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## MORPHOMETRY OF THE DIGESTIVE GLAND OF TERRESTRIAL MOLLUSKS *CORNU ASPERSUM* (GASTROPODA, HELICIDAE)

A. M. Tybinka, O. O. Zaitsev, M. V. Zakrevska, H. I. Blishch, O. M. Shchebentovska

Stepan Gzhytskyi National University of Veterinary Medicine and Biotechnologies,  
Pekarska st., 50, Lviv, 79010 Ukraine  
E-mail: a.m.tybinka@gmail.com

**Morphometry of the Digestive Gland of Terrestrial Mollusks *Cornu aspersum* (Gastropoda, Helicidae).** Tybinka, A. M., Zaitsev, O. O., Zakrevska, M. V., Blishch, H. I., Shchebentovska O. M. — Histological and histochemical techniques were used to study specifics of parenchyma and stroma in digestive gland of *Cornu aspersum*. The parenchyma had 28.8 digestive ducts/mm<sup>2</sup> on average (CV = 11.2 %) round-to-oval, with average cross-section of 27 700.9 μm<sup>2</sup> (CV = 28.1 %). The duct area (79.8 % of parenchyma) and the area of inter-duct connective tissue (20.2 % of parenchyma) were in ratio 4 : 1. The gland completely lacked elastic fibers. The muscle elements between the ducts were of different shape and arrangement, on average 59.7 μm long (CV = 42.3 %) and 1.41 μm wide (CV = 63.2 %). The gland capsule was formed by three layers: epithelial, muscular, and the layer of collagen fibers. More than 60 % of the capsule thickness belonged to the muscular layer. The proportion of calcium cells in the digestive duct wall was 15.5 % (CV = 23.5 %). Cells of digestive ducts contained a lot of vacuoles with brown granules (336.8 per 1 mm<sup>2</sup>, CV = 36.1 %). The vacuole area was 2.2 % of all area of digestive gland (CV = 15.8 %). The uneven location of muscle elements and brown granules in the glandular parenchyma indicated the functional features of the individual parts of the digestive gland.

Key words: gastropod mollusks, digestive gland, digestive ducts.

### Introduction

The terrestrial mollusks are represented in Ukraine by a significant number of species (Gural-Sverlova & Gural, 2012; Balashov, 2016). Studies of this group are multidirectional, considering the gastropods as objects of a certain ecosystem (eco-faunistical research, Guiller & Madec, 2010; Balashov, 2012; Balashov et al., 2018), as of anthropogenic pollution levels in the environment (Berger & Dallinger, 1993; Regoli et al., 2006), and as food. Morphologists are mostly interested in their digestive glands of all organ systems, because it is the heaviest part of the gastropods' visceral mass (Barker, 2001), and it is also an organ with complex digestion processes (Bogucki & Helczyk-Kazecka, 1977; Czarnoleski et al., 2008). There are many research approaches; many papers tackle the gland's morpho-physiological features, especially the interspecies comparisons (Sen Gupta,

1977; Devi et al., 1981; Arrighetti et al., 2015). The compared characters usually include the enzymatic activity of glandular cells (Lobo-da-Cunha et al., 2016), changes associated with fasting (Parcel et al., 1996), reaction to various outer factors like the environment's temperature (Naya et al., 2011; Czarnoleski et al., 2016). Biochemical and histochemical indicators of the gland are adversely affected by insecticides (Hamlet et al., 2012) and compounds of metals such as lead, cadmium and copper (Russell et al., 1981; Manzl et al., 2004; Amaral et al., 2004; Mleiki et al., 2015), iron nanoparticles (Besnaci, et al., 2016), and metal dust (Boucenna et al., 2015). The gland is reported to be the animal's main organ of immunity (Röszer, 2014).

We aimed to conduct an advanced morphometric study of the digestive gland's structural parts and establish the specifics of relationship between their relative parameters.

### Material and methods

For the study, 15 terrestrial gastropods of the species *Cornu aspersum* (Müller, 1774), Helicidae (fig. 1), were collected 03.07.2017 in Zyrkuny village of Kharkiv District (Kharkiv Region of Ukraine) (50.08555 N 36.38472 E). The mollusks were grown on a farm and were fourteen months old at the point of morphological examination. From April till October they were kept on special open plots planted with a mixture of napa cabbage and rapeseed, without additional food resources. From November to March they were kept in the fridge at 4 °C. Before culturing the mollusks were kept hungry for four days, so that their mass at the start of the study was 8.5–9 g.

Fragments of tissue were taken from the lower (larger) parts of digestive gland of each snail. Tissue samples were wholly submersed in Bouin solution for fixation for a day. Then samples were thrice washed in 70 % ethanol, kept in each solution for a day. After that they were dehydrated in ethanol series of 10 % increments from 70 % ethanol and 1 day of exposure to each successive solution. Next, the fragments were put into two baths of absolute ethanol, for six hr in each bath. Afterwards they were put into absolute ethanol: chloroform 1 : 1 mixture for two hr, then in two baths of pure chloroform (for two hr in each), and lastly in chloroform: paraffin 1 : 1 mixture at 37 °C for an hour. Then they were soaked in two changes of paraffin at 56 °C and poured into molds. The blocks were cut with a sliding microtome MC-2 into 5 µm thick sections, and stained after deparaffinization. From each sample one slide was taken for each method of staining. For general studies, we stained the sections with hematoxylin and eosin (Mulisch & Welsch, 2010), for collagen fibers we used Heidenhain's azan (Mulisch & Welsch, 2010). For elastic fibers we employed Weigert's resorcin-fuchsin and aldehyde-fuchsin after Gabe-Dyban (Falin, 1961). The latter method was also used to reveal muscular elements. Glycogen and neutral glycoproteins were studied based on PAS reaction after McManus (Mulisch & Welsch, 2010). Brown granules in cells were studied both on preparations stained as described above, and on slides stained with potassium ferricyanide after Schmorl (Pirs, 1962).



Fig. 1. Specimens of *Cornu aspersum*.

Fotofixation and morphometry research were done using microscope Leica DM-2500 with Leica DFC450C camera and Leica Application Suite 4.4 software. Morphometric computer software ImageTool and WCIF ImageJ were used to determine on histological slides the number and size of structures in different parts of the digestive gland: digestive ducts, inter-duct connective tissue, muscle elements, capsule. The data were statistically processed using StatPlus 2008 software. Sample mean ( $M$ ), standard error ( $SE$ ), and coefficient of variation ( $CV$ ) were calculated. Correlation between separate characters was evaluated using Spearman's rank correlation coefficient. The calculations were conducted on samples each containing 15 measurements (same as the number of experimental animals).

## Results and discussion

The gastropod's digestive gland had typical morphological organization. Most of it consisted of digestive ducts interspersed by the branches of liver ducts, and vessels (Tunali & Erkan, 2008) also known as haemocelic ducts (Rosenbaum & Ditzion, 1963) that branched off vessels connecting the heart and the general haemocoel of visceral mass. The supporting cells were located at the periphery of haemocelic ducts (fig. 2). The digestive gland was covered with a thin membranous capsule.

**Digestive ducts.** The ducts were located under the capsule, entering it and gradually joining with formation of a diversified three-dimensional network. The ducts after that entered the gastric tracts at various areas in unspecified way (fig. 2). Digestive ducts were of round or round to oval shape and of average variability of cross-sectional area ( $CV = 28.1\%$ ),  $27\,700.9\ \mu\text{m}^2$  on average (table 1).

The density of digestive ducts (number of ducts per  $\text{mm}^2$ ) remained the most stable character of all studied, with  $CV$  of  $11.2\%$ . The average duct density was  $28.8/\text{mm}^2$ .

The specifics of variability of duct lumens were completely different. The lumen was the most varying character of all studied, with  $CV$  of  $94.1\%$ . The widest lumen was  $9.0\%$  of cross-section area of a duct, however most lumens were  $2$  to  $4\%$  of total duct cross-section area. Ducts which lacked lumens were also found. The average lumen area was, thus,  $929.5\ \mu\text{m}^2$ , which was  $2.8\%$  of total cross-section area of ducts.  $97.2\%$  of duct area belonged to the duct walls which, thus, defined the fluctuating total cross-section area of



Fig. 2. Structure of the digestive gland of *Cornu aspersum* snail (haematoxylin-eosin): haemocoelical ducts (1), digestive ducts (2), stomach duct (3).

**Table 1. Morphometric parameters of digestive gland parenchyma (N = 15 specimens)**

Parameter	M	SE	CV
Part of parenchyma with digestive ducts, %	79.8	4.29	20.8
Part of parenchyma with connective tissue, %	20.2	1.05	20.1
Number of digestive ducts per mm <sup>2</sup> of digestive gland	28.8	0.83	11.2
Digestive gland cross-section area, μm <sup>2</sup>	27 700.9	2012.3	28.1
Digestive duct lumen area, μm <sup>2</sup>	929.5	225.8	94.1
Digestive duct wall area, μm <sup>2</sup>	26 771.4	1845.1	26.7
Length of muscular elements between digestive ducts, μm	59.7	6.52	42.3
Width of muscular elements between digestive ducts, μm	1.41	0.23	63.2
Number of Calcium cells on the cross-section of a duct	4.5	0.38	32.7
Area of all Calcium cells on the cross-section of a duct, μm <sup>2</sup>	4155.8	392.5	36.6
Share of the digestive duct's wall occupied by Calcium cells, %	15.5	0.94	23.5
Mean area of a Calcium cell, μm <sup>2</sup>	923.5	61.4	25.7
Number of vacuoles on the cross-section of a digestive duct	15.0	1.19	30.7
Number of vacuoles per mm <sup>2</sup> of digestive gland	432.0	38.9	34.9
Number of vacuoles with brown granules on cross-section of a digestive duct	11.9	1.09	35.5
Number of vacuoles with brown granules per mm <sup>2</sup> digestive gland	336.8	31.4	36.1
Share of the gland cross-section occupied by brown granules, %	2.2	0.09	15.8

digestive ducts. Conversely, changes in the duct lumen's area had the least effect on that value. The deviation of duct wall area values from the average one (26 771.4 μm<sup>2</sup>) was similar to the abovementioned parameters of total duct area, with CV of 26.7 %.

The dynamics of duct wall area (thickness) was more informative given in percentages. It made up 91 % of cross-section area in large ducts. With decreasing duct diameter, the wall thickness tended to increase up to 100 % in "closed" ducts. However, not only the smallest digestive ducts lacked lumens. Also, there were 1–2 "closed" ducts per 6 mm<sup>2</sup> of cross-section, middling in size (30 000–35 000 μm<sup>2</sup>), in all studied snails. That probably indicates their functional state.

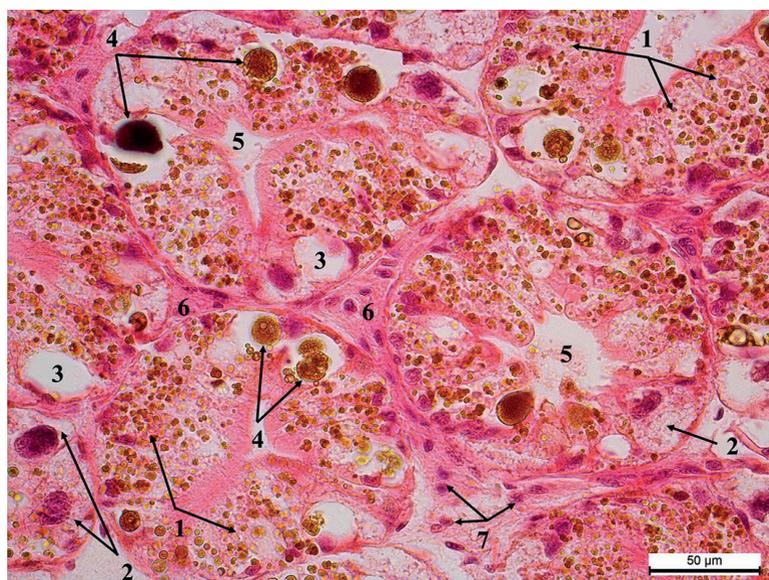


Fig. 3. Structure of ducts of the digestive gland of *Cornu aspersum* (stained by haematoxylin-eosin): digestive cells (1), Calcium cells (2), vacuoles without brown granules (3), vacuoles with brown granules (4), duct lumen (5), inter-duct connective tissue (6), cells of inter-duct connective tissue (7).

90–95 % of digestive ducts contained fairly small amounts of matter or were altogether empty.

Cell component of digestive ducts. The study of cell composition of digestive ducts revealed two main cell types: digestive cells and Calcium cells (fig. 3). Digestive cells, depending on the digestion stage, were at one of three possible functional states: adsorption, secretion and excretion. There were also undifferentiated cells. Such digestive ducts structure is in line with other findings (Leal-Zanchet et al., 1993). However, not all researchers agree with the classification and some list three main cell types: digestive, Calcium and excretory (Walker, 1970). We did not conduct an advanced study of cellular morphometry, but focused on the ratio of separate parameters. It was found that the number of Calcium cells (table 1) at the cross-section of digestive duct was to an extent connected to its size. The number of Calcium cells strongly varied ( $CV = 32.7\%$ ), with the mean of 4.5 cells per duct.

The parameter of area of single Calcium cell was less variable,  $923.5\ \mu\text{m}^2$  on average. The area of Calcium cells did not correlate with the size of digestive ducts, each of which included cells of varying size. For these two parameters, a direct ( $r = 0.284$ ) but not significant ( $p > 0.05$ ) correlation was found. It is also worth noting that Calcium cells are usually described as pyramidal in shape (Leal-Zanchet et al., 1993). However, we've consistently seen oval or irregular shapes of these cells.

Taking the Calcium cells together (numbers and areas) we found their area per duct. Absolute values of that area were expected to vary strongly for small and large ducts. CV confirmed that assumption and was rather high, and the parameter's deviation from mean ( $4155.8\ \mu\text{m}^2$ ) was  $36.6\%$ .

Conversely to the total area of Calcium cells, their percentage in a duct wall was characterized with an average variability ( $CV = 23.5\%$ ), and the percentage was  $15.5\%$  of duct wall.

Connective tissue. The amount of connective tissue in a digestive gland was assessed by the ratio of digestive duct area to the connective tissue area (fig. 3). The ratio was, on average,  $4 : 1$ , meaning that digestive ducts made up  $79.8\%$  of parenchyma, and the connective tissue formed the other  $20.2\%$ . The two parameters are complementary and

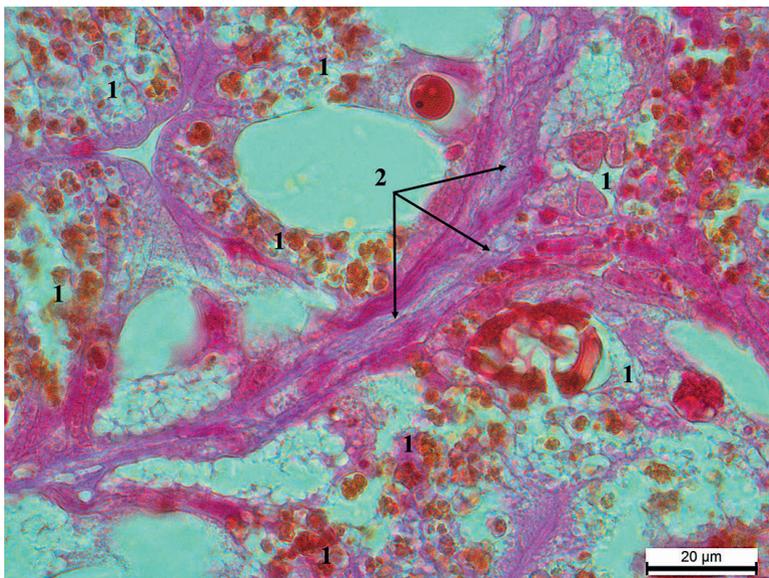


Fig. 4. Collagen fibers between digestive ducts of the digestive gland of *Cornu aspersum* (Heidenhain's azan): digestive duct (1), collagen fibers (2).

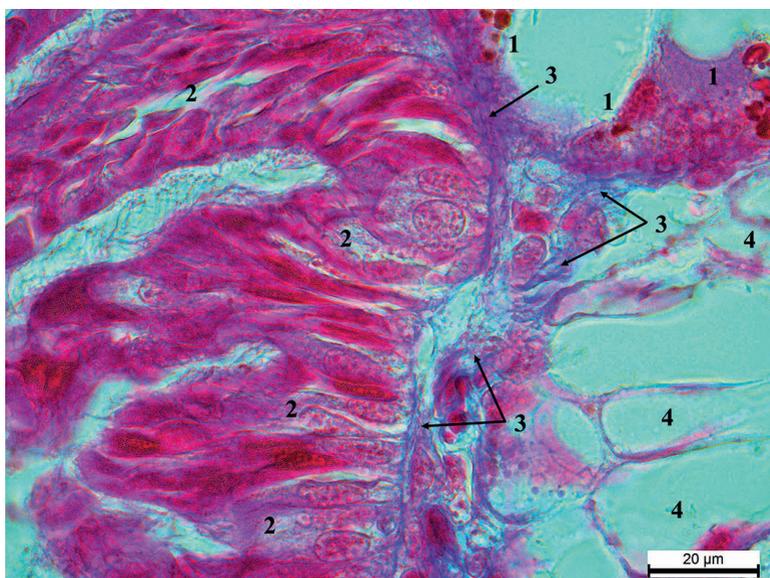


Fig. 5. Collagen fibers in the parenchyma of the digestive gland of *Cornu aspersum* (Heidenhain's azan): digestive duct (1), stomach duct (2), collagen fibers (3), supporting cells of haemocoelical duct (4).

**Table 2. Morphometric parameters of the capsule of digestive duct (N = 15 specimens)**

Parameter	M	SE	CV
Total thickness of capsule, $\mu\text{m}$	30.6	0.94	11.9
including: :			
muscle layer, $\mu\text{m}$	19.0	0.87	17.7
collagen layer, $\mu\text{m}$	4.0	0.33	32.0
epithelial layer, $\mu\text{m}$	6.6	0.31	18.2
height of epithelial microciliae of capsule, $\mu\text{m}$	1.0	0.05	19.4
Area of muscle fiber cross-section of the capsule, $\mu\text{m}^2$	10.0	1.21	46.9
Percentage of muscle fibers in the muscle layer of the capsule, %	48.2	2.76	22.2
Percentage of collagen fibers in the muscle layer of the capsule, %	15.1	0.80	20.5

were of average, almost the same level of homogeneity. The difference in their CV values was only 0.7 %.

The morphological specifics of the digestive gland's connective tissue included its fibers and cell composition. Without connective tissue (figs 3, 4), the most stable place of localization for the collagen fibers was the basal membrane of digestive ducts. Most collagen fibers were seen between digestive ducts close to the capsule, or following the walls of the stomach ducts or joining them (fig. 5). The fibers became smaller and thinner with distance from these structures.

However, the most substantial aggregation of collagen fibers was seen not between the digestive ducts, but in the gland's capsule (table 2). There, the fibers were of two kinds. One formed a clear layer between the epithelial and muscle layers and the other was sparsely seen within the muscle layer of the capsule, dividing it into muscle bundles (figs 6, 7). The thickness of collagen layer in different parts of the capsule varied significantly, deviating from the mean by 32.0 %. Correlation relationship between the two latter parameters was direct ( $r =$

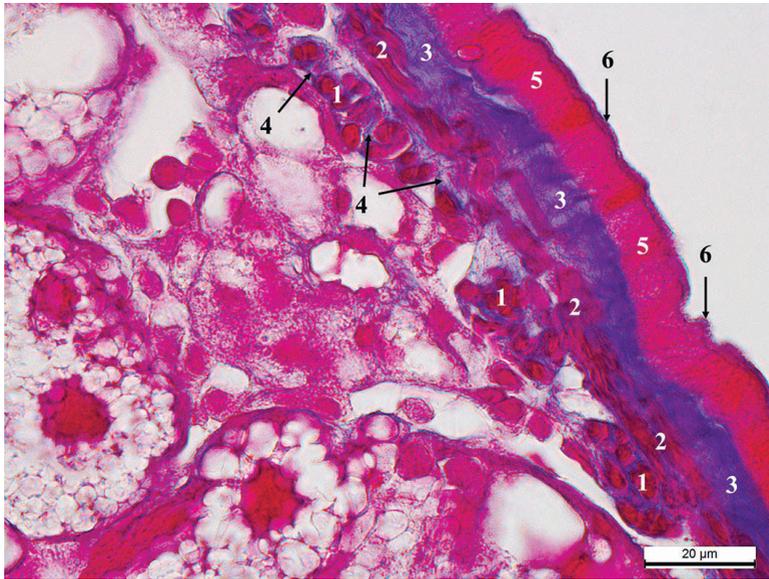


Fig. 6. Structure of the digestive gland capsule of *Cornu aspersum* with two muscle bundle layers (Heidenhain's iron-haematoxylin): internal muscle bundle layer (1), outer muscle bundle layer (2), collagen fibers layer (3), collagen fibers in muscle layer (4), epithelial layer (5), epithelial microcilia (6).

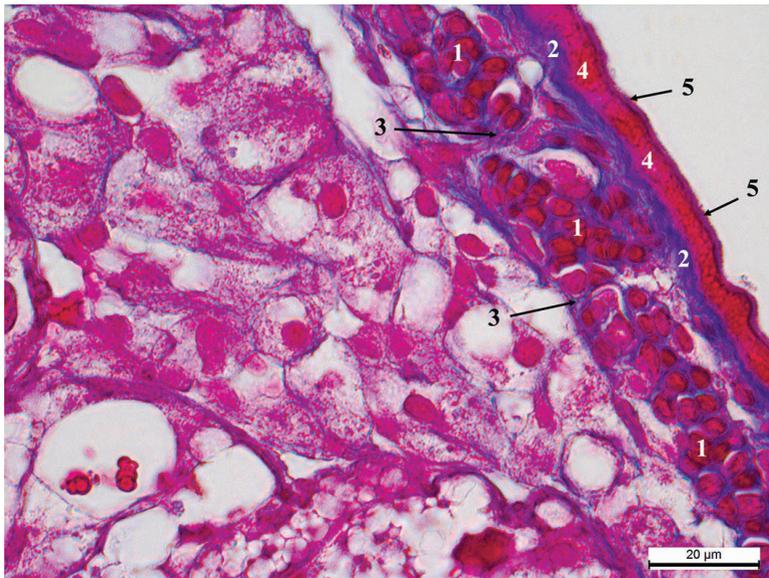


Fig. 7. Structure of the digestive gland's capsule of *Cornu aspersum* with one layer of muscle bundles (Heidenhain's iron-haematoxylin): layer of muscle bundles (1), layer of collagen fibers (2), collagen fibers in the muscle layer (3), epithelial layer (4), epithelial microcilia (5).

0.230) and not significant ( $p > 0.05$ ). We found no morphometric connection between that variability and other capsule parameters, for example, thickness of the muscle layer. Mean thickness of collagen fibers was 4.0 μm, 13.1 % of the total thickness of digestive gland's capsule. Also, the collagen fiber layer included fibers of the basal epithelial membrane.

The amount of collagen fibers varied less in the muscle layer of capsule than in the separate layer of collagen fibers, and the variability was average (CV = 20.5 %). In the muscle layer of capsule, the percentage of collagen fibers was 15.1 %.

Well-formed collagen fiber bundles were also typical for the walls of haemocelical and stomach ducts.

Notably, there were no elastic fibers in all structural components of the digestive gland, whether in the connective tissue between digestive ducts, in the walls of haemocelical or stomach ducts, or in the capsule.

Cellular component of the connective tissue. According to the literature (Barker, 2001) the tissue has two kinds of cells: globulocytes (amoebocytes) and fibrocytes.

The connective tissue cells occurred mostly in groups (fig. 3). Their number in a group varied in different regions of parenchyma from 2–5 cells to 20 and more. Smaller groups of cells were more often (55–65 %) found nearer to the gland's surface, under capsule. Larger groups of cells were more common (65 to 75 %) deeper in the glandular parenchyma. The cells in such groups were roundish-oval. There were also single, elongated (rod-like) cells between densely packed digestive ducts. Several such cells formed a chain between two large oval digestive ducts. These chains connected different cell groups, so that in 50–60 % of all cases, one cell group became another.

Muscle elements. All observed muscle elements of the digestive gland were of smooth muscle tissue, formed by myocytes in bundles of varying thickness.

Most muscle structures were concentrated in the capsule forming a layer of heterogeneous spatial organization. In some parts of the capsule the muscle layer divided in outer and internal thinner sublayers (fig. 6). On average, they were approximately equally thick and with orthogonal directions of the muscle bundles. In some areas the internal layer pierced the outer, separating it in two, and three sublayers were observed. However, there were also areas with only one muscle layer (fig. 7). We also detected differences in the structure of the muscle layers. They were mostly solid, with relatively homogeneous placement of muscle bundles. However, concentrations of bundles were also observed separately and joining into oval bundles distantly resembling distinct muscles. These specifics of muscle structures were not presented regularly and were found in all studied mollusks.

The mean thickness of muscle layer was 19.0  $\mu\text{m}$ , or 62.1 % of total capsule thickness, with variability that pointed to average homogeneity in various parts of the gland (CV = 17.7 %). The parameter varied from 12.0  $\mu\text{m}$  to 23.4  $\mu\text{m}$ . An important characteristic of

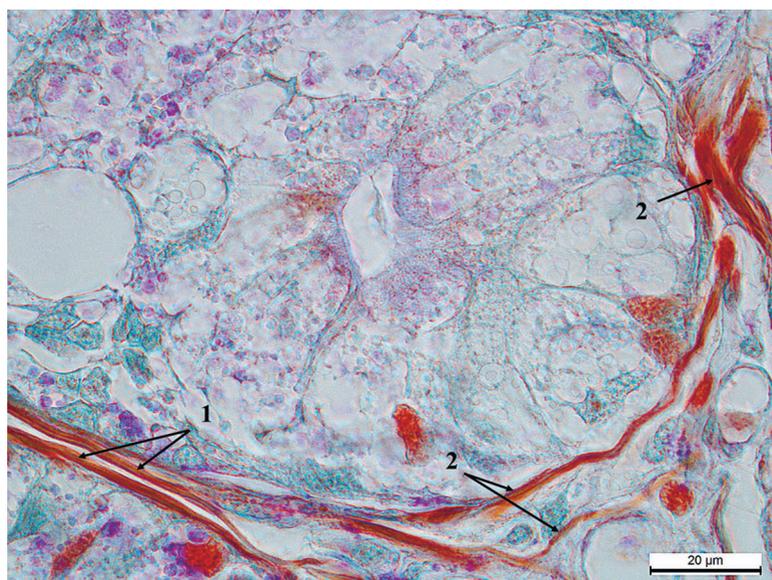


Fig. 8. Muscle bundles in parenchyma of the digestive gland of *Cornu aspersum* (aldehyde-fuchsin after Gabe-Dyban): straight muscle bundles (1), horseshoe-shaped muscle bundles (2).

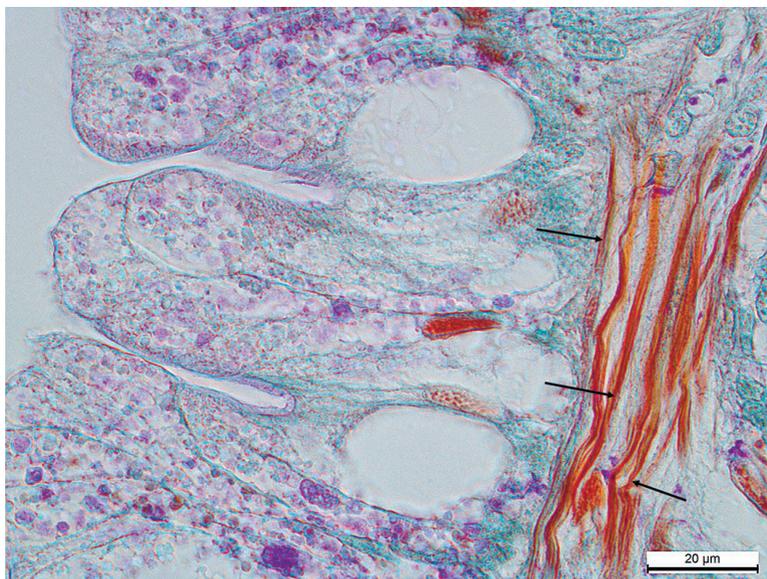


Fig. 9. Parallel rows of straight muscle bundles (arrows) in the parenchyma of digestive gland of *Cornu aspersum* (aldehyde-fuchsin after Gabe-Dyban).

the muscle layer of the capsule was its saturation with the muscle cells, since muscle bundles were separated by a lot of collagen fibers. Parameters of these two components of the muscle layer were characterized by similar level of variability, which was higher by 1.7 % in muscle bundles. Their CV was 22.2 %. The mean share of muscle elements in the muscle layer of capsule was 48.2 %.

CV of the muscle bundles' thickness was twice higher, 46.9 %, indicating the high variability of that parameter and possibly the multidirectional moving activity of capsule. The mean cross-section area of a muscle bundle was  $10.0 \mu\text{m}^2$ .

Myocyte bundles in the walls of stomach ducts were significantly fewer, yet also well-defined.

Separate muscle bundles went from the capsule and from stomach ducts into the parenchyma between digestive ducts. In some cases (12 to 15 per  $6 \text{ mm}^2$  of section area) only single muscle cells occurred around digestive ducts. In other cases (1 to 2 per  $6 \text{ mm}^2$  of section area) whole groups of digestive ducts were surrounded by single muscle cells or muscle bundles.

The number of ducts in such groups was on average two to four. That created a certain mosaicity in the topography of gland's muscle elements. There was no clear regularity to their order. However, these areas were found in all specimens and samples. We suppose that it was associated with the uneven functional activity of separate parts of the digestive gland. The localization of muscle bundles around digestive ducts also had some specific features. In some cases (1 to 2 per  $6 \text{ mm}^2$  of section area), the long thin muscle bundle (or a single myocyte) surrounded almost a whole digestive duct. In other cases (7 to 8 per  $6 \text{ mm}^2$  of section area), a shorter and thicker bundle could form a horseshoe-shaped arc around only a part of a duct (fig. 8). Alternatively (6 to 7 per  $6 \text{ mm}^2$  of section area), straight muscle bundles went between large oval densely packed digestive ducts. Also, two or more muscle bundles were located in parallel rows (fig. 9) or in a fan-like spread 1–2 times per  $6 \text{ mm}^2$  of section area in slides of all studied specimens.

That diversity of forms and topography of muscle structures was reflected in their measurements. The latter were highly variable, because the structures included both separate myocytes and muscle bundles. The highest variability was observed in the width of muscle

elements, deviating from the mean (1.41  $\mu\text{m}$ ) by 63.2 %. The variability of length measurements was lower by 20.9 % for the muscle elements, which was still quite high. The mean length of muscle elements between digestive ducts was 59.7  $\mu\text{m}$ .

There also were certain specifics of muscle elements in all specimens. For example, the middle part was frequently (7 to 8 cases per 6  $\text{mm}^2$  of section area) thicker than the ends. At the ends of many bundles (5 to 7 cases per 6  $\text{mm}^2$  of section area) the fibrils veered off into smaller bundles or single myocytes of varying length. The rate of that separation was connected to the initial thickness of the bundle. Thus, to standardize the measurements, we

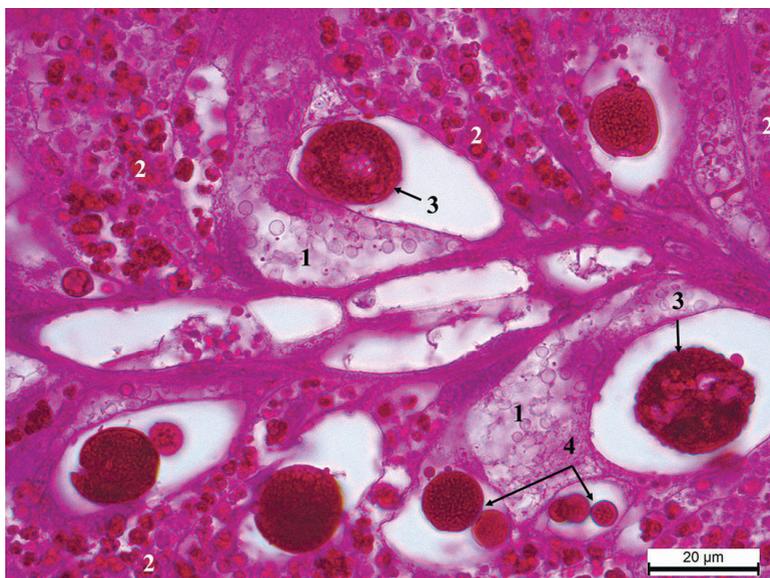


Fig. 10. Vacuoles and granules in digestive ducts of *Cornu aspersum* (PAS reaction): Calcium cell (1), digestive cell (2), large brown granules (3), small granules joining into large granules (4).

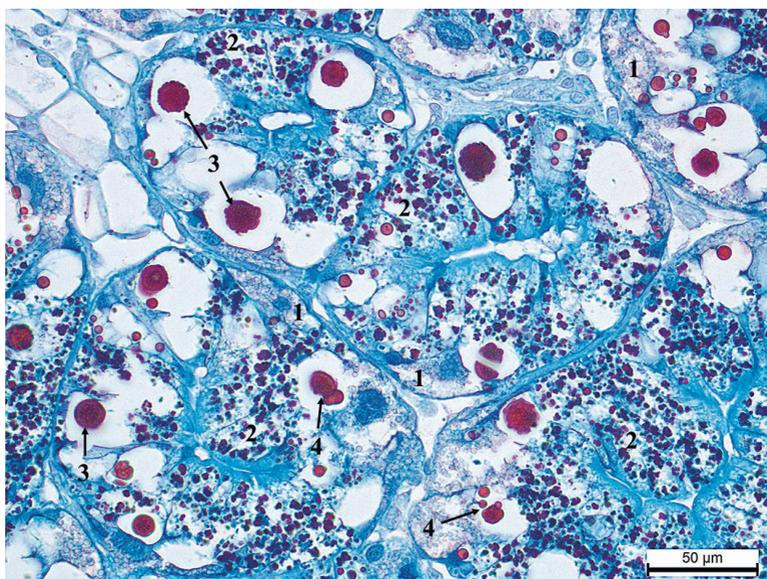


Fig. 11. Topography of vacuoles and brown granules in the digestive gland of *Cornu aspersum* (Potassium ferricyanide after Schmorl): Calcium cell (1), digestive cells (2), large brown granules (3), formation of large granules by small granules joining together (4).

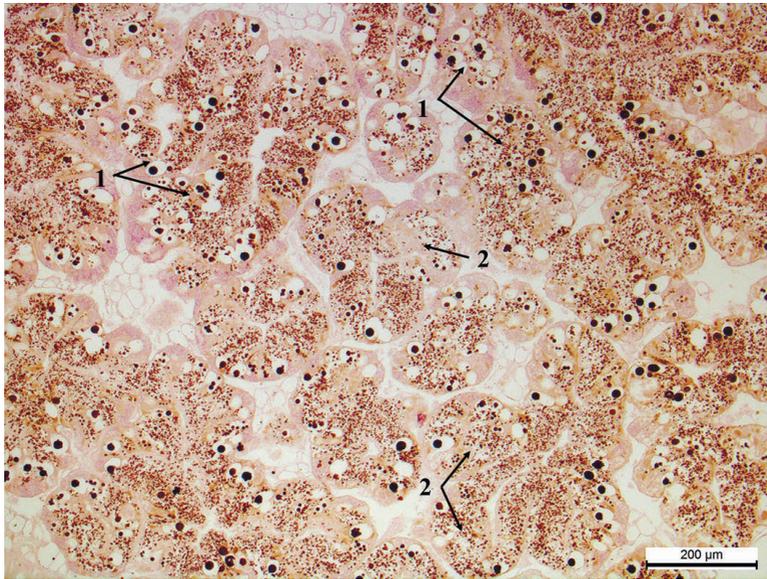


Fig. 12. Digestive ducts of *Cornu aspersum* with different amounts of brown granules (Weigert's resorcin-fuchsin): ducts with high (1) and low (2) brown granule content.

measured the thickness of all muscle structures in the middle area. At times (3 to 5 cases per 6 mm<sup>2</sup> of section area), thicker and thinner areas alternated in long muscle bundles. In these cases we took several measures within a bundle and then calculated the mean.

It is also interesting to note that there were no muscle elements in the walls of haemolymph ducts. Therefore, the vessels are passive haemolymph conductors.

**Glandular capsule.** In all specimens, the glandular surface was covered by simple cuboidal epithelium (figs 6, 7). It bore microciliae, surrounded with homogenous mass. The heights of epithelial cells and of microciliae were of similar average homogeneity, with CVs of 18.2 % and 19.4 %. The absolute value of height for the epithelial cells was 6.6 μm, 21.6 % of total thickness of the glandular capsule. The height of microciliae was 1.0 μm, 3.2 % of capsule's thickness.

The variability of thickness of capsule's individual constituents determined the changes in its total thickness. Understandably, the latter was mostly determined by the muscle layer. Sometimes, if it were of average thickness the other layers (collagen and epithelium) were also of average thickness. However, the parameters often varied asynchronously. For example, a significantly thick muscle layer could be accompanied by average or thin epithelial or collagen layers. We saw a not significant correlation between the thickness of muscle and collagen layers of the digestive gland. Even weaker correlation ( $r = 0.153$ ) was found between the thicknesses of muscle and epithelial layers. We saw no regularity or conditions that influenced the thickness of different layers of the digestive gland. However, despite the average or high variability of thickness of certain capsule layers, the total thickness was highly stable in different parts of the glandular capsule (CV = 11.9 %), with the mean of 30.6 μm.

**Cellular vacuoles and granules.** A functional digestive gland of the gastropod always had vacuoles forming in cells of the digestive ducts. Most of them contained brown granules of various sizes. However, the color of these granules, in part, was determined by the method of staining histological sections (figs 3, 10, 11). Large granules formed by small ones gradually joining. In our opinion, they contain not only lipofuscin as sometimes assumed (Arrighetti et al., 2015), but also an elaborate composition which requires complex multi-aspect studies.

We've found that the number of vacuoles and their brown granule content were highly variable in different parts of the digestive gland, with CV of 30 to 37 %. On average, there were 15.0 vacuoles in the wall of one digestive duct (CV = 30.7 %), or 432.0 vacuoles per 1 mm<sup>2</sup> of digestive gland area (CV = 34.9 %).

The number of vacuoles filled with brown granules within a duct was on average 11.9 (CV = 35.5 %), or 336.8 vacuoles per 1 mm<sup>2</sup> of digestive gland area (CV = 36.1 %). It corresponds to 78.0 % of all vacuoles. The parameter varied from 61.1 to 100 % (the latter in small ducts with few vacuoles).

The number of vacuoles and their saturation with brown granules was relatively stable in 50–55 % of section area of the gland. There were also areas with foci of granules, where groups of 3 to 6 digestive ducts contained 2 to 3 times more brown granules compared to nearby groups of ducts with much lower granule content (fig. 12). We did not find regularity to the location of such groups of ducts.

However, a direct moderate correlation was found ( $r = 0.466$ ,  $p < 0.05$ ) between the cross-section area of a digestive duct and the amount of vacuoles with brown granules in its wall. That, in our opinion, also indicated the specific functional activity in different parts of the digestive gland of studied mollusks.

The average area of brown granules was 2.2 % of section of digestive gland parenchyma. That parameter, unlike the other pertaining to vacuoles and brown capsules, was characterized by twice lower variability (CV = 15.8 %). We suppose that the concentrated location of brown granules is more obvious on the local level of separate ducts, less so in case of large areas of parenchyma (6 mm<sup>2</sup> of cross-section area). At the latter area, values of that parameter varied less.

The cells of digestive ducts also have various amounts of PAS-positive substances (neutral glycosaminoglycans, glycoproteins and glycogen) in cytoplasm. Different saturation of color (fig. 10) indicated that the digestive cells were highly saturated with the compounds, while Calcium cells were significantly less so.

As to the stomach ducts (fig. 2), we did not conduct a morphometric study, yet we observed their branching from stomach to capsule. The branches grew gradually smaller. The shape of stomach ducts was mostly roundish-oval. However, in places where the duct branched off smaller ducts or where it was joined by two or more digestive ducts, its shape became irregular (amoeboid).

## Conclusions

On average, 28.8 digestive ducts per 1 mm<sup>2</sup> (CV = 11.2 %) were noted in parenchyma of the digestive gland of *Cornu aspersum*. They were roundish-oval in shape with average area of 27 700.9 μm<sup>2</sup> (CV = 28.1 %). The area of digestive ducts (79.8 % of the parenchyma) and inter-duct connective tissue (20.2 % of the parenchyma) were in the ratio of 4 to 1.

The digestive gland completely lacked elastic fibers. The muscular elements were of various shape and placement between the digestive ducts. Their mean length was 59.7 μm (CV = 42.3 %), mean thickness 1.41 μm (CV = 63.2 %).

The glandular capsule had three layers: epithelial, muscular and collagen fibers. The muscles made up more than 60 % of capsule.

Percentage of Calcium cells in the walls of digestive ducts was 15.5 % (CV = 23.5 %).

Cells of digestive ducts contain a lot of vacuoles with brown granules, 336.8 per 1 mm<sup>2</sup> (CV = 36.1 %). Area of brown granules made up 2.2 % of digestive gland section (CV = 15.8 %).

The irregular placement of muscle elements and brown granules in the parenchyma points to certain functional specifics of separate parts of the digestive gland.

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