The Morphology of Absorption of Nutritive Substances in the Gut of Tigriopus japonicus*1

Kazuma Yoshikoshi*2

(Accepted November 2, 1987)

The absorptive function of the alimentary canal of Tigriopus japonicus was investigated using lipids, saccharated iron oxide, and ferritin as tracers. Light and electron microscopic examinations revealed that nutritive substances were absorbed only in the mid-gut. Lipids were absorbed by the non-vacuolated cells by means of transmembrane absorption and accumulated in lipid vacuoles of the non-vacuolated cells and lipid storing tissues of the body. Positive Berlin blue reaction by absorbed iron was found in all the mid-gut epithelial cells. Ferritin was absorbed only by the vacuolated cells by means of endocytosis. These tracer substances were found to be absorbed more actively in the mid-gut in the metasome than that in the urosome. These results indicate that there are differences in mode and degree of absorption between the mid-gut in the metasome and that in the urosome.

In a previous paper dealing with the ultrastructure of the mid-gut epithelium of Tigriopus,*1 it has been shown that the mid-gut epithelium in the metasome consists of two kinds of epithelial cells, strongly vacuolated cells and non-vacuolated cells, characterized by prominent endocytosis and well developed microvilli, respectively. It has also been shown that the mid-gut epithelium in the urosome consists of rather flattened non-vacuolated cells. The microvilli and cytoplasmic organelles of which are respectively shorter and less plentiful than those of the non-vacuolated cells in the metasome. These findings suggest that the two types of the mid-gut epithelial cells function as the absorptive cells by means of different absorptive mechanisms, and that there are differences in kind and degree of absorption between the mid-gut in the metasome and that in the urosome. According to this suggestion an attempt was made to demonstrate where and how nutritive substances are absorbed.

Materials and Methods

Animals

Adult females of Tigriopus japonicus were obtained from a laboratory culture.

Experimental feeding

Three kinds of artificial foods were prepared: a mixture of 2 parts of 5% gelatin and 1 part of fresh cream, 5% gelatin containing 5% saccharated iron oxide (Merck Co.), and 5% gelatin containing 5% ferritin (Sigma Chem. Co.). Test animals were starved in filtered seawater for 3 or 7 days for lipid absorption, 4 days for iron absorption, and 3 days for ferritin absorption prior to use. After starvation test animals were reared in filtered seawater in beekers containing each food and fixed at different intervals of time. Foods and rearing seawater were renewed every 12 hours. Water temperature was kept at 17–20°C in a water bath during starvation and rearing.

Light and Electron Microscopy

Animals starved for 7 days and fed with fresh cream were fixed in Champy’s fluid, dehydrated in ethanol, and embedded in paraffin. To visualize lipid droplets, sagittal sections of 5 μm in thickness were cut and cleared in liquid paraffin. Animals fed with saccharated iron oxide were fixed in cold Bouin’s fluid, dehydrated in ethanol, and embedded in paraffin. For detection of iron, the Berlin blue reaction*2 was used. Animals starved for 3 days and fed with fresh cream or ferritin were examined using an electron microscope. The methods used for preparing ultrathin sections were the same as described earlier*3 except that some specimens were dehydrated in polyethylene glycol 200 for better preservation of lipids.

---

*1 On the Structure and Function of the Alimentary Canal of Tigriopus japonicus (Copepoda; Harpacticoida) -IV.
*2 Faculty of Fisheries, Nagasaki University, Nagasaki 852, Japan (吉崎一馬：長崎大学水産学部).
Results

Absorption of Lipids

Light microscopically, no lipid droplets were seen in the mid-gut epithelial cells of animals reared for 0.5 hour with food (Fig. 1). A number of small lipid droplets were first observed in the mid-gut epithelium in the metasome of animals reared for 1 hour with food and increased in size with time (Figs. 2-4). The number and size of lipid droplets were always larger in the mid-gut epithelium in the metasome than that in the urosome. Accumulation of lipids in lipid storing tissues existing in various regions of the body was found at and after the 6th hour of the rearing. No lipid droplets were found in the fore- and hind-gut epithelia during lipid absorption.

Electron microscopically, as shown in Fig. 5, the nonvacuolated mid-gut epithelial cells in the metasome of animals starved for 3 days possess three kinds of vacuoles, larger empty lipid vacuoles, smaller lipid-laden vacuoles, and autophagic vacuoles. In an animal reared for 1 hour with food, the empty lipid vacuoles were found to be filled with lipids in the apical region of the cells (Fig. 6). At the 3rd hour of the rearing most of the empty lipid vacuoles were filled with lipids and, in some specimens, lipid vacuoles increased in number and size to a considerable degree. Lipid vacuoles increased in size by the coalescence of very small vesicles or tubules containing lipids which are probably a part of the agranular endoplasmic reticulum (Fig. 7). No appreciable changes were found in the free and lateral surfaces of the non-vacuolated cells during lipid absorption (Figs. 8 and 9). In the vacuolated cells of animals reared for 3 hours with food, numerous endocytotic vacuoles containing a moderately electron dense amorphous material similar to the gut content and voluminous residual bodies existing in the basal vacuoles were observed (Figs. 10 and 11). However, no lipid vacuoles were found in the vacuolated cells during lipid absorption.

Absorption of Saccharated Iron Oxide

In animals reared for 1 hour with food a weak diffuse staining was seen on the apical side of the mid-gut epithelium in the metasome. The staining became intense with time up to the 12th hour of the rearing and was found in the mid-gut epithelium not only in the metasome but also in the urosome. In animals reared for 12 hours with food intensely stained fine granules were found in the apical region of the epithelial cells against the diffusely stained background (Fig. 12). After the 12th hour of the rearing the staining behaviour remained almost unchanged. The morphology of absorption of saccharated iron oxide found in Tigriopus was very similar in many aspects to that observed in the freshwater crab, Atya spinipes.3)

Absorption of Ferritin

In animals reared with food numerous ferritin particles were found between microvilli and ingested only by the vacuolated cells by means of endocytosis (Figs. 13 and 14). In animals reared for 3 hours with food ferritin particles were found not only in endocytotic vacuoles in the apical region of the cells but also in the basal vacuole.

Discussion

Results obtained conclusively show that (1) the mid-gut alone participates in absorption of nutritive substances; (2) there are two absorptive mechanisms; (3) there are differences in kind and degree of absorption between the mid-gut in the metasome and that in the urosome; and (4) the mid-gut participates in storage of certain nutritive substances such as lipids.

It is likely that the mid-gut of copepods is the sole site of absorption, since, so far as is known, the mid-gut gland is absent in copepods, though there are small diverticuli or outpocketings in the anterior region of the mid-gut, and since gutless copepods seem exceptional. The mid-gut is the greatest part of the alimentary canal of copepods, occupying 81.1% of the total length of the alimentary canal in Calanus finmarchicus4) and ranging from 57.3% in Pseudodiaptomus marinus to 96.5% in Lernaea cyprinacea (unpublished data obtained from 25 species).

No ultrastructural changes were found in the free surface of the non-vacuolated cells during lipid absorption. Accordingly, it seems probable in Tigriopus that, as is well known in the mammalian intestinal absorptive cells, free fatty acids and monoglycerides which are derived from ingested lipids by enzymatic digestion in the gut lumen diffuse across the plasma membrane, and lipids are resynthesized from them in the agranular endoplasmic reticulum. It seems probable that carbohydrates and amino acids are also absorbed by means of transmembrane absorption, although
convincing evidences are lacking. Recently, Arnaud et al.\textsuperscript{5) reported that the vacuolated B cells in the mid-gut epithelium of Centropages typicus, the primary function of which is regarded as intracellular digestion, absorb the gut content and extrude the digested substances into the gut lumen in a manner analogous to that of holocrine secretion. The extruded substances are then reabsorbed by the R cells, a type of the mid-gut epithelial cells. More recently, they suggested that the B cells of calanoid copepods are not only digestive but also excretory in function.\textsuperscript{6)} If the primary function of cells is intracellular digestion, it is suspected that the luminal extrusion of digested substances and reabsorption by the absorptive cells are unnecessary physiological processes. In Tigriopus, the luminal extrusion of the vacuolated cells probably takes place as senile decay as discussed earlier.\textsuperscript{1,7,8) The primary function of the vacuolated cells of Tigriopus is regarded as absorption and intracellular digestion of nutritive substances. By means of endocytosis proteins and, possibly, lipids are absorbed by the vacuolated cells. However, the vacuolated cells are probably unable to resynthesize lipids from fatty acids and monoglycerides. The biological significance of intracellular digestion in the mid-gut is regarded as compensating a relatively insufficient luminal digestion at a rather low phylogenetic stage as discussed earlier.\textsuperscript{1,9) Gauld\textsuperscript{9) described in calanoid copepods that there is a functional division of the mid-gut into an anterior stomach in which the food mixed with digestive juices and a posterior intestine which is probably mainly absorptive in function. The partition in function of the mid-gut is, however, not the case in Tigriopus, since the anterior mid-gut, the mid-gut in the metasome, is apparently of more importance to absorption in kind and degree.

The storage function of lipids in the mid-gut epithelial cells has been suggested in Paranthessius anemontae, a cyclopoid copepod associated with snakelocks anemone.\textsuperscript{10) In Tigriopus, large lipid vacuoles are frequently found in the non-vacuolated cells and the vacuole content is consumed when animals are starved, as shown in Fig. 5. In addition, lipid vacuoles increase in size to a considerable degree in animals fed with fresh cream (Figs. 3 and 4). These findings suggest that the non-vacuolated cells possess the storage function of lipids. It is uncertain whether the mid-gut epithelial cells of Tigriopus possess the storage function of other substances.

References

Explanation of Figures

All the cells shown in the figures are the mid-gut epithelial cells in the metasome.

\begin{itemize}
  \item \textbf{Figs. 1-11.} Absorption of lipids.
  \item \textbf{Figs. 1 and 2.} Mid-gut epithelia of animals reared for 0.5 and 3 hours with food, respectively. \times 360.
  \item \textbf{Figs. 3 and 4.} Mid-gut epithelia of animals reared for 12 and 24 hours with food, respectively. \times 770.
  \item \textbf{Fig. 5.} Non-vacuolated cells of an animal starved for 3 days. l.v: empty lipid vacuole, Mv: microvilli, Pv: autophagic vacuole, \times 15,000.
  \item \textbf{Fig. 6.} Non-vacuolated cells of an animal reared for 1 hour with food. N: nucleus, \times 9,000.
  \item \textbf{Figs. 7-11.} Animals reared for 3 hours with food.
  \item \textbf{Fig. 7.} Basal region of the non-vacuolated cell. Arrows indicate the coalescence of small vesicles into larger lipid vacuoles. \times 50,000.
  \item \textbf{Fig. 8.} Apical region of the non-vacuolated cell. \times 30,000.
  \item \textbf{Fig. 9.} Lateral surface of the non-vacuolated cells. Plasma membranes of opposing cells are seen between arrows. \times 30,000.
\end{itemize}
Fig. 10. Apical region of the vacuolated cell. ×15,000.

Fig. 11. Central to basal region of the vacuolated cell. Arrows indicate indigestible residues in the basal vacuole. ×5,000.

Fig. 12. Absorption of iron. Mid-gut epithelium of an animal reared for 12 hours with food. Berlin blue reaction. ×1,500.

Figs. 13 and 14. Absorption of ferritin.

Fig. 13. Apical region of the non-vacuolated cell. Arrows indicate ferritin particles. ×50,000.

Fig. 14. Apical region of the vacuolated cell. ×50,000.