The Comparative Study of the Digestive Tract of Teleost Larvae—III. Epithelial Cells in the Posterior Gut of Halfbeak Larvae

Tamotsu IWAI and Masaru TANAKA*

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The previous papers have dealt with fine structure of ciliated cells and columnar absorptive cells of the gut epithelium in the larvae of salmonids and their relatives¹⁻³⁾. However, the exact nature of the columnar absorptive cells in teleost larvae is still far from being satisfactorily understood. It seems better to approach to the problem by taking extensive data from various kinds of larvae rather than by studying a few species intensively. The purpose of this paper is to describe the possible functional significance of the peculiar absorptive cells found in the posterior gut epithelium of halfbeak larvae, particularly at the period of active feeding.

Materials and Methods

The materials used in this study were about 20 larvae of the halfbeak, *Hemiramphus sajori* (TEMMINCK and SCHLEGEL), hatched in laboratory tanks. After hatching, they were fed live nauplii of the brine shrimp. For light microscopy, 1-day larvae (7.0~7.5 mm long), 2-day larvae (7.5~8.0 mm long), and 8-day larvae (11.5~12.0 mm long) were fixed in 10% formalin, and sectioned at 5~8 μ by the usual paraffin method. Stains used were Mayer's hemalum and eosin, and safranin and fast green. For electron microscopy, small pieces of the intestine from living 2-day larvae were fixed for 90 minutes in chilled 2% 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

In the larval stages of the halfbeak, the gut is a straight tube, provided with a simple columnar epithelium. The epithelium consists of two principal cell types; the columnar cells with a striated border and a few goblet cells with mucigen droplets.

In the 2-day larvae, the posterior gut is clearly demarcated from the mid gut by the valvular projection of mucosa at the level 700~750 μ before anus. Of particular interest is the peculiar epithelial cells found in this region of well-fed larvae. These columnar cells possess large acidophilic granules in their cytoplasm which displace

* Department of Fisheries, Faculty of Agriculture, Kyoto University, Maizuru. (岩井 保・田中 克, 京都大学農学部水産学教室)

their nuclei basally. They become more prominent in the 8-day larvae (Fig. 1).

The detail of the granular cells is demonstrated more clearly by electron microscopy. From the evidence of fine structure they are identical with the columnar absorptive type. The striated border is resolved into a brush-like formation of myriad

Fig. 1. Photomicrograph of columnar cells with acidophilic granules in the posterior gut epithelium of 8-day larva. $\times 1,300$.

Fig. 2. Electron micrograph of section through the supranuclear part of granular cells in 2-day larva. $\times 12,000$.

Fig. 3. Electron micrograph showing vigorous pinocytosis. $\times 36,000$.

ag, acidophilic granules; *f*, fine filaments; *m*, mitochondria; *mv*, microvilli; *pv*, pinocytotic vesicles; *v*, vacuoles with less dense materials.

Fig. 4. Electron micrograph of section through the basal part of granular cells in 2-day larva. $\times 12,000$. *G*, Golgi apparatus; *ls*, lamellar structure; *m*, mitochondria; *n*, nuclei; *v*, vacuoles with less dense materials.

microvilli which are finger-like projections of apical cytoplasm covered by the plasma membrane. They average about 1.6μ in height and 0.1μ in diameter. Clustered microvilli are supported by the underlying terminal web which is characterized by a meshwork of fine filamentous materials. The terminal web is frequently indiscernible, because numerous intermicrovillous invaginations and pinocytotic vesicles crowd there (Figs. 2 and 3). The supranuclear part of the cell is mostly occupied by small vesicles and large vacuoles containing less dense materials (Fig. 2). The vacuoles, $1.2\sim 8.2\mu$ by $0.8\sim 5.8\mu$ in major and minor axes respectively, are spherical or ovoid in shape, and are enclosed by well defined membranes separating them from the surrounding cytoplasm. Apparently they are derived from the intermicrovillous invaginations. These vacuoles seen by electron microscopy are in the same location as the acidophilic granules seen by light microscopy (cf. Figs. 1~4).

Mitochondria are scattered throughout the cytoplasm. Most of them are rod-shaped, tending to be oriented parallel to the long axis of the cell. In the supranuclear cytoplasm, they are displaced near the lateral plasma membrane by conspicuously large vacuoles (Fig. 2). Well developed Golgi apparatus is evident above

and beside the nucleus (Fig. 4). A moderate amount of endoplasmic reticulum is seen in the remainder of the cytoplasm. The granular endoplasmic reticulum in the granular cell is not as well organized as that of the secretory cell. Membranous lamellar structures are located in the infranuclear cytoplasm in close association with mitochondria. They are flattened sac lined by two regular parallel membranes running parallel to the long axis of the cell.

The nucleus is basally located and its upper margin often appears concave by the distension of large vacuoles. The lateral plasma membrane is more or less smooth and apposed to that of adjacent cells with thin intercellular space. There is a junctional complex of the lateral plasma membrane at the level of the terminal web, that makes a tight junction between adjacent cells. The basal plasma membrane is smooth and rests on a thin homogeneous basement membrane.

Discussion

There is a considerable literature dealing with the granulation and vacuolation of the columnar epithelial cells in the intestine of adult fishes⁴⁻⁹. In particular, the epithelium of the posterior intestine of the goldfish has intrigued some investigators by its interesting feature. McVAY and KAAN⁶ reported conspicuous vacuoles located in the supranuclear cytoplasm of the columnar cells in the goldfish, and they postulated the relationship between the appearance of vacuoles and the presence of food in the intestinal lumen. In making an electron microscopic study, YAMAMOTO⁹ regarded these vacuoles as related to protein absorption. A similar condition can be seen in the epithelial cells of the posterior gut of well-fed halfbeak larvae, though they possess intense granules instead of vacuoles. One may assume the granules to be of secretory substance. However, there is good evidence suggesting that these granular cells are implicated in absorption. The granules correspond in location to the conspicuous vacuoles viewed by electron microscopy. Furthermore, the granular endoplasmic reticulum in these cells is not so prominent as in cells actively engaged in secretory function such as pancreatic acinar cells and goblet cells. Vigorous pinocytosis, vesicles, and vacuoles containing less dense materials show a strong resemblance to those described by YAMAMOTO⁹ for the goldfish. Working with suckling rat and mice, CLARK¹⁰ demonstrated that large vacuoles are formed in the absorptive cells of the small intestine following the administration of protein or colloidal materials. Those are comparable to unusually large vacuoles found by the present observation. It is therefore plausible to suppose that the granular cells in the halfbeak are related to protein absorption in function. This interpretation is strengthened by the fact that similar cells with acidophilic granules showing positive reaction on both phenol and indole groups, have been observed in the same region of the gut epithelium of well-fed larvae of the puffer (Unpublished data). Since some suckling mammals are capable of absorbing intact protein during early stages

of life¹¹), there seems no reason to doubt that absorptive cells of the gut epithelium ingest such substance as protein in teleost larvae. That these cells are limited to the posterior gut in the larvae of the halfbeak suggests that vigorous absorption of protein is occurring there. It is generally conceded that in the teleost larvae having a straight gut, ingested food materials are passed rapidly to the posterior end of the gut and accumulated there¹²). Therefore, the posterior gut may probably play an important role in digestion of nutrients in these larvae.

On the other hand, the columnar cells in the mid gut of halfbeak larvae are filled with massive fat droplets, by which their nuclei are displaced basally, as is the case with many teleost larvae¹³). As was pointed out by AL-HUSSAINI⁶), the fat droplets appear as vacuoles in the preparation made by the paraffin method. A detailed account of fat absorption in these larvae will be given in the forthcoming paper.

Summary

Peculiar columnar cells with acidophilic granules are found to occur in the posterior gut epithelium of well-fed larvae of the halfbeak. By electron microscopy, the granules are identifiable as vacuoles containing less dense materials which are formed by pinocytosis. The posterior gut epithelium marked by such peculiar cells may be implicated in absorption, probably ingestion of protein.

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