K. K. Akhmetov et al /J. Pharm. Sci. & Res. Vol. 10(8), 2018, 2037-2040

ISSN:0975-1459
Journal of Pharmaceutical Sciences and Research
www.jpsr.pharmainfo.in

Ultrastructural Study of the Morphology and Functions of the Digestive System in Trematodes Azygia lucii (Muller, 1776)

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Abstract

This article discusses morphological traits of the trematodes' digestive system. Studying semifine slices of helminth throat has shown that its inner surface is lined with highly thinned elongation of the outer tegumental layer, and is essentially a thin layer of syncytium, which, unlike integuments, has no nuclei. The materials also describe in more detail the methods used by the authors. The trematode's digestion process starts in the digestive cavity, and involves secretions of the parapharyngeal glands and electron-dense secretory granules of the intestine.

Keywords: *Azygia lucii*, electron microscopy, tegument, functional morphology of flukes.

INTRODUCTION

Functional and morphological studies are focused on studying organisms as integral systems of organ-related, tissue-related, and cell-related adaptations that correspond to certain living conditions. Traditionally, such studies are made on free-living organisms. Functional morphology of parasitic animals has been the least studied. Particular interest was raised by the studies of the parasite and the host organism direct contact zones, including representatives of the class of trematodes. Many researchers consider the digestive system of trematodes as one of the morpho-functional complexes of adaptations to the parasitic lifestyle [1; 2; 3; 4; 5]. Various conditions for trematodes' existence in the organism of the ultimate host undoubtedly contribute to the formation of certain morphological features that may be characteristic of the species or the taxa of higher rank. In trematodes, the processes of feeding through the digestive system coexist with tegumentary processes, therefore, there are issues associated with correct interpretation of this phenomenon.

MATERIALS AND METHODS

For ultrastructural studies, trematode *Azygia lucii* (Muller, 1776), family *Azygiidae* (Odhner, 1911) was taken, which was parasitizing in the stomachs of pikes (*Esoxlucii*).

The ultrastructure was studied using the method of transmission electron microscopy. Ultrafine slices were prepared according to the method of B. Weekly [6].

For this purpose, the tissue was fixed in a 1.5-2.5% solution of gluteraldehyde buffered with 0.1 M cacodylate buffer (pH 7.4) for 2 hours at 4^0C. Then, it was rinsed twice with cacodylate buffer (pH 7.4) for 10-15 min., and post-fixed in 1% solution of osmium tetroxide (in 0.1 M cacodylate buffer) for 2 h, followed by washing twice in cacodylate buffer (10-15 min each time). Then the material was dehydrated in ethyl alcohols with increasing concentration: 50% alcohol for 15-20 min, 70% overnight, then 80%, 90%, 96% – 15-20 min each, in absolute alcohol or acetone for 20-30 min twice.

Dehydrated preparations were placed into a mixture of epon-araldite resin. For this purpose, a mixture of resins with the following ratio was prepared:

Epon 812 – 4 g,
Araldite 502 – 2 g,
Epon DDSA – 9 g, and
DMP-30 catalyst – 8 drops.

The preparations were impregnated according to the following scheme:

mixture of resins: absolute acetone 1:3 – 4 hours;
mixture of resins: absolute acetone 1:1 – 4 hours;
mixture of resins: absolute acetone 3:1 – 4 hours;
mixture of resins - 12 to 24 hours; and
a new mixture of resins in other vessel – 12 to 24 hours.

After that, the preparations were transferred to a fresh mixture of resins for polymerization. Polymerization lasted for 1.5–2 days at 60^0C.

The 60-100 nm thick ultrafine slices were prepared on Ultrotome III (LKB, Sweden). The obtained slices were placed on the formvar-coated substrate grid and contrasted in a 2% solution of uranyl acetate on 50% ethanol (for 10-20 min at 37^0C) and lead citrate (for 3 to 10 min at room temperature) according to E. Reynolds. The obtained preparations were viewed in electronic microscope JEM-100 CXII (JEOL, Japan) with the aperture diaphragm of 25-30 microns under accelerating voltage of 80 kV.

RESULTS

General morphology of the digestive system

The general structural organization of trematodes' digestive system corresponds to the same of most flukes. The system begins with the mouth opening in the center of the oral sucker, followed by the pharynx, and a small, compared to the size of the body, esophagus, which ends with intestinal bifurcation into two digestive branches.

Studying semifine slices of helminth throat has shown that its inner surface is lined with highly thinned elongation of the outer tegumental layer, and is essentially a thin layer of syncytium, which, unlike integuments, has no nuclei (Figure 1).

The walls of the upper part of the pharynx contain developed muscular elements, which are attached to electron-dense layer under the syncytial layer on the opposite side; the muscles are also attached to the electron-dense layer. Based on the above, one can assume that the main function of the forward sections of the pharynx is mechanical compression of food.

The lower half of the trematode's pharynx contains unicellular glands in the layer of the radial muscles of the walls. The glands are large, and have oval-ovate shape on the slices; the nuclei of the glandular cells occupy the basal or central positions, and have rounded shape, their cytoplasm has granular structure. Based on the morphological features of the structure of the lower part of the pharynx, it may be assumed that food particles in this section are for the first time exposed to the secretions of the helminth. At the same time, the food processed with secretions is also shrunk and pushed further into the esophagus by means of reduction of developed longitudinal muscles located on the periphery of the outer part of the pharynx.

For understanding the general morphology and topography of intestinal branches in trematodes, histological specimens were prepared and examined at the light-optical level. By the results of

the research it has been found that the intestine twists and does not show any pattern with regard to confinement to a certain plane.

The histological specimens that were studied using a light microscope did not show any peculiarities of the intestinal epithelium organization, thus, it cannot be attributed unequivocally to the cellular or the syncytial structure.

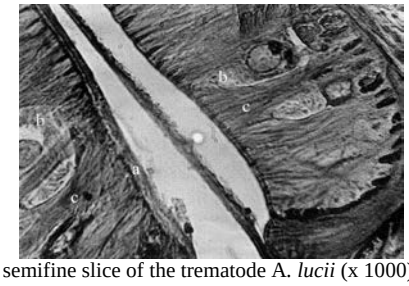

Figure 1. A semifine slice of the trematode A. *lucii* (x 1000) pharynx: *a* - tegumental lining of the pharynx; *b* - unicellular glands in the wall of the pharynx; *c* - pharynx muscles.

Intestinal ultrastructure of the *Azygia lucii* trematodes

Studying the X-ray diffraction pattern of trematodes' intestinal has shown that epithelium of the intestine is syncytial along its entire length. The syncytial structure of the epithelium is characteristic of parts of the digestive system, which includes the pharynx (Lat), the bifurcation (Lat) of the intestines, and the intestine branch itself. The apical membrane of the intestine has one layer; it continuously covers straight sections of the luminal surface without microvilli and the body of the microvilli (microfibers) itself. Microvilli in the sections with few or no food particles in the gap have smaller height, compared to the intestine sections with microvilli that contain food.

Glycocalyx of the apical membrane of the intestinal syncytium morphologically varies in various areas of the epithelium. In the area of the intestines with shorter microvilli in the intestine without feed, glycocalyx is weakly manifested, and its fringed structure in only distal sections is more visible. A pronounced glycocalyx is present on the surface of the microvilli in the sections of the intestine with feed. In these areas, microvilli appear to be more mobile structures. Morphologically, mobility of microvilli may be assessed by their participation in the formation of anastomotic interactions. Anastomotic are the distal sections of the microvilli, forming surface digestive vacuoles, where the process of surface above-membrane digestion apparently starts. The process of anastomosing probably includes two or more adjacent microfibers. The bodies of microvilli that participate in the anastomosis contain granular electron-dense material.

On the surface of anastomosing microvilli, the height of the glycocalyx greatly increases, and it forms visible mucoid "fringe"; it is apparently a characteristic functional feature associated with the activation of digestive activities.

Morphological symptoms of functional substances that ensure the process of digestion in the syncytial intestine of helminths are associated with secretory granules, which are mainly located in the apical part of the syncytium, in the layer located under the apical membrane of the syncytium. In syncytium of the intestines, two types of secretory granules with different electron density are found. The first type of secretory granules mainly has electron density higher than moderate, but not too high; granules of this type contain scarce electron-dense granules.

The same layer contains granules of the second type with high electron density, which makes them different from granules of the first type. Studying X-ray diffraction patterns allows talking about electron-dense secretory granules that make one third of all secretory granules, their accumulations are rather local, and only affect individual sections; these granules are localized on the microvilli that do not anastomose between themselves. Secretory granules of both types are covered with membranes.

Apparently, secretory granules of both types are destroyed, and release the contents into the apical layer of syncytium cytoplasm, or upon contact with the apical membrane of the intestine; after that, the material contained in the granules enters the composition of the axial part of the microvilli; electron-dense grains of secretory granules of the first type become part of the morphologically active anastomosing microvilli without being destroyed. Secretory granules of the second type (electron dense) are destroyed and egest their content into the composition of the microvilli that are not anastomosing. In the opinion of the authors, electron-dense granular material in the axial part of the described microvilli that forms a continuous "chain" comes from electron-dense secretory granules and is the morphological symptom of their operation. Electron-dense secretory granules (granules of the second type) are concentrated directly in the base of the described microvilli.

Based on the fact that secretory granules are not egested undestroyed to the luminal surface of the intestinal syncytium, one can assume that they are destroyed upon direct contact with the apical membrane during exocytosis.

In the opinion of the authors, the morphological difference between secretory granules in terms of electron density is associated with functional difference. Electron-moderate and electron-dense secretory granules have round shape on many electron-diffraction photographs made on slices in various planes; hence it may be asserted that their true configuration is spherical.

Emergence of secretory granules of both types is associated with well-developed granular endoplastic apparatus (GEA) that is present over the entire thickness of the syncytium. Synthesis of secretory granules with moderate electron density is started in the GEA of basal layers of syncytium.

Cisternae located in the basal layers of syncytium intestinal cytoplasm are surrounded by a developed system of GEA channels. Initially, cisternae contain electron-light material, after which they are filled with a substance with low electron density.

The content of the cisternae of this type is more or less homogeneous. Later, the material in the cisternae is differentiated, which is manifested by the fact that the material becomes more electron-dense in one section of the cisterna. Section of the cisterna with thickening material is separated, and a layer with very low electron density, almost electron-light, is formed at the periphery of the thickening material. Next, with increasing electron density of the material the secretory granules are formed from, and formation of their boundaries, cisternae migrate into the median layers of the cytoplasm of the syncytium. Secretory granules of both described types are egested closer to the apical layers of the intestinal wall cytoplasm. "Ripe" secretory granules are localized in one or two rows so that only a very thin layer of cytoplasm remains between the secretory granules and the apical membrane of the intestinal syncytium. "Ripening" of secretory granules is apparently related to the cisternae of the Golgi's apparatus.

In X-ray diffraction patterns of the intestinal walls of the studied species of helminth, one can see that the endoplasmic reticulum is associated with cisternae of the Golgi's apparatus. The motion vector of the secretory granules, as they are "ripening", is directed from the basal part towards the apical layers of the syncytium. Secretory granules, remaining undestroyed, are localized in the layer of cytoplasm adjacent to the apical membrane of the syncytium, and are mainly concentrated in the areas adjacent to microvilli, which form anastomoses. Anastomosed microvilli do not have developed "fringe" of glycocalyx on the surface. An ultrastructural feature of microvilli internal morphology is the fact that they include rare electron-dense granular material.

Intestinal syncytia contain nuclei of elongated shape. The long axis of the nuclei is oriented parallel to the length of the intestine. Most often, the nuclei have irregular shape.

The karyolemma on X-ray diffraction patterns is well differentiated and looks double. Nucleochylema contains 2 to 4 condensed chromatins. Nucleochylema is rich in electron-dense granular material.

The basal membrane of gastrodermis is smooth or has a smooth undulating curvature. In the ultrastructural images, the membrane is double. Channels of both smooth and rough endoplasmic reticula come close to it.

Analysis of the obtained X-ray diffraction patterns allows stating that the basal membrane of the intestine does not show the presence of “basal labyrinths”.

The basal plate of the intestine is very thin; sometimes it almost merges with the basal membrane of the intestinal syncytium. Muscle fibers with associated mitochondria are directly adjacent to the basal plate. The complex of muscle fibers and mitochondria is surrounded by the membrane. X-ray diffraction patterns show the presence of more than two mitochondria in the complexes.

DISCUSSION

The digestive system of trematodes *A. lucii* has well-known topography of the structure and consists of the mouth opening that is located on the bottom of the oral sucker, the pharynx, the front non-branching part of the intestine, a bifurcation, and two blind branches of the intestine.

Walls of the cavity of the sucker and the pharynx are the continuation of the epithelial tissue of the helminth and are presented by a typical tegument; similar facts have been noted in the literature for other types of *Cotylophoron cotylophorum* [7], *Brachylaemus aequans* and *B. fuscatus* [8] helminths. The well-developed muscles of the front sections of the digestive system in the studied trematodes are due to participation in helminth fixation to the wall of the organ of localization - stomach of predatory fish (pike), since stomach serves mainly for storing food before it is accepted by the intestines, and for physical processing of food and initial chemical exposure. The conditions for trematodes in the localization organ are periodically complicated by the fact that the host procures food irregularly, large portions are supplied at once, and should be stored until they move into the intestine. Pike stomach has well-developed muscles, the inner surface of the stomach is highly folded and studded with mucous cells; there are also immersed glands that secrete digestive enzymes. In the opinion of the authors, the foregoing substantiates the presence of the tegumental lining in the front-most sections of the digestive system of the helminth. The chemical effect associated with the glands of the host's stomach has the same effect on the lining of the oral sucker and the pharynx, and on the fluke's skin coverings. Apparently, the mechanisms of tegument that protect the parasite from chemical, immunological and mechanical stress of the host, fully “work” in the front digestive system of trematodes as well.

Unicellular glands in the wall of trematode's pharynx, with the morphological characteristics inherent to glandular structures, may indicate secretion of substances into the pharynx cavity. Secretions of the trematode pharynx glands affect integuments and deeper tissues of the host stomach lining. Therefore, secretions of pharynx glands are the first agents that trigger cavity digestion in helminths. The well-developed muscles of the pharynx may be a symptom of not only a single act of suction in a specific place of localization, but also of participation in the processes of multiple swallowing of the host's intestinal walls lysed by the enzymes of trematodes’ pharynx glands. The front sections of the alimentary system of the helminth start cavitary digestion in the intestine and are its markers. Apart from the syncytial tegumental lining in the oral sucker and pharynx cavity, the discussed part of the digestive system also has cellular structures which are glandular cells in the wall of the trematode's pharynx. Chemically processed tissues of the host's walls, and partially lysed blood elements get into the cavity of the intestines of helminths first.

The wall of the intestine of the *A. lucii* trematode has syncytial organization, which has been confirmed by many X-ray diffraction patterns obtained in studying the ultrastructure. According to literature sources, the problem of morphological organization of trematodes’ intestine has not been definitely solved [9]. The syncytial form of gastrodermis organization has been described for a number of species of flukes that belong to various systematic groups and gostal confinedness (locations), and hence ecological confinedness of the helminth itself: *Schistosoma mansoni*, fam. *Schistosoma tidae* [10]; *Gorgoderina orientalis*, fam. *Gorgoderidae* [11]; *Paramphistomum microbotrium*, fam. *Paramphistomatidae* [12] *Paragonimus westermani*, fam. *Troglotrematidae* [13] *Schisthogonimus rarus*, fam. *Prosthogonimidae*, *Apharingostrigea cornu*, fam. *Strigeidae* [14]. Cellular epithelium of the intestine has also been detected in trematodes of various systematic and ecological groups: *G. attenuata* [15]; *Fasciola hepatica Fasciolidae* [16]; *Gorgodera amplicava* fam. *Gorgoderidae* [17] and others.

The presence of glandular cells in the walls of the pharynx of the studied trematode appears to be associated with secretion of substances involved in decomposition of substances coming from the oral cavity, since the intestinal cavity of the trematode contains partially lysed tissues, and little elements of the host's blood. The latter fact may indicate that blood cells of the host are random components of the feed. Acid phosphatase activity was detected in the intestine and apical membrane of the intestine of fluke *A. lucii* [18]. In our opinion, the appearance of this enzyme is also due to peripharyngeal glands and is an evidence of cavitary digestion. The presence of morphologically developed glycocalyx and acid phosphatase in the apical membrane suggests that the products of the digestive cavity are further splitting due to membrane. In another trematode of genus *Azygia* (A. robusta), Nacheva [19] identified the type of digestion that she called parietal digestion. Apparently, this refers to membrane [20, 21] digestion.

The apical surface of the intestinal epithelium of the trematode, being the well-developed set of cytoplasmic outgrowths of microvilli, is the evidence of its activity. According to the opinion of Fujino, Ishii, the shape of trematodes’ intestinal microvilli does not depend on the host and on the helminth localization; at the same time, he suggested that this might be related to the systematic position of the parasite, in particular, with the taxa of higher rank than species or genus [13].

Ultrastructure of the intestinal syncytium cytoplasm indicates that it is physiologically active in the process of secretions synthesis, as evidenced by numerous GEA channels; the channels often form extensions; cisternae of the Golgi's apparatus are also present. By their morphological properties, by their ability to let through electron beams, the synthesized secretions are divided into two types: the first type includes electron-medium secretory granules; the second type includes electron-dense secretory granules. Previously, Davis, Bogitsh, Liferea, and Shaimardanov also distinguished two types of secretions in the composition of the intestinal walls of other trematodes. Evidently, after secretion, electron-moderate secretory granules supply substances that penetrate anastomosing microvilli, and their secret may penetrate digestive vacuoles formed on anastomoses, on the apical membrane of gastrodermis, and on the glycocalyx layer. Secretory granules of the second type, in the opinion of the authors, excrete the content into the apical membrane layer and the intestinal lumen [22].

Since secretory granules of both types are restricted by the membrane, and their ultrastructural properties are represented by an amorphous substance, they may be referred to one of lysosome types. This opinion is also indirectly confirmed for other species by the studies of Davis, Bogitsh, and Akhmetov.

Determining the type of secretion of the contents of secretory granules is important. During the electron microscopic research, the process of excreting the content into the apical membrane and the intestinal cavity was not observed. On this basis, it has been assumed that the process of exocytosis of the secretions contained in secretory granules of the trematode takes very short time, and occurs instantly, so it has been impossible to obtain the X-ray diffraction patterns that would record directly the moment of exocytosis. The above is consistent with the data obtained by K. De Duve. Exocytosis of the secretory granules' contents occurring in the studied species of trematodes has the merocrine type of secretion.

The digestive vacuoles formed by anastomoses of the microvilli in gastrodermis are indicators of intracellular digestion type.

Based on the study of the ultrastructure of gastrodermis in the intestinal of trematode *A. lucii* from pike stomach, indicators of recessed, membrane and intracellular digestion have been differentiated. This may be due to the fact that the parasitizing helminth feeds on the tissues of the inner surface of the host's stomach; blood may partly get into the feed. The feed of the host in the place of parasite's localization does not serve as food for the trematode.

Analysis of X-ray diffraction patterns of the intestinal walls of trematodes indicates that the basal plate of the intestine is not developed. Therefore, filling the intestines and nutrients' movement in the intestinal cavity are mainly associated with the movement of microvilli of gastrodermis, and active process of "pumping" food performed by developed muscles of trematode's pharynx. In our opinion, the lack of developed basal plate is the functional basis for mechanical activity of pharynx walls, and this provides nutrition of the parasite. According to Rees, Williams, Reynolds, well-developed basal plates provide mechanical stability to the structures where they are present, and thus, in different degrees may be obstacles to changing the shape of the organs and their relative size [23; 24]. The poor development of the basal plate characterizes functional ability to actively change the shape and size of the intestinal wall lumen in course of filling with feed or excreting undigested feed residues.

CONCLUSION

As a result of the electron microscopic study of trematodes *Azygia lucii* (family Azygiidae) digestive system, it has been found that the helminth, after localizing in the stomach of predatory fish (pikes, perches), feeds on the tissues of the intestinal walls, rather than on the contents of host's stomach. The muscular wall of the pharynx plays an important role in the feeding process and food movement along the intestines of the helminth.

The trematode's digestion process starts in the digestive cavity and involves secretions of the parapharyngeal glands and electron-dense secretory granules of the intestine. High-molecular compounds are further digested on the membranes by the membrane type digestion; intracellular digestion is also present.

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