of lipid particles (CLp). M, mitochondria. ×21,000.

and the cores of Golgi vesicles were usually electron-translucent (Figs. 2～5). Some granular structures, about 0.2～0.3 μm in diameter and carrying numerous fine particulate materials in their cores, were sometimes observed at the periphery or the interior of the conglomerates of lipid particles (Figs. 3, 4). Pterinosomes increased in size, measuring about 1.0～2.0 μm in diameter (Figs. 2～4). Micropinocytotic vesicles were not observed at the plasma membrane of the xanthophore in this stage of larva.

Two weeks after hatching: stage 27+. A large part of the cytoplasm of the xanthophore was filled with lipid particles. They mostly formed conglomerates of varying sizes near the nucleus (Fig. 5), but a considerable number were distributed freely in the dendrites of the xanthophore (Fig. 6). Pterinosomes were few in the cytoplasm neighboring the nucleus (Fig. 5), but they were found in large numbers in the dendritic portion (Fig. 6). Sporadic micropinocytotic vesicles were observed at the plasma membrane (Fig. 5).

Discussion

In the xanthophores of the adult goldfish, we have reported that numerous electron-dense particles of 60～80 mμ in diameter were observed after the fixation of the tissue with Dalton's chrome-osmium solution. These particles were never observed when the tissue was fixed with Millonig's double fixation method using a phosphate-buffered glutaraldehyde and osmium solution (Millonig, 1961); moreover, these particles were not observed



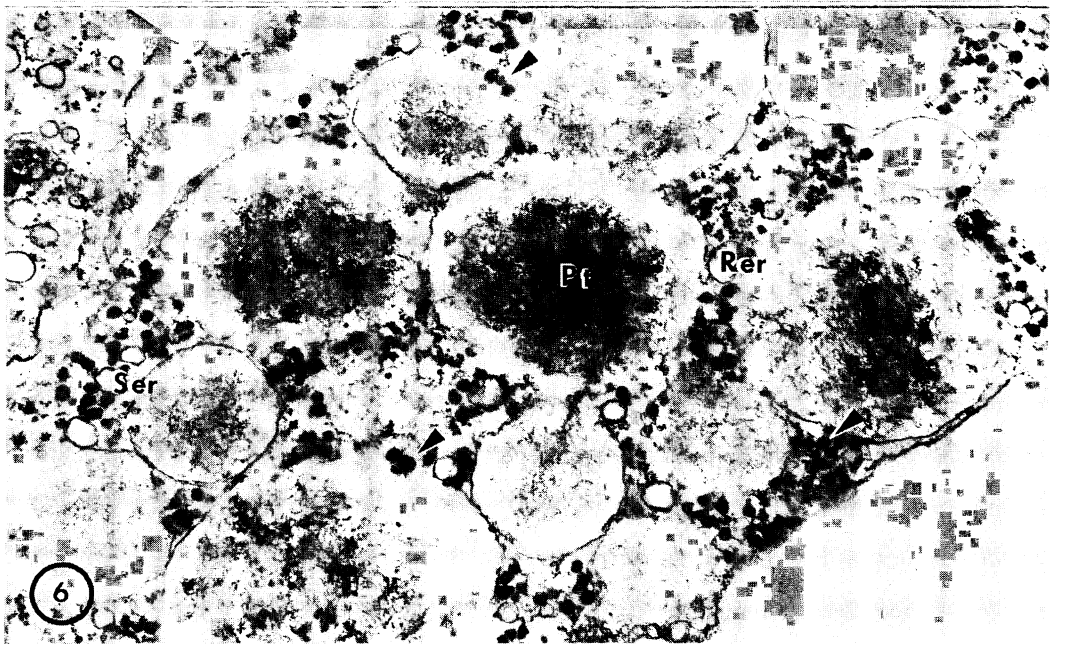


Fig. 5. Xanthophore in larva two weeks after hatching. Golgi apparatus (G) are intermingled with lipid particles in the conglomerate (CLp). Sporadic micropinocytotic vesicles (Mpv) are observed at the plasma membrane. Nu, nucleus; Pt, pterinosome. ×19,000.

Fig. 6. Dendritic portion of the xanthophore in the same material as in Fig. 6. Numerous lipid particles (arrow-heads) are distributed freely. Pt, pterinosome; Rer, rough-surfaced endoplasmic reticulum; Ser, smooth-surfaced endoplasmic reticulum. ×23,000.

when the tissue was pre-treated with acetone before the fixation with Dalton's solution. These results have led us to conclude that these particles were lipid in nature and lipid-soluble carotenoid pigments might be dissolved in them (Takeuchi and Kajishima, 1972).

The present study revealed that the xanthophores in the hatching larva of a goldfish contained numerous pterinosomes, but no lipid particles were observed in the cytoplasm. Lipid particles appeared in the cytoplasm neighboring the nucleus of the xanthophore in the larva 5 days after hatching. By light microscopy, Hama and Fukuda (1964) have reported that the xanthophores of the hatching larva of the goldfish contained only pterinosomes carrying yellow sepiapterins, and lipids and carotenoid pigments were first observed in the central parts of the cells of the larva at ca. 6 days after hatching. In general, the present study agrees with their observations, although the accumulation of lipid particles in the xanthophores occurred slightly earlier than the stage Hama and Fukuda (1964) have observed.

At present, the way these lipid particles are accumulated in the xanthophores remains a matter unresolved. In the present study, micropinocytotic vesicles, which are known as a means for transport of exogenous materials into the cell and have been reported to be numerously present in the xanthophores of the adult goldfish (Takeuchi and Kajishima, 1972), were not found in the xanthophores of the larva at the stage of onset of accumulation of lipid particles. This observation indicates that micropinocytosis does not play a role in the accumulation of lipid particles in the xanthophores. In various cell types, it has been shown that lipids penetrate the plasma membrane as micelle and diffuse into the cytoplasmic matrix, where they are accumulated in the cisternae of smooth-surfaced endoplasmic reticulum (SER) and Golgi apparatus; thereafter they are formed into lipid particles or droplets (e.g., Cardell et al., 1967; Stein and Stein, 1967). It is probable that lipids will penetrate the plasma membrane of the xanthophores as micelle, as in the case of these cells. However, the cisternae of the SER and Golgi apparatus in the xanthophores were usually electron-translucent, suggesting that no lipid materials were accumulated in these structures. Moreover, it must be noticed that, contrary to the well-known fact that the cytoplasmic organelles derived from SER or Golgi apparatus are generally delineated with a clear membrane (Favard, 1969), the delineating membrane was indiscernible all over the surface of the said lipid particles. In the xanthophores of the goldfish, the condensation from micelle into particles of lipids may occur on the surface of the SER and/or Golgi apparatus, but not in the cisternae of these structures.

Acknowledgments

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キンギョ稚魚期の黄色素胞における脂質性顆粒の蓄積

竹内 郁夫・梶島 孝雄

キンギョ稚魚期の黄色素胞の微細構造を、特に細胞質内における脂質性顆粒の出現と蓄積に焦点を置いて調べた．孵化直後の稚魚の黄色素胞細胞質には多数のプテリノソームが存在したが，脂質性顆粒はまったく認められなかった．孵化後5日目では，直径約60～80 mμ で内腔は均質に電子密度が高い脂質性顆粒が，核の周辺の細胞質に出現した．これらの顆粒は種々の大きさの集塊を形成していた．稚魚の発生の進行とともにこれらの集塊は増大し，また多くの脂質性顆粒は黄色素胞の樹枝状突起の細胞質内にも分布した．これらの脂質性顆粒の細胞内蓄積機構について，他種の細胞における知見を参考にして推論した．

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