The Comparative Study of the Digestive Tract of Teleost Larvae—V. Fat Absorption in the Gut Epithelium of Goldfish Larvae

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Working with various kinds of teleost larvae which were fed daily on plankton organisms, TANAKA¹⁾ found numerous fat droplets in the epithelial cells of the mid gut. Following this work, similar result was obtained by electron microscopy in the *Artemia*-eating larvae of the halfbeak²⁾. However, no experimental study has hitherto been made on fat absorption in the gut of larval fish. The aim of this paper is to present cytological evidence of fat absorption in the gut of goldfish larvae by means of administration of corn oil, and to discuss the process of fat absorption in the gut of teleost larvae.

Materials and Methods

More than 30 larvae of the goldfish, *Carassius auratus* LINNAEUS, at 7 days after hatching (5.5~6.0 mm in total length) were used for this study. These larvae were reared by feeding with nauplii of the brine shrimp, *Artemia salina* (LINNAEUS), in laboratory tank, and were fasted for 24 hours prior to experiment. Then boiled egg-yolk emulsions with corn oil were administered orally. At 6 hours after administration of the experimental diet, these larvae were fixed in several fixatives. Some fasting control larvae were also fixed in various fixatives.

For general observations of the gut, larvae fixed in Bouin's fluid were sectioned by the usual paraffin method and stained with Mayer's hemalum and eosin. For demonstration of lipids, the larvae were fixed in Baker's formol-calcium, embedded in gelatine, and sectioned on a freezing microtome. Sections of these blocks were stained with Sudan black B. For electron microscopy, small pieces of the gut were fixed for 2 hours in 2% chilled osmium tetroxide buffered at pH 7.3 with phosphate buffer, dehydrated by acetone, and embedded in Epon 812. Thin sections were double-stained using uranyl acetate and lead citrate, and examined in a JEM-7A electron microscope.

Observations

General morphology. In the larvae measuring 5.5~6.0 mm in total length, the digestive tract is a nearly straight tube occupying the ventral portion of the body

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cavity. It is briefly divided into the esophagus, mid gut, and posterior gut (Fig. 1). Anteriorly, the esophageal part is distinguished from the successive mid gut by a weak circular constriction and also by the sudden enlargement of the lumen. The diameter of the gut proper is more or less uniform throughout, becoming slightly narrower toward the anus. A circular constriction, by which the gut is divided into the mid gut and posterior gut, is observed in the gut wall approximately $800\sim900\mu$ anterior to the anus.

Internally, the lining epithelium is thrown into narrow longitudinal ridges. In the esophagus, the inner surface is covered with a stratified epithelium consisting of cuboidal secretory cells (Fig. 1). At the junction of esophagus and mid gut, the epithelium changes abruptly from the stratified cuboidal type to the simple columnar type which is common to the gut.

The cuboidal cells arranged in the surface of the esophageal epithelium are uniformly filled with oval mucigen droplets. Due to the distention of mucigen

Fig. 1. Diagrammatic drawing of gut in 7-day goldfish larva, especially showing fine structures of three epithelial cells: cuboidal secretory cell in the esophagus, columnar absorptive cell loaded with fat droplets in the mid gut, and columnar absorptive cell containing vacuoles with less dense materials (probably protein) in the posterior gut.

droplets in the supranuclear cytoplasm, the nucleus is displaced latero-basally. The microvilli formed by finger-like projections of apical plasma membrane, are rather short and scarce. They do not form the striated border. At the base of the cell are crowded the mitochondria and endoplasmic reticulum.

The epithelium of the gut consists of two types of cells, simple columnar cells and goblet cells. The columnar cells basally rest on a basement membrane and are apically covered by a peculiar striated border. The striated border is formed by numerous closely packed microvilli which serve important absorptive function. The lateral surfaces of these cells exhibit few plication. Lying below the level of the microvilli is a layer of terminal web having fine filaments. The nucleus lies in the lower half of the cell. Many cell organelles such as vesicles, endoplasmic reticulum, Golgi complex, ribosomes, and mitochondria are clustered throughout the cytoplasm. Membranous lamellar structures, often associated with mitochondria, are also prominent in the infranuclear cytoplasm. Various changes occur in the condition of cell organelles of the columnar absorptive cells during the period of feeding.

As compared with the columnar cells, the goblet cells are very few in the gut, though they slightly increase in number in the posterior gut. The supranuclear part is much distended due to the accumulation of oval mucigen droplets. The nucleus is pushed toward the basal half of the cell. The Golgi apparatus and granular endoplasmic reticulum are well developed along the mass of mucigen droplets. Mitochondria and ribosomes are also scattered in the cytoplasm. Basally, the goblet cell rests on a basement membrane as is the case with the columnar cell. Apically, the microvilli are similar to those of the columnar cell in arrangement, but become blunt and irregular as cell becomes fully charged with mucigen droplets.

Experimentally induced fine structural changes. In those larvae which ingested egg-yolk emulsions with corn oil, the gut was found to be fully filled with a creamy yellowish mass. This active feeding caused the gut to change much of its epithelium in cytological features. The most conspicuous change in the epithelial cells was the appearance of numerous Sudan-stained fat droplets in the mid gut before the constriction (Fig. 1). The series of experiments showed that fat absorption took place abundantly in the mid gut. Electron microscopy also clearly showed evidence of fat absorption. The fat droplets filled not only the supranuclear cytoplasm but the infranuclear cytoplasm as well. They were provided with enveloping membrane and varied widely in size from 0.6 to 3.2μ in diameter. Generally, the droplets were larger and more numerous in the distal half of the cell, often reached a diameter of 3μ or even more. During fat absorption, the microvilli of the striated border of the epithelial cells resembled those of the epithelial cells of fasting larvae. There was no apparent increase of intermicrovillous invaginations. Moreover, few fat droplets were observed in the terminal web layer. The cytoplasm immediately beneath the terminal web contained numerous large fat droplets (Fig. 1). The fat droplets were

often associated with the Golgi apparatus and mitochondria in the supranuclear cytoplasm, and closely associated with mitochondria and membranous lamellar structures in the infranuclear cytoplasm. The intercellular spaces between the epithelial cells were not evident after fat feeding, and fat droplets were not common in these regions. The control materials presented few fat droplets in the gut epithelium. The droplets were few in number and generally much smaller than those occurring during fat absorption.

In contrast to the mid gut, fat droplets were nearly absent in the epithelium of the posterior gut after administration of egg-yolk emulsions with corn oil. But the epithelium showed a moderate loading with acidophilic granules. These granules were detected as less dense materials in membrane-enclosed vacuoles by electron microscopy (Fig. 1). These vacuoles, $0.9\sim 2.2\mu$ by $0.6\sim 1.6\mu$ in major and minor axes respectively, were oval in shape. The most striking feature in fine structure of these columnar cells was vigorous pinocytosis occurring in the apical edge (Fig. 1). The apical plasma membrane was deeply invaginated at intermicrovillous spaces. Some tubules and numerous vesicles were distributed in the terminal web layer and apical cytoplasm. Among these tubules and vesicles, filamentous structures run parallel to the long axis of the cell. Lying below the zone of vesicles were vacuoles with less dense materials which might be considered as being derivatives from the pinocytotic vesicles. These vacuoles were much alike to those found in some mammalian intestinal epithelium following the administration of protein.

Discussion

The observations recorded in this paper confirm the findings from previous studies with teleost larvae feeding on plankton organisms, which indicate that the mid gut is chiefly implicated in fat absorption^{1,2)}. Such evidence is not confined to larval fish alone. In the adult fish, some investigators demonstrated marked absorptive capacity of fat in the pyloric caeca and/or the antero-mid part of the intestine, but nobody regarded the rectum as active part of fat absorption³⁻⁶⁾.

The columnar absorptive cells of the gut epithelium of larval goldfish are virtually the same in fine structure as those of the intestinal epithelium of adult goldfish described by YAMAMOTO⁷⁾. YAMAMOTO⁸⁾, by feeding the goldfish on casein and corn oil, found that much absorptive activity of protein is seen in the posterior part of the intestine, and that fat is absorbed mainly by the intestinal bulb. In that material, vigorous invaginations of the intermicrovillous plasma membrane occurred in the former, but no such changes were found in the latter. Such sectional differences of absorptive function in the digestive tract are in agreement with those found in the larvae of the same species. It seems worth noting that similar pattern of absorptive activity is known to occur in the gut epithelium of various kinds of teleost larvae^{1,2,9)}. This may be a common occurrence for teleost larvae.

Although the process of fat absorption in the columnar absorptive cells has been extensively studied by many investigators, two major theories of fat absorption have been the subject of debate over the past decade. The first is the diffusion of fat across the plasma membrane, and the other is the engulfment of fat by a process of pinocytosis. According to those who support the first theory, hydrolysis of fat is mostly completed in the intestinal lumen, and resulting fatty acids and monoglycerides diffuse across the plasma membrane into the cell where they are resynthesized to triglycerides¹⁰⁻¹²). Those who uphold the second theory demonstrated that a significant amount of fat is absorbed by pinocytosis in a form of droplet¹³⁻¹⁵). SENIOR¹⁶) was of the opinion that these two mechanisms possibly occur during fat absorption though the former may be more efficient and normally predominant mode of entry of fat. Under the experimental conditions in the present study, no increase of pinocytotic vesicles containing fat droplets was visible after administration of corn oil, and absorbed fat was first detected as droplets within vesicles located below the terminal web layer. These facts do not support pinocytosis as an important process in fat absorption in the gut of goldfish larvae. YAMAMOTO¹⁷) pointed out that pinocytosis is seen during fat absorption in the newborn cat, but that pinocytosis is indiscernible in relation to fat absorption in the adult rat. One may thus expect the possibility of fat absorption by pinocytosis in the gut epithelium of fish at least in larval stages when the gut is presumably in poor condition in digestive secretions. However, we failed to find evidence for the expected occurrence of pinocytosis during fat absorption in the mid gut of halfbeak larvae³) and goldfish larvae. In the rat jejunum, fat absorption and transport through the epithelium are said to be extremely rapid, and fat droplets can be seen in the endoplasmic reticulum and the Golgi apparatus of the epithelial cells within 1 minute after administration of fatty-chyme¹⁸). Since these observations in the goldfish larvae were made 6 hours after administration of fat containing diet, further experiments are necessary to clarify the mechanism of fat absorption in the epithelial cells immediately after administration of fat.

Summary

In the goldfish larvae, numerous fat droplets were detected in the cytoplasm of columnar epithelial cells of the mid gut after administration of boiled egg-yolk emulsions with corn oil. No conspicuous changes of invaginations were observed in the intermicrovillous spaces of the cells loaded heavily with fat droplets. On the other hand, vigorous pinocytosis occurred in the columnar epithelial cells of the posterior gut, which was probably implicated in protein absorption.

Observations supported the hypothesis that in teleost larvae fat is mainly absorbed by the mid gut before the constriction by which it is distinguishable from the posterior gut.

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