

Original papers

Basic morphofunctional features of pharmaceutic leech (*Hirudo verbana* Carena, 1820) tissues in various forms of response after hirudotherapeutic procedures

Aleksandr K. Frolov, Raisa A. Litvinenko

Faculty of Biology, Zaporizhzhia National University, Zhukovskogo 66, 69600 Zhaporizhzhia; Ukraine

Corresponding author: Aleksandr K. Frolov; e-mail: a_frolov@ukr.net

ABSTRACT. It is analyzed morphofunctional features of *Hirudo verbana* tissues on histological sections in order to determine the possible causes of their partial death after hirudotherapeutic procedure. In the study was taken 4 groups of leeches: hungry (control), healthy well-fed, well-fed that vomited blood and well-fed dead. Morphofunctional changes in midgut of healthy well-fed *H. verbana* compared to the hungry are the changes of epithelium height through stretching eaten blood. In well-fed that vomited blood and well-fed dead leeches in the intestinal epithelium were observed degenerative processes that are accompanied by a decrease in the height of the epithelium, its partial desquamation. Botryoidal tissue of hungry leeches is in an inactive state, in healthy well-fed ones it responds with an increased physiological vascularization, activation of some botryoidal granulocytes with an increase in their average size, on receipt of the products of digestion. Activation of some botryoidal granulocytes, without a significant increase in their sizes, in well-fed leeches that vomited eaten blood and well-fed dead leeches, with increased infiltration of the adjacent connective tissue lymphocyte- and macrophage-like cells, amebocytes and granulocytes indicates the development of pathological processes, which are resulting in immunological conflict between eaten human blood and leeches' tissue microenvironment.

Key words: medicinal leech, hirudotherapy, intestinal epithelial cells, botryoidal tissue, botryoidal granulocytes, lacunar channels

Introduction

Over the past decade due to successful research in the field of hirudology and hirudotherapy (HT), the use of medicinal leeches has increased significantly [1,2], especially in chronic forms of disease and complications after prolonged intake of pharmaceutical drugs [3,4]. In this regard, the need for biotechnological medicinal leech is growing, but we [5] and other researchers [6,7] found that some medicinal leeches after feeding on the blood of cows or pigs in terms of biotechnology and human blood during hirudotherapy are dies. But the mechanism of such leeches death is unknown. It should be noted that the blood of warm-blooded mammals, except plastic material, contains highly efficient cellular as well as humoral immune factors. Also, during HT procedure and under laboratory breeding conditions, medicinal leech is saturates with blood to the entire satisfaction, without interference with the increase of initial weights for 5–7 times, whereas in the natural environment, while eating, the medicinal leech has a limited period due to watering place of an animal [3]. Therefore, before the medicinal leech, as an obligate hematophagous naturally arises the problem of immune suppression properties of consumed blood. It can be assumed that not all medicinal leeches are overcome this problem, as a result they may have an immunological complications such as „graft versus host reaction" (GVHR) [8,9], in which the graft acts as a significant blood amount of host-feeder and host is the microenvironment of the medicinal leech digestive system. Therefore, the purpose of work is to establish the basic morphological features of pharmaceutic medicinal leech (*Hirudo verbana*

Carena, 1820) tissues in various forms of response after HT in order to determine the involvement of immune mechanisms among the possible causes of death.

Materials and Methods

The object of the study were 400 specimens of hungry and 400 specimens of well-fed medicinal leeches *H. verbana* age of 7 months, grown in Cellular and Organismal Biotechnology Scientific Research Lab of the Faculty of Biology of Zaporizhzhia National University (TU U 05.0-02125243-002: 2009 „Medicinal leeches, sanitary-epidemiological conclusion of the Ministry of Health of Ukraine No. 05.03.02-06/49982 of 12.08.2009). The last blood-feeding of hungry medicinal leeches, taken for the experiment with cattle blood was 4 months ago. Well-fed medicinal leeches received after the HT treatments at relatively healthy donors and patients with various chronic cardiovascular diseases in remission. Donors and patients during the study have not taken medical preparations and other toxic substances. Informed consent was obtained from all patients after explanation for the objectives of the research. On the human blood medical leeches are fed to full saturation. Medicinal leeches are kept in a 3-liter bottles with settled dechlorinated tap water volume of 2-liter (fed by 10–15 individuals, hungry by 15–25 individuals) at ambient temperature +22°C. Observation of medicinal leeches lasted for more than 2 months with a periodic change of water every 3–4 days. It is analyzed morphological and physiological condition of medicinal leeches. In the case of deviation from the normal condition (inactivity, lack of contractile and suction reflexes, vomiting of blood and the appearance of constrictions on the body) medicinal leeches were deposited into individual 1-liter bottles with the subsequent daily observation. Dead medicinal leeches that were found among the well-fed ones which previously vomited blood and had a constriction on the body, were fixed immediately. Out of the two groups observations of medicinal leeches for histological studies were isolated 4 groups with 15 individuals in each: 1) control group – hungry commodity medicinal leeches; 2) intact healthy well-fed medicinal leeches, which spent a feeding on human blood held at HT procedures after 4 months of starvation, and fixed 12 days after feeding; 3) well-fed medicinal leeches after consumption of human blood at HT procedure with symptoms of vomiting blood at 9–14 days after feeding; 4) well-fed medicinal leeches who died at 11–14 days after feeding and before that were vomited blood.

Histological sections were prepared from the middle part of the body all investigated groups of leeches, stained with hematoxylin-eosin [10]. It is analyzed histological features of the leeches body structure. Particular attention paid to morpho-functional characteristics of the intestinal epithelium (height, μm), connective (the number of free granulocytes, amebocytes, lymphocyte-, macrophage-like cells per unit area of 0.1 mm^2 tissue) and botryoidal (size of botryoidal granulocytes in μm, the percentage of activated and non-activated botryoidal granulocytes per unit area of tissue 0.1 mm^2, diameter of lacunar channels (blood vessels) in μm) tissues. Botryoidal granulocytes with flattened, half-moon shaped cells that mainly surround the intermediate lacunar channels were considered as activated; botryoidal non-activated granulocytes are rounded, not forming lacunar channels [11–13].

Statistical analysis of experimental data was carried out using application package IBM SPSS Statistics 20.0, using parametric and nonparametric statistical methods. The test data for normality of distribution was carried out using Kolmogorov-Smirnov test. At normal distribution were used parametric statistical methods, statistical significance of differences between the study groups was assessed by the Student criterion (T-test for independent samples). The differences of the results were considered valid if P>95%, $p<0.05$.

Results and Discussion

Over the period of observation (2 months) for the group of hungry and well-fed medicinal leeches the expressed pathological morphophysiological features demonstratively manifested in the last group (Fig. 1). At 9–14 days after feeding on human blood in some well-fed animals (9.5±0.57%) are manifested aforementioned pathological features. It is noteworthy that during the observation period of cannibalism [14] or cannibalistic tendency [15] in the group of well-fed medical leeches were not observed. Ability of cannibalism was excluded at the beginning of the study due to complete saturation of all the leeches taken in the experiment, and the alimentary reflexes which may appear in the

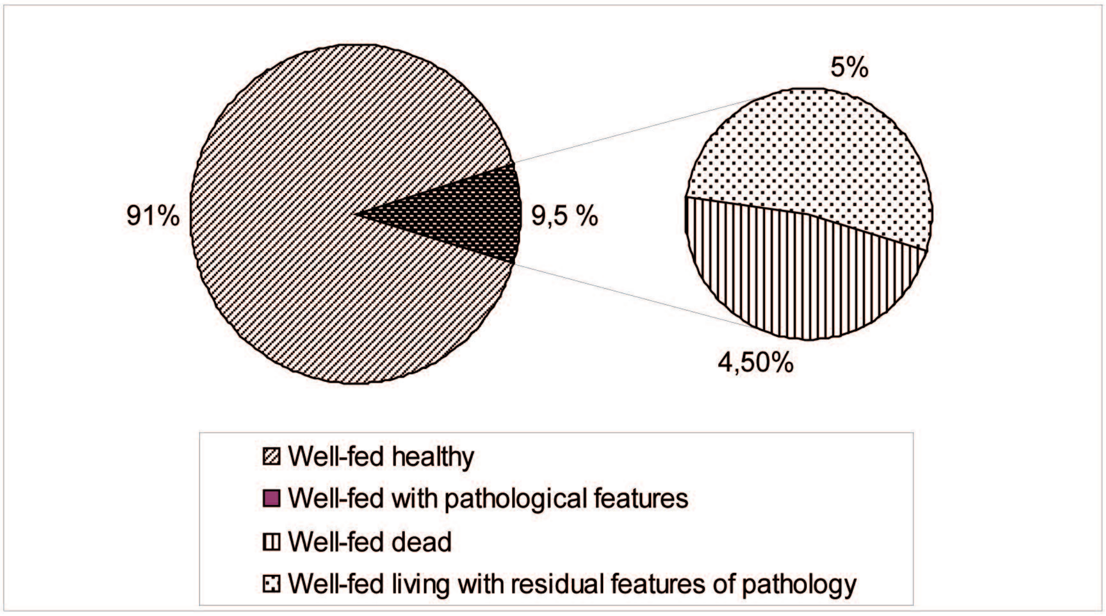

Fig. 1. The relative proportion of healthy well-fed medicinal leeches and well-fed with pathological morpho-physiological features followed by death among them

future exceed in the period of our observation. In a substantial part (4.5±0.48%) among the deposited after development of pathological morpho-physiological features medicinal leeches observed fast death after 3–5 days. The rest of the deposited specimens remained viable throughout the observation period; although a substantial part (5.0±0.49%) had residual pathology manifestations in the form of varying width constrictions, but without a further vomiting of eaten blood.

In order to ascertain the possible causes of morphophysiological pathological manifestations in

Table 1. The height of the epithelium of the crop (stomach) *H. verbana* at different physiological conditions, μm

	Group of leeches	Central chamber of the crop		Crop diverticulum (caecal pockets)	
		Dorsal epithelium, μm	Ventral epithelium, μm	Smooth surface, μm	Folds, μm
1	Hungry, n=15	10.1±0.28	9.9±0.24	9.8±0.56	10.0±0.27
2	Healthy well-fed, n=15	9.5±0.90	7.9±0.53*1Δ	9.5±0.57	10.3±0.37
3	Well-fed that blood vomiting, n=15	5.7±0.24*2Δ,*4Δ	5.3±0.25*2Δ,*4Δ	7.75±0.34*2Δ,*4	9.0±0.28*2,*4Δ
4	Well-fed dead, n=15	4.7±0.25*3Δ,*5Δ,*6Δ	3.5±0.18*3Δ,*5Δ,*6Δ	7.4±0.45*3Δ,*5Δ	9.2±0.34*5

*1 the differences between hungry and healthy well-fed leeches were considered valid if $p < 0.05$, *1Δ if $p < 0.01$

*2 the differences between hungry and well-fed that blood vomiting leeches were considered valid if $p < 0.05$, *2Δ if $p < 0.01$

*3 the differences between hungry and well-fed dead leeches were considered valid if $p < 0.05$, *3Δ if $p < 0.01$

*4 the differences between healthy well-fed and well-fed blood vomiting leeches were considered valid if $p < 0.05$, *4Δ if $p < 0.01$

*5 the differences between healthy well-fed and well-fed dead leeches were considered valid if $p < 0.05$, *5Δ if $p < 0.01$

*6 the differences between well-fed blood vomiting and well-fed dead leeches were considered valid if $p < 0.05$, *6Δ if $p < 0.01$

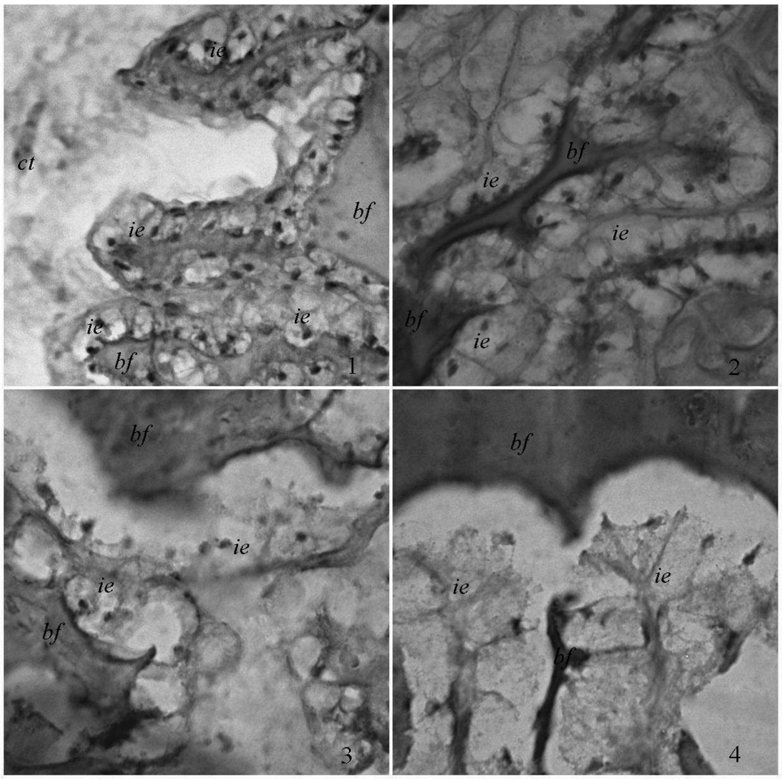

Fig. 2. Morphological features of intestinal epithelium in the crop of *H. verbana* (hematoxylin-eosin staining): 1– hungry leech (lens 20×); 2 – healthy well-fed leech (lens 40×); 3 – well-fed that blood vomiting leech (lens 40×); 4 – well-fed dead leech (lens 100×). *Conventional signs: ie* – intestinal epithelium; *bf* – blood feeder; *ct* – connective tissue.

the immediate period after feeding we performed selective histological examination. Hungry *H. verbana* has a typical for the genus *Hirudo* structure of the digestive system [3,4] and, accordingly, the intestinal epithelium [16]. Thus, the intestinal epithelium of the crop is presented by a single layer of cylindrical/cubic limbic epithelium (Fig. 2.1) in the apical and basal parts of which are small vacuoles and liposomes. The nuclei of epithelial cells are found mainly in the basal parts of the cells. The height of the epithelium in various parts (central chamber, diverticular chambers) of the crop is varied from 9.8 to 10.1 μm (Table 1). Living well-fed medicinal leeches, fixed in 12 days, as opposed to hungry, the crop has flattened epithelial cells, probably due to the consumption of a significant amount of blood, and their average height in different parts of the crop is varied from 7.9 to 10.3 μm (Table 1, Fig. 2.2). There is a statistically significant reduction in the height of epithelial cells of the ventral epithelium of the central chamber of the crop (7.9±0.53 μm in healthy well-fed leeches, compared to 9.9±0.24 μm in hungry, $p<0.01$) and a tendency to decrease the height of the dorsal epithelium (9.5±0.90 μm in healthy well-fed leeches, compared with 10.1±0.28 μm to hungry ones, $p>0.05$). In well-fed *H. verbana*, that vomited some portion of the consumed blood is observed a significant lifetime degenerative processes in the intestinal epithelium (Fig. 2.3). So, most of the epithelial cells of the crop are with pyknotic nuclei located mainly in the apical part of the cells, their statistically significantly reduced height is compared to the hungry and well-fed leeches, not only in the central chamber of the crop: 5.3±0.25 μm (ventral epithelium) and 5.7±0.24 μm (dorsal epithelium), with $p<0.01$, and in the caecal pockets (diverticular chambers) at $p<0.05$: 7.75±0.34 μm (smooth surface) and 9.0±0.28 μm (fold), Table 1. In addition, in the central chamber of the crop there are areas of desquamation of the epithelium. Even more pronounced signs of destruction are observed in intestinal epithelial of well-fed dead *H. verbana* (Fig. 2.4). Most of the intestinal epithelial cells are low; there are cells of different heights. Epithelial cells were vacuolated; the nuclei of irregular shape, pyknotic, placed in the apical part, there are non-nuclear fragments of cells and areas of epithelial desquamation. The height of the epithelial cells statistically significantly reduced, mainly in the central chamber of the crop to 3.5±0.18 μm (ventral epithelium), 4.7±0.25 μm (dorsal epithelium) are compared to the hungry, healthy well-fed, and well-fed that vomited blood leeches ($p<0.01$), the height of epithelial caecal pockets of well-fed dead leeches close to these values of well-fed *H. verbana* that vomited blood (Table 1).

Relevant morphological changes are observed in the connective and botryoidal tissues adjacent to the digestive system *H. verbana*. In hungry medicinal leech, the space between internal organs filled with coarse-fibered connective tissue, which is associated with botryoidal tissue, as in other species of leeches [16,17]. Connective tissue is represented by elongated supporting connective tissue cells, including rarely seen few free granulocytes, amebocytes, lymphocyte- and macrophage-like cells (10.7±0.77 cells per 0.1 mm^2 tissue), Table 2. In the living healthy well-fed *H. verbana* in connective tissue is compared to hungry, increases the number of lymphocyte- and macrophage-like cells, amebocytes and free granulocytes (22.2±1.50 cells per 0.1 mm^2 tissue, $p<0.01$). Infiltration of

Table 2. The number and functional status of botryoidal granulocytes and lymphocyte-, macrophage-like cells, amebocytes, free granulocytes per unit area (0.1 mm^2) of botryoidal and adjacent connective tissue of *H. verbana* at different trophic states

	Group of leeches	Granulocytes of botryoidal tissue							Lacunae of botryoidal tissue	Lymphocyte-, macrophage-like cells, amebocytes, free granulocytes in connective tissue, pcs
		Total, pcs	Including							
			Activated			Nonactivated				
			Pcs	%	d, μm	Pcs	%	d, μm	d, μm	
1	Hungry, n=15	123.6±4.71	13.2±1.01	10.6±0.51	6.8±0.21	110.4±3.93	89.4±0.51	7.3±0.23	7.2±0.23	10.7±0.77
2	Healthy well-fed, n=15	186.5±7.56 *1Δ	156.1±6.52 *1Δ	83.7±1.14 *1Δ	8.6±0.11 *1Δ	30.4±2.44 *1Δ	16.3±1.14 *1Δ	11.3±0.34 *1Δ	15.8±0.98 *1Δ	22.2±1.50 *1Δ
3	Well-fed that blood vomiting, n=15	161.5±10.07 *2Δ	75.9±5.21 *2Δ,*4Δ	46.9±1.14 *2Δ,*4Δ	5.3±0.23 *2Δ,*4Δ	85.7±5.50 * 2Δ, *4Δ	53.1±1.14 *2Δ, *4Δ	8.5±0.19 *2Δ,*4Δ	12.2±0.53 *2Δ,*4Δ	43.1±1.62 *2Δ ,*4Δ
4	Well-fed dead, n=15	170.7±9.45 *3Δ	145.4±7.86 *3Δ,*6Δ	85.3±0.97 *3Δ,*6Δ	4.4±0.14 *3Δ,*5Δ,*6Δ	25.4±2.34 *3Δ,*6Δ	14.7±0.97 *3Δ,*6Δ	5.5±0.15 *3Δ,*5Δ,*6Δ	14.8±0.91 *3Δ,*6	62.8±1.84 *3Δ,*5Δ,*6Δ

Abbreviations: see Table 1.

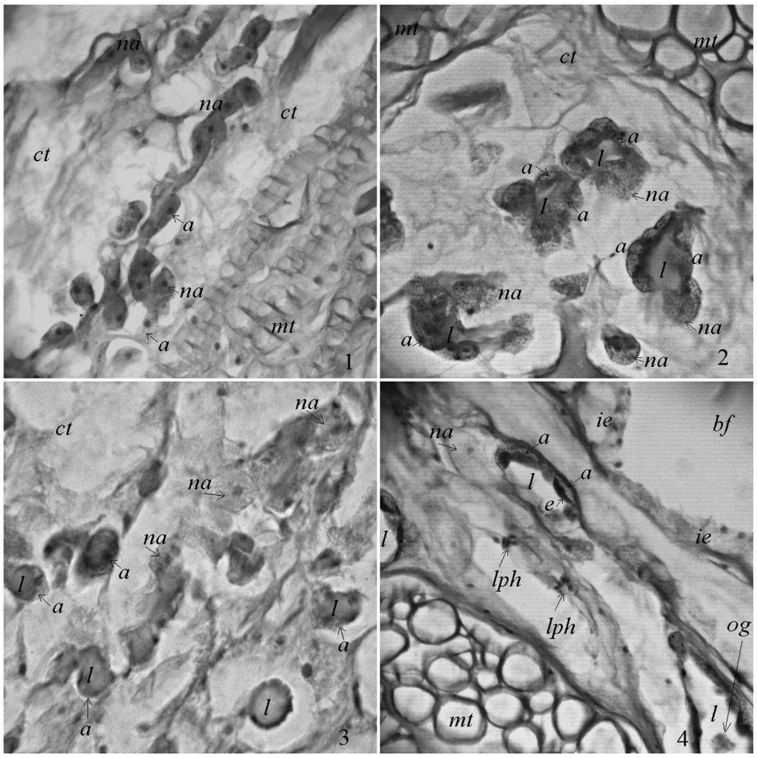

Fig. 3. Structural and functional features of botryoidal and adjacent connective tissue *H. verbana* (hematoxylin-eosin staining, the lens 40×): 1 – hungry leech; 2 – healthy well-fed leech; 3 – well-fed that blood vomiting leech; 4 – well-fed dead leech.
Conventional signs: a – activated granulocytes of botryoidal tissue; *na* – non-activated granulocytes of botryoidal tissue; *e* – endothelium-like cells of botryoidal tissue; *ct* – connective tissue; *lph* – lymphocyte-, macrophage-like cells, amebocytes, free granulocytes in connective tissue; *l* – intermediate lacunae in botryoidal tissue; *ie* – intestinal epithelium; *mt* – muscle tissue; *bf* – blood feeder; *og* – own hemocytes of leeches hemolymph.

connective tissue and surrounding tissues (botryoidal tissue) and organs (midgut) with such an immunocompetent cells in the well-fed leech that vomited blood significantly increased, are compared to the hungry, and healthy well-fed *H. verbana* (43.1±1.62 cells per 0.1 mm^2 tissue p<0.01). Available granulocytes, which are found in the connective tissue, are probably granulocytes type I. They are capable of migration, and degranulation of antimicrobial agents, can respond to multiple antigenic stimuli [18]. In well-fed dead *H. verbana* in connective tissue are the number of free granulocytes, amebocytes, lymphocyte- and macrophage-like cells that surround the midgut (crop) and botryoidal tissue (Fig. 3.4, Table 2) and are maximum among the studied groups (62.8±1.84 cells per 0.1 mm^2 tissue, p<0.01) as well as they are closer to the intestinal epithelium compared to other leeches.

Botryoidal tissue (Fig. 3) is an oxyphilic hollow structure and consists of rounded cells of two types – granular botryoidal (granulocytes) and flat endothelium-like cells [11,13]. Most cells of this tissue tightly surround channel of the capillary bed of lacunar (circulatory) system that combines dorsal, ventral and 2 lateral lacunar channels. It is known that botryoidal tissue undergoes functional and structural changes in response to different needs that arise during the life cycle of animals [11,19,20]. In healthy leeches, botryoidal cells are arranged in clusters and in experimentally injured, there is a transition structures of botryoidal tissue from the cluster (cord-like) to the hollow (tubular) architecture typical for vascular structures [11–13]. In addition, botryoidal granular cells is provided by nutrients adsorption [11,16,19], which come in the connective tissue from the midgut, and also metabolites, they are chloragogen cells, and are involved in secretory function [16]. Together with endothelium-like cells, they form additional (intermediate) lacunae and capillaries. It is believed that the granular cells of botryoidal tissue perform protective (immune) function as well [11]. In addition, botryoidal tissue is involved in angiogenesis, its celothelium performs myelo/erythroid function [11,20].

In the investigated hungry *H. verbana* intermediate bed of lacunar channels is barely noticeable, almost absent, botryoidal tissue of cluster (cord-like) type, consists mainly of non-activated botryoidal granulocytic cells (89.4±0.51%, the average diameter of the cells 7.3±0.23 μm), with a weakly pronounced graininess or without, the fraction of activated cells is small (10.6±0.51%, the average diameter of the cells 6.8±0.21 μm), Table 2, Fig. 3.1. Lacunae of botryoidal tissue are mostly in the constricted state (average diameter of 7.2±0.23 μm).

In a living well-fed *H. verbana* botryoidal tissue is large collared, it has the most hollow (tubular) architecture, typical for vascular structures, rarely clustered (cord-like) type. It is significantly increased the number of activated granulocytes (83.7±1.14%, the average diameter of the cells 8.6±0.11 μm), which form a cluster around the pockets, folds and walls of crop and around lacunar vessels (mostly dorsal). In the connective tissue is increased the number of capillaries and lacunae and their average diameter (15.8±0.98 μm, is compared to the hungry medicinal leech, $p<0.01$), Table 2. They, like as reticular botryoidal tissue are well filled with oxyphilic fluid – hemolymph, Fig. 3.2. Also it is statistically significantly increased the average size of both activated (8.6±0.11 μm) and non-activated botryoidal granulocytes (11.3±0.34 μm) compared to the hungry *H. verbana*, at $p<0.01$, apparently as a response to reception of metabolites of nutrients.

In well-fed blood vomiting leeches compared to hungry and well-fed *H. verbana* with no pathological changes in the connective and botryoidal tissues a growing number of lymphocyte-like cells similar to the small and large human lymphocytes, and in connective tissue further is increased the number of free granulocyte- and macrophage-like cells, enhances the processes of tissue vascularization – intermediate capillary bed and lacunas is more expressed compared to the hungry *H. verbana* (Fig. 3.3). In botryoidal tissue is compared with healthy well-fed *H. verbana*, the number of activated botryoidal granular cells and their fractions in absolute and in relative values decreased (46.9±1.14%) with a decrease of the mean diameter (5.3±0.23 μm, is compared to healthy well-fed and hungry leeches, $p<0.01$), they resorted to a domed skylight lacunae, which expanded (average diameter of 12.2±0.53 μm, is compared with a hungry leech, at $p<0.01$, but slightly lower compared to healthy well-fed, at $p<0.01$, Table 2) and filled with self-homogenous gray-orange hemolymph (Fig. 3.3).

In the well-fed dead *H. verbana* enhanced degenerative processes in the connective and botryoidal tissues. Connective tissue has large cellular structure in cells of which are present a significant numbers of amoeba-like cells with homogeneous and fine-grained cytoplasm. In the connective tissue are present lymphocytes-like cells, and in individual cells there are macrophage-like ones. They are especially numerous under the folds of crop. Lacunae of botryoidal tissue are sharply expanded (average diameter is 14.8±0.91 μm), Fig. 3.4. The relative content of activated granulocytes of botryoidal tissue are maximum among all studied groups of leeches (85.3±0.97%, $p<0.01$– compared with hungry and well-fed that vomited blood, $p>0.05$ – compared to healthy well-fed leeches). This type of granulocytes has a minimal diameter (4.4±0.14 μm, compared with other groups of leeches, at $p<0.01$), whereas the content and the average size of non-activated granulocytes are minimal among the studied groups, both in absolute (25.4±2.34 cells per 0.1 mm^2 tissue), and relative (14.7±0.97%) indices ($p<0.01$ – compared with hungry leeches and well-fed that vomited blood, $p>0.05$ – compared with healthy well-fed leeches), mean cell diameter – 5.5±0.15 μm, with $p<0.01$, Table 2. Also, in some intermediate lacunae of botryoidal tissue are found clusters of own hemocytes (Fig. 3.4), which probably indicates the active migration of immune cells to the site of injury, such as intestinal epithelium.

Thus, it is identified the major adverse morphological changes in the tissues of *H. verbana* during posttrophical period of well-fed leeches that vomiting eaten blood and dead ones, which may indicate immunological conflict type „GVHD" – feeder blood (human) destroyed tissues of medicinal leech. This assumption is confirmed by a number of factual evidences. First of all, the time of pathology occurrence in the second week, which coincides with the known in immunology maximum time of cellular immune responses [21]. Secondly, one of the most important signs of pathological changes in the medicinal leech should be considered distinct atrophy of the intestinal mucosa, as one of the most common signs of immunodeficiency [21,22]. For example, according to other researchers atrophy of the intestinal mucosa occurs in adult patients and children with disorders of the immune system and is accompanied by enhanced cellular infiltration, enlargement of lymphatic vessels [23,24]. In leeches, most likely, the role of lymphatic vessels carries lacunar channels of botryoidal tissue. Thus, this atrophy, even desquamation of cells, accompanied by increased infiltration of activated immune cells. Thirdly, in the connective and botryoidal tissues observed significant cellular changes. Thus, in the connective tissue of well-fed dead leeches significantly increases the number and activity of immune cells (lymphocyte- and macrophage-like cells, amebocytes, free granulocytes, and activated botryoidal granulocytes), which is accompanied by significant infiltration of their surrounding midgut (crop) tissues. Growing tissue vascularization due to activation of botryoidal granulocytes, resulting in changing architectonics of botryoidal tissue from their usual cord-like (cluster) to the tubular (hollow) structure. However, probably that in the well-fed dead leeches such activation of botryoidal tissue is not accompanied by activation of recycling hemolymph, and ends with the extension (paralysis, stagnation) of lacunar channels in the histological preparations expressed in their expansion, central location in their own hemocytes and subtle content of hemolymph.

Thus, it is identified morphological changes in the tissues of well-fed leeches with blood vomiting and well-fed dead leeches pointing to the development of pathological processes in vivo, presumably as a consequence of immunological conflict between eaten human blood and tissue microenvironment of leeches. Therefore, further research is considered perspective for the more detailed understanding of the protective mechanisms of the medicinal leech from immuno-competent tissue of feeder (blood) in the posttrophic period, and mechanisms of its disorders at death, in the case of immunological conflict, which may be genetically mediated.

Conclusions

1. At 9–14 days after HT in 9.5±0.57% medicinal leeches pathological manifestations of morphophysiological features were observed, which is resulted the death of the medicinal leeches (4.5±0.48%).
2. Morphofunctional changes in midgut (crop) of well-fed healthy *H. verbana* 12 days after feeding by human blood are in changes of the epithelium height and level of its vacuolization, and in well-fed leeches with blood vomiting and died these changes are accompanied by degenerative processes (pyknotic changes of nucleus, its fragmentation, disturbance of plasmolemma integrity).
3. Botryoidal tissue of hungry leeches is inactive, of healthy well-fed ones – responds with increased vascularization, activation of botryoidal granulocytes on receipt of the products of digestion, in well-fed vomiting and dead leeches activation processes of botryoidal tissue are associated with immune mechanisms of protection.
4. Identified structural and functional properties of tissues of healthy well-fed *H. verbana* are the result of the normal physiological processes – absorption and accumulation of plastic substances, while the destructive processes in the intestinal epithelium, botryoidal and connective tissues of well-fed leeches that vomited eaten blood and well-fed dead leeches indicate the development of immunopathological processes by type of GVHD.

References

[1] Abdullah S., Dar L.M., Rashid A., Tewari A. 2012. Hirudotherapy/leech therapy: applications and indications in surgery. *Archives of Clinical and Experimental Surgery* 1: 172-180.

[2] Sobczak N., Kantyka M. 2014. Hirudotherapy in veterinary medicine. *Annals of Parasitology* 60: 89-92.

[3] Kamenev O.Ju., Baranovskij A.Ju. 2006. Treatment by leeches: theory and practice of hirudotherapy: a guide for physicians. IG «Ves'», St. Petersburg (In Russian).

[4] Savinov V.A. (Ed.). 2004. Hirudotherapy: guide. Medicina, Moscow (In Russian).

[5] Frolov O.K., Kopijka V.V., Fedotov Je.R., Litvinenko R.O. 2011. Trophic properties ecotypes medicinal leeches in natural reservoirs and in biotechnology. *Visnyk of Zaporizhzhya National University. Biological Sciences* 1: 117-122 (In Ukrainian).

[6] Rassadina E.V. 2006. Environmentally sound biotechnological reproduction of *Hirudo medicinalis* L. under laboratory conditions. Thesis for PhD (Biology): 03.00.23, 03.00.16. Ulyanovsk (In Russian).

[7] Dul'nev G.N., Krashenjuk A.I. 2010. From the synergetics to the informational medicine. Institute of Biosensory Psychology, St. Petersburg (In Russian).

[8] Tkachev V.O., Gojman E.V., Lykov A.P., Kudaeva O.T., Kolesnikova O.P. 2006. Indicators of hematopoiesis in the dynamics of development of chronic graft versus host disease. *Immunologiya* 27: 168-170 (In Russian).

[9] Gojman E.V. 2011. Homeostatic proliferation in the development of autoimmune pathology induced by chronic reaction „graft versus host". Thesis abstract for PhD (Medicine): 14.03.09. Novosibirsk (In Russian).

[10] Romejs B. 1953. Microscopic technique: monograph. (Ed. I.I. Sokolova). Izd-vo inostr. lit., Moscow (In Russian).

[11] de Eguileor M., Grimaldi A., Tettamanti G., Congiu T., Protasoni M., Reguzzoni M., Valvassori R., Lanzavecchia G. 2001. Ultrastructure and functional versatility of hirudinean botryoidal tissue. *Tissue and Cell* 33: 332-341.

[12] Tettamanti G., Grimaldi A., Rinaldi L., Arnaboldi F., Congiu T., Valvassori R., de Eguileor M. 2004. The multifunctional role of fibroblasts during wound healing in *Hirudo medicinalis* (Annelida, Hirudinea). *Biology of the Cell* 96: 443-455.

[13] Grimaldi A., Ferrarese R., Tettamanti G., Valvassori R., de Eguileor M. 2013. Ras activation in *Hirudo medicinalis* angiogenic process. *Invertebrate Survival Journal* 10: 7-14.

[14] Kutschera U., Roth M. 2005. Cannibalism in a population of the medicinal leech (*Hirudo*

medicinalis L.). *Biology Bulletin* 32: 626-628.

[15] Ceylan M., Erbatur I. 2012. A study on nutrition of medicinal leech (*Hirudo verbana* Carena, 1820): Cannibalism? *Ege Journal of Fisheries and Aquatic Sciences* 29: 167-170.

[16] Ivanov A.V., Monchadskij A.S, Poljanskij Ju. I., Strelkov A.A. 1983. Large workshop in Invertebrate Zoology: handbook. 3th edition. Part 2: Types: Annelida, Arthropoda. Vysshaia shkola, Moscow (In Russian).

[17] Rahemo Z.I.F., Hamad N.R. 2013. Histology of the body wall of the piscine leech, *Cystibranchus mastacembeli* (Hirudinea: Piscicolidae). *Journal of Agricultural Science and Technology* 3: 136-143.

[18] de Eguileor M., Grimaldi A., Tettamanti G., Valvassori R., Cooper E. L., Lanzavecchia G. 2000. Different types of response to foreign antigens by leech leukocytes. *Tissue and Cell* 32: 40-48.

[19] Bradbury S. 1959. The botryoidal and vaso-fibrous tissue of the leech *Hirudo medicinalis*. *Quarterly Journal of Microscopical Science* s3-100: 483-498.

[20] Vetvicka V., Sima P. 2009. Origins and functions of annelide immune cells: the concise survey. *Invertebrate Survival Journal* 6: 138-143.

[21] Drannik G.N., Drannik A.G. 2011. Clinical immunology and allergology: guide for students, medical interns, immunologists, allergists, medical therapeutic profile of all spec. Responsible for the release V.P. Pshenichnyj. 5th edition. Poligrafpljus, Kiev (In Russian).

[22] Ivashkina V.T., Lapina T.L. (Eds.). 2012. Gastro-enterology. National manual: short edition. GJeOTAR-Media, Moscow (In Russian).

[23] Ovsyannikova V.V., Kozlova I.V. 2010. Clinicodiagnostic value of functional morphological changes of intestine in encephalopathy development in cirrhosis. *Saratov Journal of Medical Scientific Research* 6: 365-369 (In Russian).

[24] Moes N.D., Ruemmele F.M., Rings E.H. 2011. Autoimmune enteropathy in children. *Nederlands Tijdschrift voor Geneeskunde* 155: 32-46.

Received 28 January 2015
Accepted 26 February 2015