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The Ultrastructure of Muscular Cells of the Body Musculature of the Trematode *Dyplostomum huronense* (La rue, 1927).

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ABSTRACT

The issue of the ultrastructure of muscular cells of the trematode *Dyplostomum huronense's* (La rue, 1927) body is considered in the article. The work is devoted to the detailed analysis of the trematodes' organs of localization, study of the electron microscopic peculiarities of the muscular system of the trematode from the suborder Strigeata [1], in particular. Typical peculiarities of the trematode *Dyplostomum huronense's* [2] body musculature, separate organs and systems are marked out and described by the author. The electron microscopic researches of the trematode *Dyplostomum huronense's* body musculature discovered the fact that muscles of different layers have common traits of the ultrastructure.

Keywords: Ultrastructure, Circular Musculature, Longitudinal Musculature, Parenchymal Musculature, Protofibrils, Fibrous Elements.

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INTRODUCTION

Significant attention was paid to the study of spatial organization of the trematodes' muscular system in works of different authors [3-5]. In this regard M.V. Yastrebov and I.V. Yastrebova's monographic composition [6] is analytical comprehension and systematization of all researches on anatomy and morphology of the flukes' muscular system. Issues on the ultrastructure of the muscular elements and the muscular system as a whole have been an insufficiently explored problem tillthepresent moment. The given work is devoted to the study of electronic microscopic peculiarities of the muscular system of the trematode from the suborder *Strigeata* [1].

MATERIALS AND METHODS

Adult trematodes *Dyplostomum huronense* (La Rue, 1927), *Diplostomatidae* (Poirier, 1886) were gathered from the intestine of a herring gull (*Larusargentatus*). Examination of the ultrastructure was conducted according to the method of transmission electronic microscopy [7]. Ultrathin sections were prepared on B. Weekly's system [8].

For that the taken tissue was fixed in 1.5-2.5 % Glutaraldehyde solution, buffered by 0.1M Cacodylate buffer (pH 7.4), within 2 hours at a temperature of 4 ^{0}C. Next the tissue was flushed by Cacodylate buffer (pH 7.4) twice for 10-15 minutes each time, and later it was afterfixed in 1% Osmium Tetroxide solution (on 0.1M Cacodylate buffer) during 2 hours with following double flushing by Cacodylate buffer (for 10-15 minutes). Then material was dehydrated in the ethyl alcohols of ascending concentration: in 50% ethanol – within 15-20 minutes, in 70% ethanol – during a night, later in 80%, 90%, 96% ethanol - for 15-20 minutes in each one, in absolute alcohol or aceton – for 20-30 minutes twice.

The dehydrated specimens were putintoa mixture of Epon Araldite resins. For that the mixture of resins was prepared in the following proportions [6]:

- Epon 812 – 4 g;
- Araldite 502 – 2 g;
- Epon DDSA – 9 g;
- Catalyst DMP-30 – 8 drips.

Soakage of the specimens was accomplished in accordance with 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 – from 12 to 24 hours;
- new mixture of resins in other glassware – from 12 to 24 hours.

Next the specimens were put into a fresh mixture of resins for polymerization. The polymerization was conducted within 1.5-2 days at a temperature of 60 ^{0}C.

The ultrathin sections 60-100 nanometers thick were prepared on "Ultrotome III" ("LKB", Sweden).The derived sections were applied on net-substrates with a formvar film-substrate and were contrasted with 2% Uranyl Acetate solution on 50% ethanol (for 10-20 minutes at a temperature of 37 ^{0}C) and Plumbum Citrate (at a room temperature for 3-10 minutes) on the instructions of E. Reynolds [9]. The derived specimens were examined in theelectron microscope "JEM-100 CXII" ("JEOL", Japan) with theaperture diaphragm of 25-30 microns under theaccelerating voltage of 80 kilovolt.

RESULTS

Circular musculature

Circular muscular fibers, the most external ones, are immersed in the depth of a layer consisting of collagenous fibers, spread under the basal membrane of the trematode's tegument. The basal lamina of the

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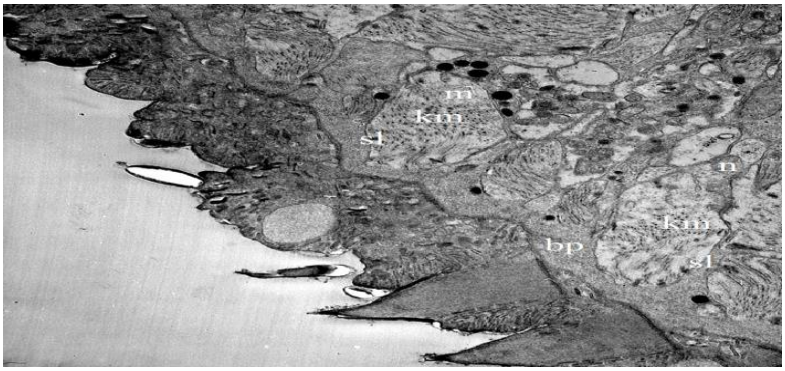
trematode's tegument is a layer containing circular muscles. The collagenous fibers are oriented in different directions; therefore in general the basal lamina has an electron density, which may be characterized as a higher than moderate. Judging from the density of the basal lamina of the tegument, it can be said that this structure is firm and participates in imparting elasticity to the helminth's body. Both single glycogenous granules and their linear clusters are registered in the layer of the collagenous fibers, enclosing the circular muscles (Picture 1).

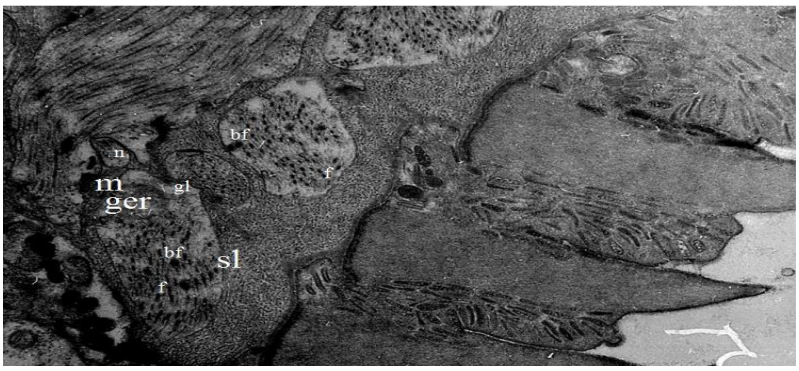
Picture 1: "The circular musculature of the trematode *Dyplostomum huronense*".



km - circular musculature; sl - sarcolemma; n - nucleus; bp - basal lamina of tegument; m - mitochondrion.

The morphological peculiarity of localization of the layer of the circular musculature in the composition of the basal lamina is the fact that the layer of the collagenous fibers between the basal membrane of the tegument and the sarcolemma is larger than thedepth of the layer to the sarcolemma of elements of the longitudinal musculature. The thickness of the layer of the collagenous fibers, situated between the sarcolemma of the circular fibers and the basal membrane of the tegument, is bigger than the thickness of the layer of fibers between the sarcolemmas of the circular and the longitudinal muscles in 5-6 times (Picture 1).

Picture 2: "The circular musculature of the trematode *Dyplostomum huronense*".



ger - granular endoplasmic reticulum; sl - sarcolemma; n - nucleus; bp - basal lamina of tegument; gl - glycogenous granules; bf - large protofibril; f - fibril; m - mitochondrion.

The layer of the circular musculature near the tegument with well-developed integument spinelets is situated as uniform bundles, which are distant from each other in the same manner. All bundles are bounded by thewell-differentiable sarcolemma of a moderate electron density. The sarcolemma tightly contacts with the basal lamina, this communication is held due to strong conformity of the collagenous fibers in the composition ofthe lamina to the sarcolemma and due to numerous hemidesmosomes. Mostly, form of the muscular fibers on a transverse section is ovoid-oval. The nuclei of the muscular cells are situated at the periphery of a cell. The mitochondria on the ultrastructural sections are also localized at the periphery of fibers; in each of them 2-3 mitochondria are registered. The endoplasmic reticulum is attached to theperipheral part of the sarcoplasm having a common lucid electronic density. Single ribosomes are present

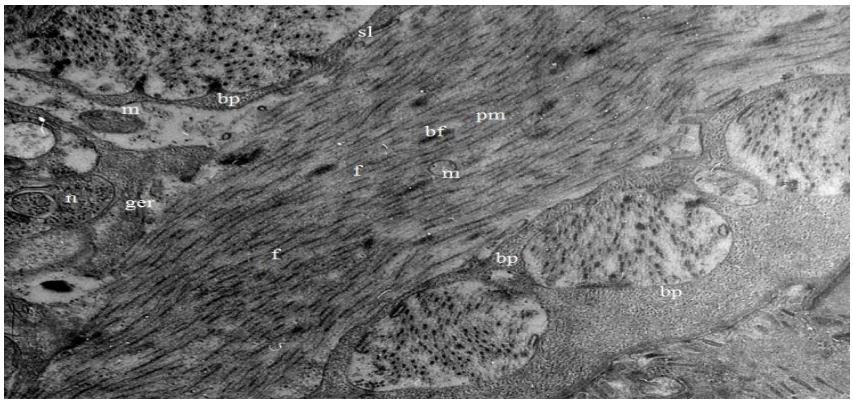
at the periphery of the muscular cells. The bortyoidal clusters of glycogen adjoin the internal surface of the sarcolemma by separate drafts (Picture 2).

The thin protofibrils form a ring of 6-7 fibrils around a larger fibril. In the composition of 1 fiber of the trematode's circular musculature 1-3 muscular cells are marked on the sections. But the peculiarity is the fact that one muscular cell has a larger amount of protofibrils in its own composition. On the electron diffraction patterns, in most cases, amongthe fibers of circular musculature there are protrusions, bounded by the sarcolemma, which mostly does not possess myofibrils, but has canals of the sarcoplasmic reticulum. Here most of the lipidic clusters in the form of homogenous body of a lucid electron density are registered. Sometimes such protrusions with lipidic bodies border immediately with neighboring fibers of the circular musculature.

Longitudinal musculature:

Fibers of the helminth's longitudinal musculature are situated under a complex of fibers of the circular musculature. The sarcolemma of fibers of the longitudinal musculature on the ultrastructural sections is not flat. There is a small layer of densely interwined collagenous fibers of the basal lamina of the trematode's tegument between the sarcolemma of the longitudinal muscular fibers, situated on the external surface, and the sarcolemma of the circular musculature, closely approaching to it.

Numerous connections being accomplished through desmosomes play a special part in attachment to the basal lamina of the tegument. In muscular cells of the longitudinal musculature situated at the periphery between the sarcolemma and protofibrils there are electron lucid areas, where single electron dense granules of glycogen as well as their clusters are present and the sarcoplasmic reticulum is marked, in addition to this, there are the mitochondria at the periphery of fibers in these areas (Picture 3). In the median part of muscular fibers rare mitochondria can be met.



Picture 3: "The longitudinal musculature of the trematode *Dyplostomum huronense"*.

ger - granular endoplasmic reticulum; sl - sarcolemma; n - nucleus; bp - basal lamina of tegument; bf - large protofibril; f - fibril; m - mitochondrion.

The sarcolemma of muscular fibers, which is directed to the median layers also is not flat, turns into canals with electron lucid properties in the lumen. In these canals free ribosomes are marked. In the canals single sufficiently large mitochondria, separate granules and botryoid clusters of glycogene, homogenous lipid rafts are marked. Apparently, these canals provide the substances being consumed in the process of muscular work.

Localization of the nuclei of muscular cells is marked in attaching to the sarcolemma parts of the section. The nuclei are oval-elongate along the axis of fibers. The chromatin in the nuclei is despiralized, the nucleoluses are poorly contrasted. Perhaps, such situation is related to the absence of a vigorous synthetic activity for the synthesis of the actyn-myosin proteinic protofibrils.

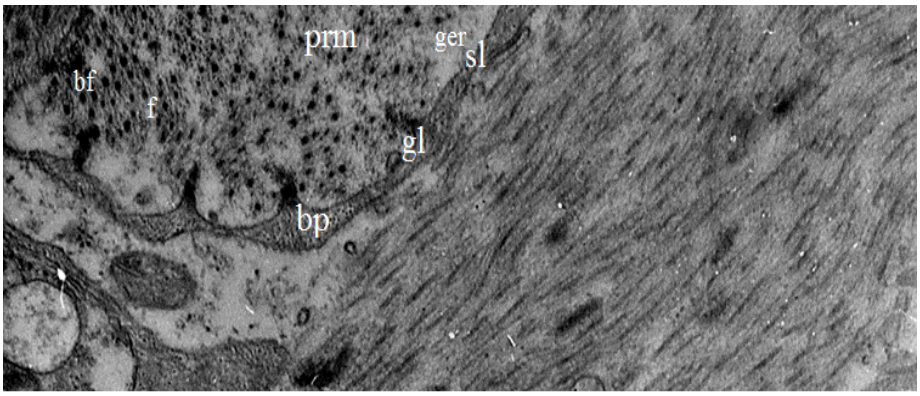
In the nuclear part, at the peripheria of a cell, presence of the rough endoplasmic reticulum is

marked, in bubbles of the non striated endoplasmic reticulum the thin fibrillar material is differentiated (Picture3). Major volume of the sarcoplasma of muscular cells does not contain cellular organoids, except for the fibrillar threads, generally oriented parallel to theaxis of thelongitudinal musculature. In fibers of the longitudinal musculature there are 4-5 fibrils around a larger one.

Parenchymal musculature:

The parenchymal musculature attaching immediately to fibers of thelongitudinal musculature (Picture 4) is separated by a thin layer of theparenchyma possessing fibrous elements. The parenchyme holds a great quantity of electron dense granules of glycogene and single lipid drops. Common morphology of thetransverse section of a muscular fiber consisting of muscular cells on theexternal side is scalloped. Areas of formation of thescalloped hollows in the"mouth" of scallops contain the electron dense material, which obtains electron moderate characteristics with moving away.

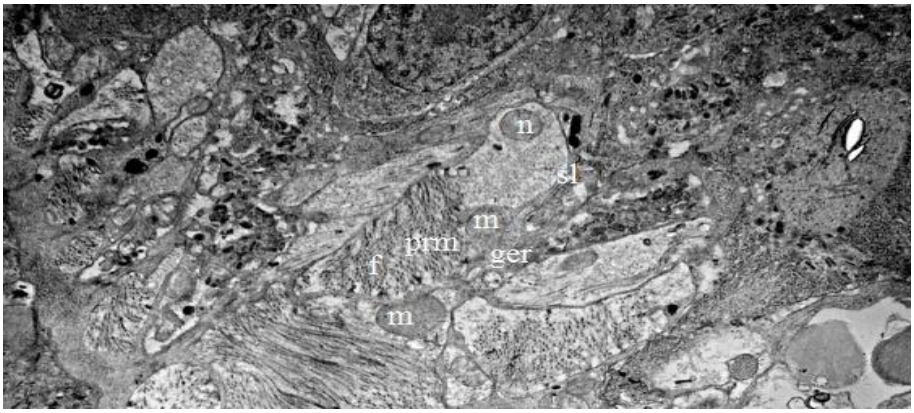
Picture4: "The parenchymal musculature of the trematode *Dyplostomum huronense*".



prm - parenchymal musculature; ger - granular endoplasmic reticulum; sl - sarcolemma; bp - basal lamina of tegument; bf - large protofibril; f - fibril.

Membranes of the sarcolemma form embolies, reminiscent of the pinocytos is embolies, sometimes pass sufficiently deep, making resemblance of canals. In the sarcoplasma of muscular fibers the small canals of the non striated endoplasmic reticulum and the mytochondria, usually concentrating immediately close to the nucleus, are observed. The chromatinic material of the nucleus is decondensated. The hyaloplasm of muscular cells is filled with protofibrils (Picture 5). The mitochondria, localized in thearea in front of thesarcolemma, are large in comparison with sizes of these subcellular structures in thecomposition ofthe circular and thelongitudinal musculature.

Picture 5: "The parenchymal musculature of the trematode *Dyplostomum huronense*".



prm - parenchymal musculature; ger - granular endoplasmic reticulum; sl - sarcolemma; n - nucleus; m - mitochondrion; f - fibril.

It is impossible to mark out large and small protofibrils in the composition of the parenchymal fibers in most of them; inthe composition of single fibers four or less fibers of protofibrils are situated equian gularly around less electron lucent fibers.

CONCLUSIONS

It is common knowledge that conditions of life and existence of the living organisms have an effect on thepeculiarities of organization of all their organs and tissues. For parasitic multicellular animals theadaptive transformations, first of all, concern thezones of immediate contact with thehost's organs. The parasite's organs, which contact with conditions of thehost's organ, in the process of theevolutional adaptive transformations obtained morphological and physiological peculiarities, allowing more thorough assimilating possibilities of habituation. In this regard the trematode's skin and muscular sac, perceiving conditions in theorgans of localization, and these conditions are connected with cavities of thehost's viscera and their physiological functions, correspondingly with the chemism of specialized tissues and thehost's protective functions, play a particular adaptive function. The trematode's musculature, in accordance with theclassic data, consists of theexternal circular, thesubjacent longitudinal layer of muscles, theparenchymal musculature, which may be called as the oblique muscles, here the dorsoventral muscular fibers in the composition of the parenchyme are referred to [3; 4].

It is generally known that the trematodes' in tegumentary tissue is a part of the special complex named as the skin-muscular sac. Structure of the complex includes the integumentary tissue proper, which covers the helminth's body and consists of a special type of the epithelium histologicallly presented by the submerged epithelium and the muscular system [10].

From the classic researches it is known thatthe muscular system in thecomposition of theskin and muscular sac of Plathelminthes is represented by the smooth musculature [4; 11]. In comparison with data of Zh.V Korneva, V.G. Davydov and N.M. Bisserova [12], who studied the ultrastructure of separate groups of muscles of three species of cestodes and discovered in their muscular cells nuclear-containing and contractile parts, we did not ascertain such situation. Perhaps, this is connected with the fact that we examined the body musculature, but not the musculature of the attaching apparatus. The subcellular elements: mitochondria, nets of the granular endoplasmic reticulum are localized at the periphery of cells, immediate close to the plasmalemma. And major volume of cells is occupied with the contractile elements, quantity of the larger center fibrils and the situated around protofibrils is different depending on belonging to a certain layer of muscles.

Discussing peculiarities of the ultrastructure of the trematode's muscular cells, it is possible to talk about its correspondence to descriptions of slow fibers of the nonstriated musculature. As is well known, energy extraction in such fibers is connected with the process of the anaerobic glycolysis – earlier it was marked in researches of F.F. Soprunov [13]. Perhaps, this is connected with the helminth's low-activity, attached existence. In our opinion, in this fact there are common traits typical for muscular cells of certain classes inside the species Plathelminthes, for the classes of trematodes and cestodes, in particular. The above described peculiarities were discovered at the tape worms by R.D. Lumsden and M.B. Hildrecht [11].

The muscular system of trematodes in the composition of the skin and muscular sac is localized beneath the syncytial layer of tegument, more median of the basal membrane. In accordance with our observations, the external layer, the circular fibers and fibers of the longitudinal musculature, in particular, at the studied species of trematodes are closely connected to the basal lamina of tegument. The basal lamina of helminth's tegument is very well developed, about that we can judge by its electron moderate density. Solid mechanical properties of the basal lamina of the tegument are confirmed by the fact that the multidirectional, as if "interwined", collagenous fibers stand out in this layer. The circular muscular fibers are situated in the thickness of the basal lamina. Attention should be paid to the fact that the diameter of all fibers is approximately similar, and they are situated as flat layer and do not leave the thickness of the basal lamina. Fibers of the circularmusculature and fibers of the longitudinal muscular fibers forma functional block. This functional block allows active accomplishing movements of the back part of the helminth's body, diminishing and increasing the diameter of the part; perhaps, such movements provide for the process of "inflation" and filling of the heminth's intestine. The peculiarity of the ultrastructure of the parenchymal musculature, which we studied at the discussed helminth, is the fact that its fibers are sufficiently rare. But its ultrastructural

organization is the evidence of its development. The functional destination of the parenchymal musculature is connected with providing for the circular movements in the complex with contraction of the longitudinal muscles. Such arrangement at certain Plathelmintes was marked in works of M.V. Yastrebov [3], M.V. Yastrebov and I.V. Yastrebova [4]. In accomplishment of the circular movements of the back half of the trematode's body all groups of muscles, which we studied on the level of the electron microscopy, participate. In our opinion, the circular andthe longitudinal layers of the helminth's musculature are connected with the parenchymal fibers through a linking element, which the basal lamina is. The basal lamina of the trematode's tegument is represented by the tightly twisted collagenous threads. Ultrastructural characteristics of the nucleus in fibers of thelongitudinal musculature, perhaps, are theevidence of reduction ofthe synthetic activity of the synthesis of at hectyn and myosin proteins in themuscular cells.

Thus, the electronic microscopy researches of the musculature of the trematode *Dyplostomum huronense's* body tell about the fact that muscles of different layers have common traits of the ultrastructure. Modification peculiarities of the muscular elements of different layers of the helminth's muscles appeared and were formed on the basis of possibilities of a nonstriated muscular cell. At the same time, common is the fact that, the nuclei, the granular endoplasmic reticulum, the mitochondria are localized at the periphery of cells.

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