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# Structure of the digestive tract of tornaria larva in *Enteropneusta* (Hemichordata)

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ABSTRACT: The ultrastructure of the digestive tract of tornaria larva of enteropneusts was investigated. It showed that the digestive tract consists of three parts: esophagus, stomach, and intestine. The esophagus epithelium consists of two types of multiciliated epithelial cells and solitary muscle cells. Axonal tracts and neurons were found in the ventral wall of the esophagus. The cardiac sphincter contains an anterior band of strongly ciliated cells and a posterior band of cells with long vacuolized processes which partition the sphincter lumen. The stomach consists of three cell types: (1) cells with electron-opaque cytoplasm, bearing a fringed border on their apical sides; (2, 3) sparse cells with electron-light cytoplasm and different patterns of apical microvilli. Cells of the pyloric sphincter bear numerous cilia and almost no microvilli. The intestine consists of three parts. The anterior part is formed of multiciliated cells which bear the fringed border. The middle part consists of flattened cells bearing rare cilia and vast numbers of mace-like microvilli. The posterior part of the intestine is formed of cells bearing numerous cilia and few microvilli. Muscle cells were not found in either stomach or intestine epithelium. One noticed that the structure of the digestive tract of enteropneust tornaria larva differs from that of echinoid pluteus larva.

## INTRODUCTION

Various species of enteropneusts have similar planktonic larva-tornariae. After the free-swimming period, the tornariae settle at the bottom and metamorphose.

Tornariae are morphologically similar to the echinoderm larvae and are united with them as dipleurula-type larvae (Ivanova-Kazas, 1978). The feeding process of tornariae has much in common with echinoderm larvae and is accomplished through the use of ciliary bands (Strathmann, 1975; Strathmann & Bonar, 1976). As for the ultrastructure of the digestive tract of echinoderm larvae, it was shown to be tripartite (muscular esophagus, spherical stomach and small tubular intestine) and consists of various types of specialized monociliated and unciliated cells (Ryberg & Lundgren, 1975; Burke, 1981). The digestive tract of a tornaria also consists of three parts, but very little is known about the structure of these larval organs. Comparison of the fine structure of these organs in tornaria and echinoderm larvae is of some interest, since they have similar morphology and behaviour but different fates of the larval body during metamorphosis (Ivanova-Kazas, 1978).

This paper presents the results from an ultrastructural study of the digestive tract of

tornaria larva of the enteropneust *Balanoglossus proterogonius*. Possible functions of different cells and comparisons of the echinoderm larvae are discussed.

## MATERIALS AND METHODS

Tornariae belonging to *Balanoglossus proterogonius* Belichov, 1930 (Van der Horst, 1933) were collected at the Vostok Bay of the Sea of Japan. Larvae were fixed in a solution of 2 % glutaraldehyde (Sigma) in 0.05 M cacodylate buffer (pH 7.4) with 0.142 M NaCl and 0.238 M sucrose for 2 h at 10 °C, rinsed for 15 min in the same buffer and postfixed in 2 % osmium tetroxide in the same buffer with 0.393 M NaCl. The material was dehydrated in ethanol and acetone series. The samples were embedded in Epon-Araldite. Sections were stained with uranyl acetate and lead citrate, and examined with a JEM-100B electron microscope (Japan). The work was done at Vostok Biological Station of the Institute of Marine Biology, FEB, Russian Academy of Sciences (Vostok Bay, Sea of Japan).

## RESULTS

### General morphology of digestive tract

The digestive tract of a tornaria consists of three parts: esophagus, stomach, and intestine (Fig. 1).

The mouth-opening is located on the ventral body side and connects with an elongated esophagus. The esophagus is formed by one layer of cylindrical and mace-like multiciliated epithelial cells and solitary muscle cells. The esophagus is separated from the stomach by a cardiac sphincter. The sphincter epithelium is much thicker than that of the esophagus and stomach, and consists of elongated cells with long vacuolized processes filled with many electron-light vesicles. These processes differentiate the sphincter lumen.

The stomach is spherical. The ventral ciliary field, just behind the sphincter in the ventral part of the stomach epithelium, is formed by a group of multiciliated cells. The anterior part of the stomach consists of cuboid cells which are flattened in the middle. From the observation of semithin sections, three cell types were found: (1) cells with electron-dense cytoplasm that form the base of the stomach epithelium, (2) cells with electron-light cytoplasm (2–4 cells per sagittal section of the stomach), (3) flattened, intensively vacuolized cells (1–2 per sagittal section).

The stomach is separated from the intestine by the pyloric sphincter. The sphincter epithelium is thickened and consists of elongated cells with a great number of cilia.

The cylindrical posterior intestine consists of cuboid and flattened cells. Near the anus region, the epithelium becomes thicker and bears many cilia. The anus opens terminally. Muscle cells were not found in the cardiac sphincter, stomach, pyloric sphincter, or intestine epithelia.

### Fine structure

Esophagus. The esophageal epithelium consists of two ultrastructural cell types. Type 1 cells form the major component of the epithelium (Fig. 2) and are distributed all

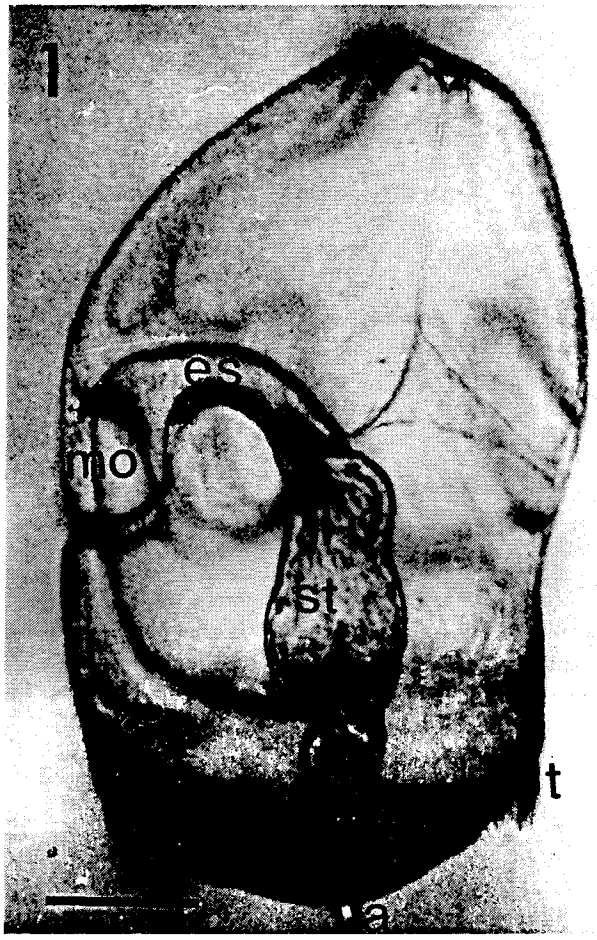



Fig. 1. Fully-developed tornaria larva. a – anus, es – esophagus, in – intestine, mo – mouth opening, st – stomach, t – telotroch. Scale bar: 100 μm

along the esophagus. All cells have many cilia with a 9 + 2 set of axoneme. The cilia have a basal body and a striated rootlet. Rare microvilli at the apical side of the cells are long, slightly branched and rosary-like (Fig. 3). Many mitochondria with tubiform cristae and few vesicles with electron-dense material are located in the apical region of the cytoplasm. In addition, Golgi bodies and cisternae of rough endoplasmic reticulum are found in the cytoplasm. A single nucleus occupies the basal part of the cell. Rare mitochondria can be found in the basal region also.

Type 2 cells have cytoplasm of higher electron density and less mitochondria (Fig. 4). The microvilli on their surface are cylindrical. These cells also have cilia, Golgi complexes and rough endoplasmic reticulum.

Epithelial cells are attached by gap junctions in the apical region. The entire epithelium is underlined by basal lamina consisting of several layers. The esophageal

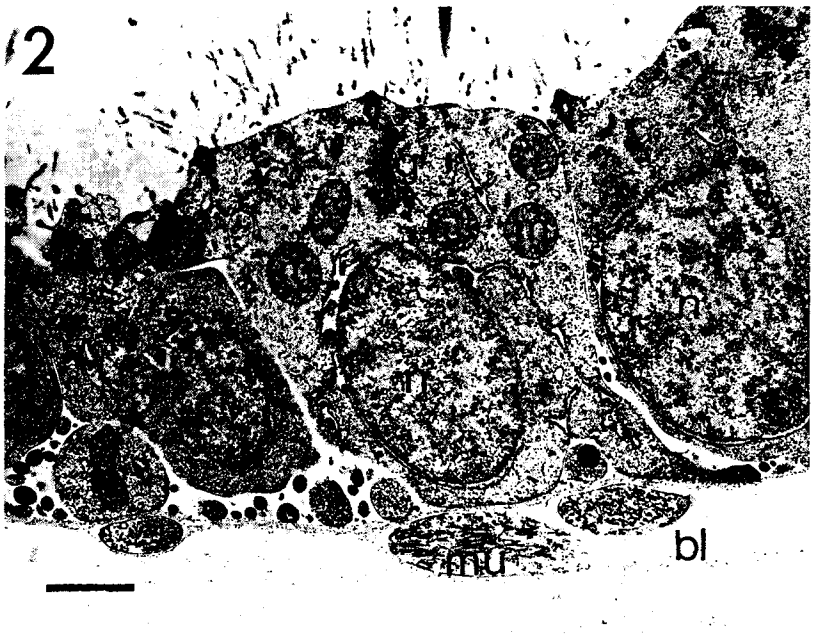



Fig. 2. Section of the esophagus epithelium with cells of type 1. bl – basal lamina, g – Golgi complex, m – mitochondria, mu – muscle cells, n – nucleus. Scale bar: 1 μm

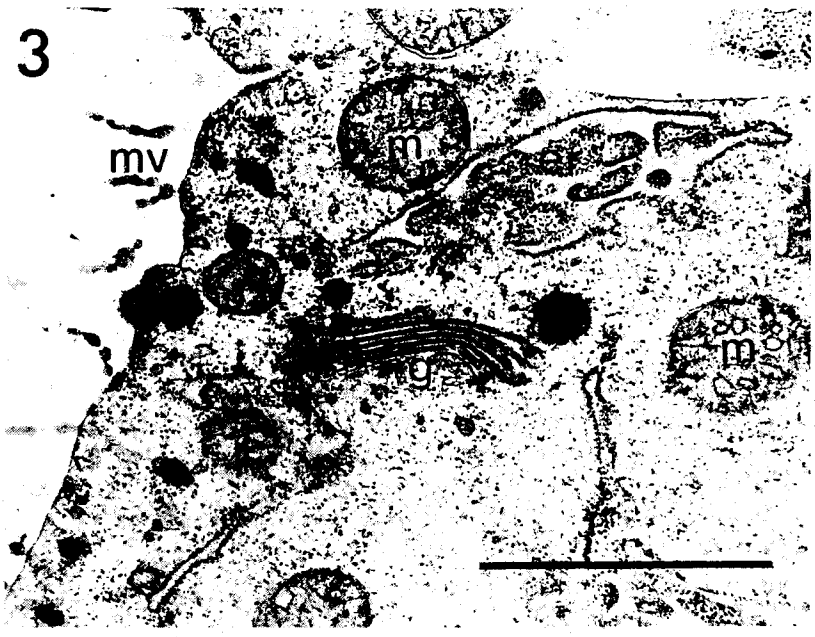



Fig. 3. An apical region of the cell of type 1 of the esophagus epithelium. Rosary-like microvilli are shown. er – endoplasmic reticulum, g – Golgi complex, m – mitochondria, mv – microvilli. Scale bar: 0.5 μm


Fig. 4. Section of the esophagus epithelium. Cell of type 2 is shown in upper right. bl – basal lamina, g – Golgi complex, n – nuclei, mu – muscle cell. Scale bar: 1 μm


Fig. 5. Neuron-like cell (arrow) in the esophagus epithelium. g – Golgi complex, m – mitochondrion, mu – muscle cell, n – nuclei. Scale bar: 1 μm

muscles are situated between the layers of the lamina (Figs 2, 4). Solitary axons or axonal tracts run between the lamina and epithelial cells presumably surrounding the esophagus. Cell bodies of what appear to be neurons with electron-dense cytoplasm and a large nucleus of irregular shape are found inside the ventral wall of the esophagus. These cells have processes and are closely associated with axonal tracts (Fig. 5).

Cardiac sphincter. The type 1 cells of the esophagus are far more ciliated in front of the sphincter, so that a ciliary ring appears in front of the stomach (Fig. 6). The sphincter consists of epithelial cells with electron-dense cytoplasm and long vacuolized processes on their apical surface which partition the sphincter lumen (Fig. 7). The vacuoles are large, electron-transparent and contain flocculent material. Each cell has few cilia and many mitochondria. Behind these cells, the stomach epithelium can be seen. Just behind the cardiac sphincter in the ventral part of the stomach epithelium there is a group of cells, each having many cilia (Fig. 8) forming a highly ciliated field.

The cells of the ventral ciliated field have long branched microvilli and numerous mitochondria in their electron-dense cytoplasm. Dorsally and laterally behind the sphincter, there are typical stomach epithelial cells. No muscle cells were detected in the cardiac sphincter. Several neuron-like cells and axonal tracts occur at the base of the sphincter epithelium. Numerous rounded electron-transparent cavities are situated between the cells of the cardiac region.

Stomach. The stomach epithelium consists of three ultrastructurally distinct cell types (except the cells of the ventral ciliary field). Type 1 stomach cells form the major


Fig. 6. Sagittal section of the cardiac sphincter. cr – ciliary ring, se – stomach epithelium, vc – vacuolated cells, vf – ventral ciliary field, arrow shows empty cavities between cells. Scale bar: 2 μm

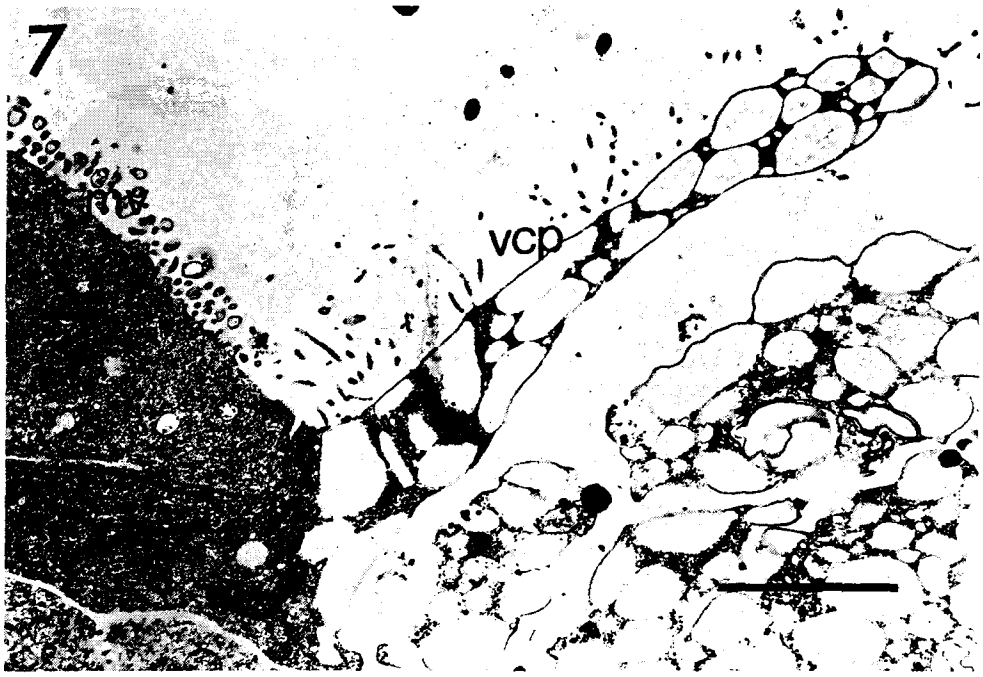



Fig. 7. Vacuolated growings of the cardiac sphincter cells. mv – microvilli, vcp – vacuolated cell process. Scale bar: 2 μm

component of the epithelium. They are cuboid near the region of the cardiac and pyloric sphincters, and flattened in the middle part of the stomach. The cytoplasm is highly electron-dense, nearly opaque and contains many mitochondria (Fig. 9). Numerous microvilli are straight. Some of them are slightly branched. Together they form a fringed border on the apical surface (Fig. 10).

Two types of vesicles concentrate in the apical part of the cytoplasm: small electron-dense vesicles, which occupy most of the cytoplasm, and large ones with electron-dense or transparent contents. Nucleus and extensive, rough, endoplasmic reticulum occur in the basal region of the cell.

We found few type 2 cells in the stomach. Type 2 cells have transparent cytoplasm with basally situated nucleus and a few short mace-like microvilli (Fig. 11). Their cytoplasm contains mitochondria, cisternae of rough endoplasmic reticulum and small transparent vesicles.

Type 3 cells have rare short microvilli of irregular shape and few cilia (Fig. 12). Cytoplasm of these cells is electron-light and filled with small and coarse electron-light vesicles.

Pyloric sphincter and intestine. In the posterior part of the stomach the cells bear many long cilia directed towards the pyloric sphincter and intestine lumen. Near the pyloric sphincter constriction, where the cells form the fringed border, a band of cells of similar ultrastructure but almost without microvilli can be seen (Fig. 13). Still

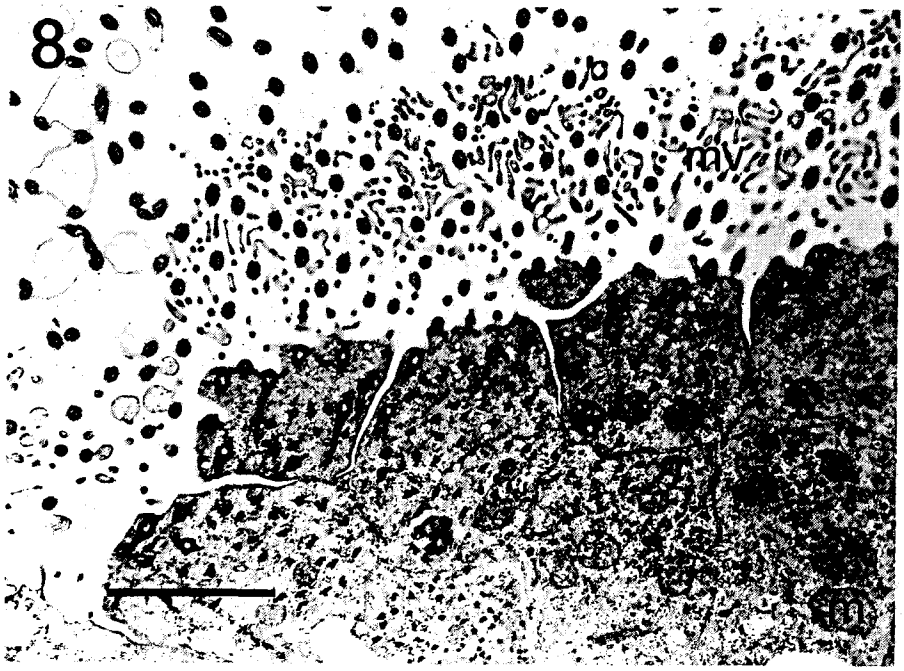



Fig. 8. An apical part of the ventral ciliary field behind the cardiac sphincter. Ciliation and branched microvilli (mv) are shown. m – mitochondrion. Scale bar: 2 μm

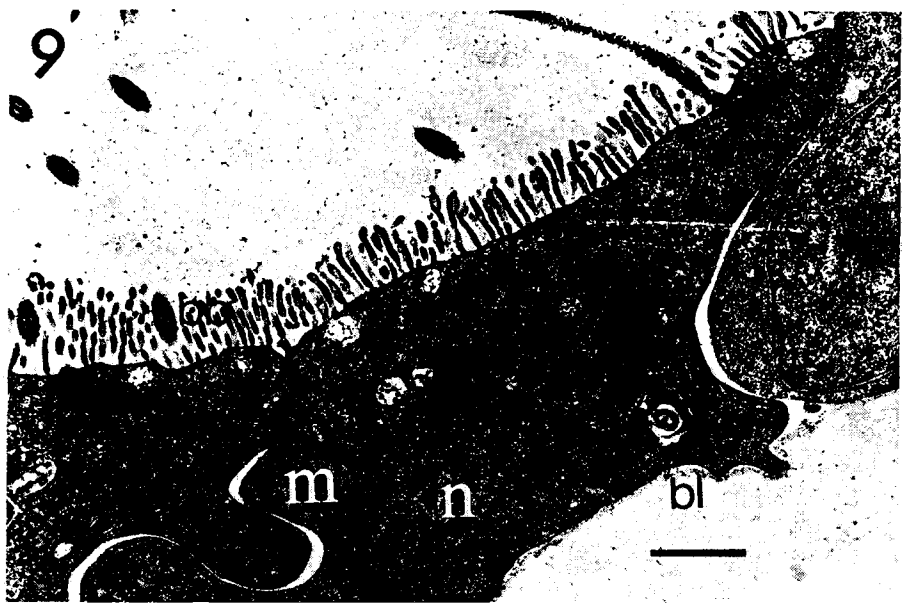



Fig. 9. Cell of type 1 of the stomach epithelium. bb – fringed border, bl – basal lamina, m – mitochondrion, n – nucleus. Scale bar: 1 μm

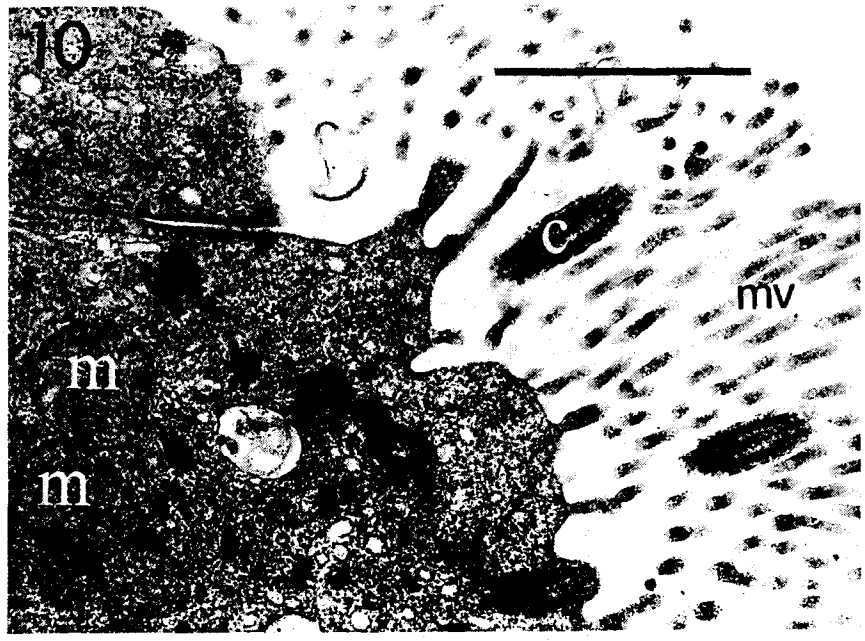



Fig. 10. An apical part of type 1 cell of the stomach epithelium. c – cilium, m – mitochondria, mv – microvilli. Scale bar: 0.5 μm

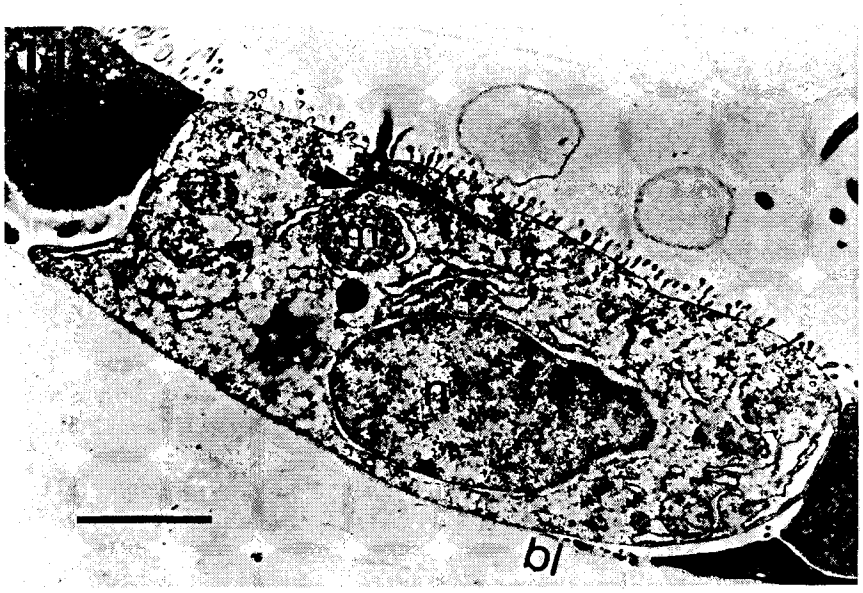



Fig. 11. Type 2 cell of the stomach epithelium. bl – basal lamina, er – endoplasmic reticulum, m – mitochondrion, n – nucleus, arrows show ciliar rootlets. Scale bar: 1 μm

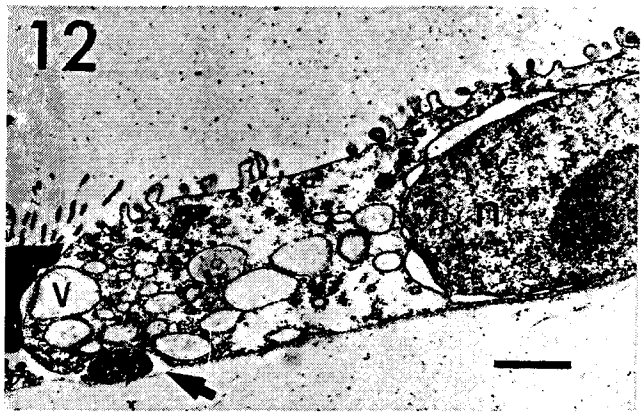



Fig. 12. Type 3 cell of the stomach epithelium. n – nucleus, v – vacuole, arrow shows basal lamina. Scale bar: 1 μm


Fig. 13. Sagittal section of the pyloric sphincter. a – axonal tracts, bl – basal lamina, m – mitochondrion, n – nucleus. Scale bar: 1 μm

further, another band of cells with electron-light cytoplasm is located. These cells have a smooth apical surface without microvilli, and bear few cilia. Then comes a band of cells with relatively dense cytoplasm, few cilia and microvilli. These cells have a variety of shapes. They form folds which resemble tight junctions. The intestine is divided into three parts, depending on the type of epithelial cells. The anterior part is lined with cuboid cells having a relatively light cytoplasm and well-developed fringed border composed of elongated slightly branched microvilli (Fig. 14). These cells bear many cilia

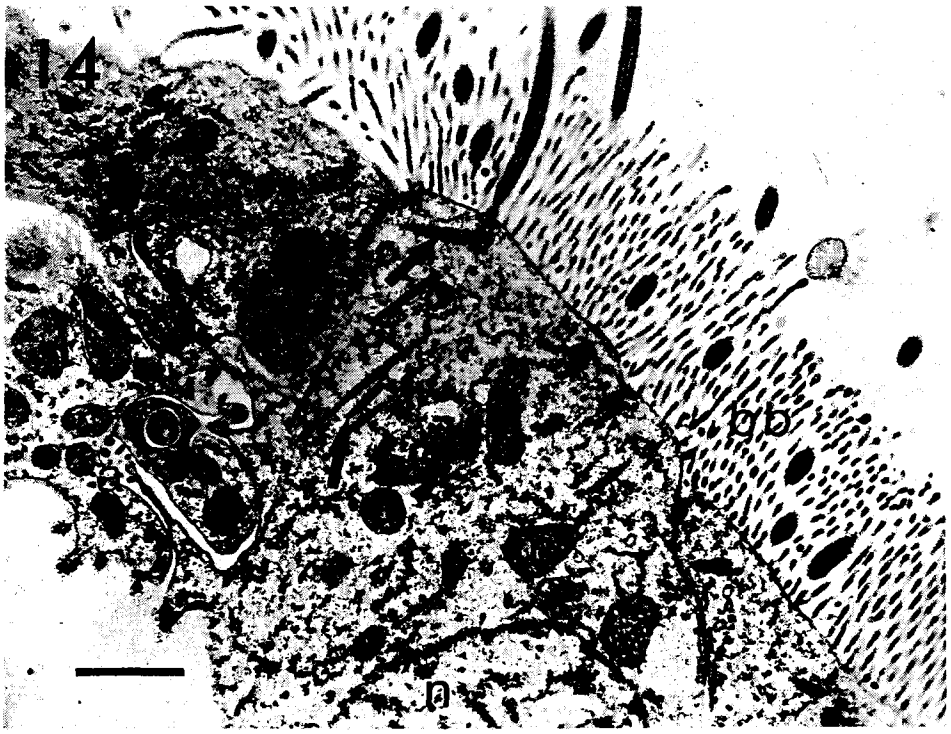



Fig. 14. Section of the anterior part of the intestine. a – axonal tract, bb – fringed border, m – mitochondrion, n – nucleus, arrows – ciliar rootlets. Scale bar: 1 μm

and contain numerous mitochondria in their cytoplasm. The middle part of the intestine consists of flattened cells with short mace-like microvilli (Fig. 15). Such cells bear few cilia. There are rounded electron-light cavities between the cell bodies. In the pre-anal part of the intestine, the cells have many cilia and have no fringed border (Fig. 16). On their surface, only rare, unbranched microvilli are visible. Muscle cells are not found.

In the cardiac and pyloric parts of the stomach, and in the preanal part of the intestine, peculiarly structured cilia can be seen (Fig. 17, 18). Their distal end is greatly widened and the axoneme is spirally curved, forming one or two loops. Hence, the cross section gives an impression of cilia with two-four axonemes.

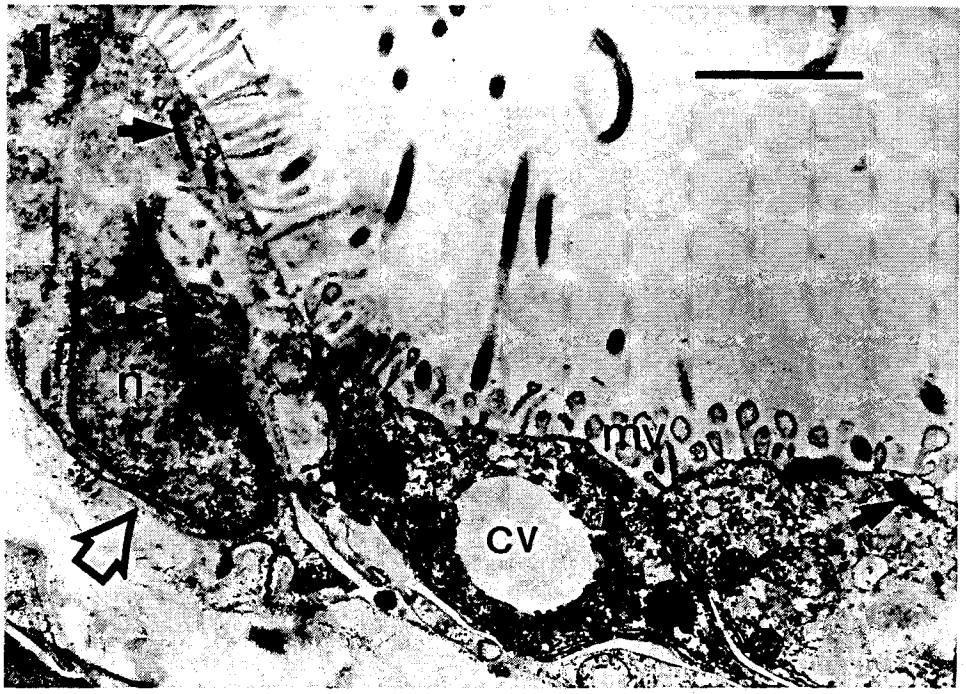



Fig. 15. Section of the middle part of the intestine. cv – cavity, mv – microvilli, n – nucleus, black arrows show ciliary rootlets, black-bordered white arrow shows basal lamina. Scale bar: 1 μm

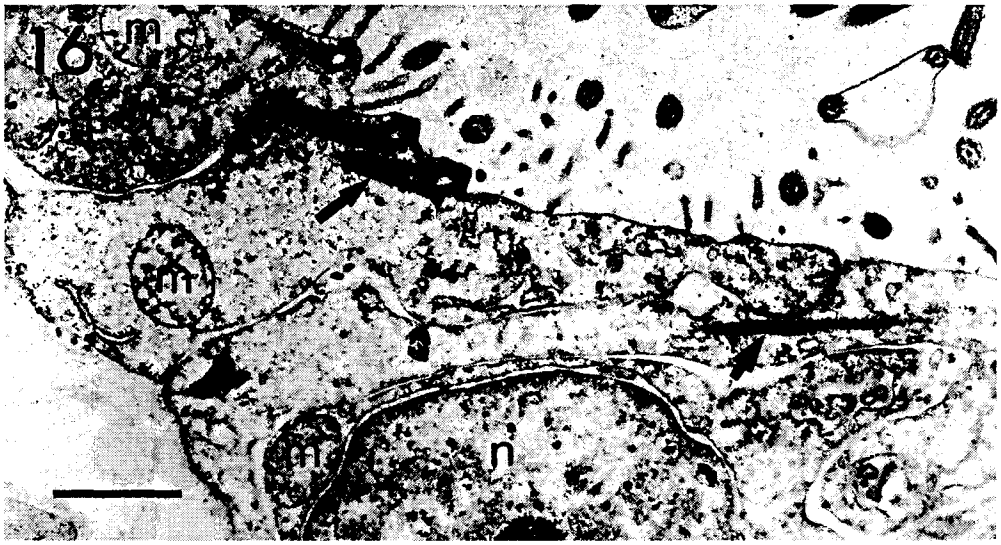
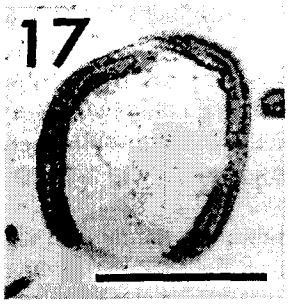



Fig. 16. Section of the pre-anal part of the intestine. m – mitochondria, n – nucleus, arrows show ciliar rootlets. Scale bar: 1 μm



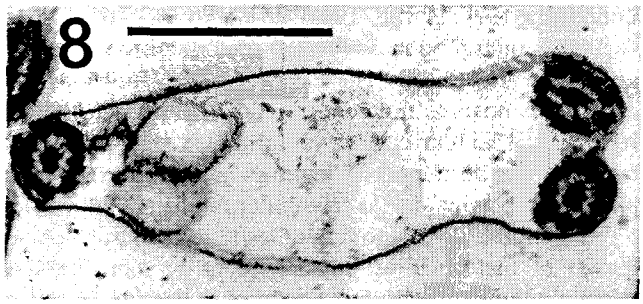




Fig. 17. Tangental section of the cilium with curved axoneme. Scale bar: 1.5 μm
Fig. 18. Cross-section of the cilium with curved axoneme. Scale bar: 0.5 μm

## DISCUSSION

The feeding process of tornaria includes the collecting of particles in the oral cavity, their passage down the esophagus, formation of a food-bolus by mucus, its transition to the stomach where digestion takes place, rejection of the undigested remains into the posterior intestine and excretion through the anus (Strathmann & Bonar, 1976). These processes are mediated predominantly by the ciliar activity of epithelial cells of the digestive tract – unlike the echinoderm larvae where the main role in feeding is done by contractions of the ring-muscles of the esophagus and sphincters (Strathmann, 1971). Food particles are rejected from the esophagus mainly by ciliary pulsations rather than by reversal peristaltic of the esophageal muscles, as in the echinoderm larvae.

This fully agrees with our data, i.e. the discovery of regions with powerful ciliation in the digestive tract of tornaria – more powerful than in the echinoderm larvae. An especially high number of cilia is found near the cardiac and pyloric sphincters and in the pre-anal part of intestine. At the same time, muscles of the digestive tract are not well-developed in tornaria. Single muscle-cells are found only in esophageal epithelium.

The cilia with spirally curved axonemes found in tornaria are similar to discocilia first described in polychaete larvae *Lanice conchilega* (Heimler, 1978). In discocilia, the axoneme is spirally curved distally and the disc-shaped widening forms terminally. It is supposed that discocilia are modified kinocilia involved in causing strong water displacement during swimming of the larvae (Heimler, 1978). However, Ehlers & Ehlers (1978) decided that discocilia of epidermal cells of marine Turbellaria may not be a genuine structure, and are an artifact of fixation.

The cells bearing many cilia are mainly (if not exclusively) found in the digestive tract of tornaria. Perhaps more developed muscles in the digestive tract of the echinoderm larvae are related to the presence of only monociliated cells, i.e. the impossibility of ciliary fields with sufficient effectiveness developing in it; so that the translocation of food particles along the digestive tract is provided for by ciliary pulsations.

Single cells of the esophageal epithelium in tornaria are ultrastructurally similar to those in the echinoid larvae. So the cells with vacuolized apical processes in the cardiac sphincter in the tornaria resemble the epithelial cells of the lower part of the esophagus in the echinopluteus of *Dendraster excentricus* and *Psammechinus miliaris* (Ryberg &

Lundgren, 1975; Burke, 1981). In both cases, the long processes are filled with coarse vacuoles containing loose fibrillar material directed towards the esophagus lumen. It was suggested that these cells have secretory functions, considering that this part of the esophagus ensures the collection of food particles and formation of a bolus by mucus. Apparently, similar cells in tornaria also perform secretory functions, but are localized only near the border between the esophagus and the stomach, rather than along the main part of the esophagus (as in echinopluteus).

The finding of neuronal bodies in the ventral stomach epithelium conforms with the results of histochemical investigations of monoamine-containing neurons in tornaria (Nezlin & Dautov, 1988). A group of such cells in the region of the ventral part of the esophagus has been found. Evidently the neuronal bodies are situated in the epithelium and their processes run along the body of tornaria between the epithelium and basal lamina.

In echinopluteus, food particles remain for a long time in the lower part of the esophagus where the bolus is forming. Then the cardiac sphincter opens and the bolus passes to the stomach (Strathmann, 1971). In tornaria, muscle cells have not been found in the cardium region. Moreover, it is known that food particles do not remain for long in the esophagus of tornaria (Strathmann & Bonar, 1976). It can be supposed that the cell processes provided in the sphincter lumen, and almost partitioning it, contain many vesicles, and that these ensure the adhesion of food particles by mucus and their transition in doses to the stomach, thus compensating for the absence of muscle sphincter.

Three well-defined types of cells were found in the stomach epithelium. The type 1 cells with electron-dense cytoplasm and fringed border on their surface probably consume and store nutrients cleaved by enzymes. The type 2 and 3 cells with light cytoplasm, well-developed rough endoplasmic reticulum and numerous vesicles, apparently fulfil secretory functions. Similar cells having secretory functions are found in the echinopluteus stomach, but they are not distributed all over the stomach epithelium and are concentrated in its anterior part. Besides, these cells in echinopluteus (in contrast to tornaria) appear not to have cilia (Burke, 1981).

In the region of the pyloric sphincter in tornaria, muscle-cells were not found. However, there is a band of cells bearing many elongated cilia which are directed towards the intestine lumen. Apparently these ciliated cells play the role of the muscle sphincter.

At the border between the stomach and the intestine epithelial, cells are almost lacking in microvilli but the cells of the posterior intestine wall bear well-developed microvilli. In the anterior part of the intestine, microvilli form a well-developed fringed border. In the middle part of the intestine, cells bear less microvilli and are shorter, but these cells are highly specialized. They have well-developed rough endoplasmic reticulum and Golgi complex. In these cells, coarse electron-light vacuoles and regions which lack any organelles are found. Similar regions were described in the echinopluteus stomach and they are considered to be the remains of consumed algal cells (Burke, 1981). Thus, the tornaria intestine apparently takes part in food consumption in contrast to echinopluteus of *Dendraster excentricus* in which the intestinal epithelium is composed of unspecialized cells which lack microvilli, well-developed endoplasmic reticulum and Golgi complex (Burke, 1981).

The data obtained demonstrate that the ultrastructure of the digestive tracts of tornaria and echinoderm larvae are essentially different. First of all, it is the presence of multiciliated cells, lack of muscles in the main part of the digestive tract, and the structure of the intestinal epithelium in tornaria.

Earlier, we investigated the ultrastructure of the nervous system of tornaria and the topography of catecholamine-containing cells in tornaria (Nezlin & Dautov, 1988; Dautov & Nezlin, 1992). Comparison of these data with the information obtained from literature also demonstrates essential differences. For example, in the echinoderm larvae, monoamine-containing cells are located all over the ciliary band epithelium, whereas in tornaria they are concentrated in the aboral and esophageal ganglia (Nezlin et al., 1984; Bisgrove & Burke, 1987).

Our data confirm the suggestion that the similarity of embryonic and larval development of the echinoderms and enteropneusts bears convergent features.

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