



PUBLICATION OF YOUNG SCIENTISTS

THE EFFECT OF SALIVARY GLAND SECRETION OF THE  
MEDICINAL LEECH *HIRUDO MEDICINALIS* ON THE  
MORPHOFUNCTIONAL STATE OF THE MESENTERY  
OF THE EXPERIMENTAL ANIMALS.

MOVSISYAN M.R.<sup>2\*</sup>, MATINYAN S.V.<sup>2</sup>, GHARAGYOZYAN T.A.<sup>2</sup>, MKRTCHYAN V.S.<sup>2</sup>,  
CHAKMISHYAN G.V.<sup>2</sup>, AVAGYAN S.A.<sup>1</sup>, ZILFYAN A.V.<sup>1</sup>

<sup>1</sup> Scientific-Research Center of Yerevan State Medical University, Yerevan, Armenia

<sup>2</sup> Students' Scientific Society, Yerevan State Medical University, Yerevan, Armenia

Received 11/05/2014; accepted for printing 02/28/2015

ABSTRACT

Structural changes in the connective tissue and vessels of microhemocirculation of mesentery have been studied from morphofunctional (morphological, morphometric and fluorescent microscopy) positions. This study involved placement of medicinal leeches *Hirudo medicinalis* on the skin surface of intact rats.

It was found that in a relatively early period of the experiment (after 20 minutes and 4 hours after placement of leeches) catabolic reactions appeared. Their structural manifestations appeared in the process of increased permeability of the arterioles and venules which led to perivascular edema and infiltration of the connective tissue matrix by lymphoid cells. The signs of increased permeability of microcirculatory vessels of mesentery were expressed by degranulation of tissue basophils and extensive release of histamine in the pericapillary space. It should be pointed out that microcirculatory disorders were temporary, as far as 24 hours after using leeches; the condition of microhemocirculatory ways was normalized. Indicators of the histamine levels in the tissue basophils of mesentery were also normalized.

An important role in this process belongs to the «mast cell-eosinophil association», whose activity prevented high intake of histamine in the perivascular space.

**Keywords:** microhemocirculatory vessels, tissue basophils, medicinal leech, histamine release, mast cell-eosinophil associations.

INTRODUCTION

It has been long believed that medicinal leech *H. medicinalis* is a highly effective remedy of symptomatic therapy against many diseases and primarily the diseases of cardiovascular system. The first data on the use of medical leeches belongs to ancient Egypt. Hirudotherapy was used for the treatment of various diseases in many countries, including China, India, Greece, Rome, Persia, Egypt, etc., in the days of Hippocrates,

Pliny, Galen, Antillius, Avicenna and other physicians [Iveson-Iveson J, 1978; Payton BW, 1981, 1984; Leake LD, 1983].

Leech treatment was also highly appraised by ancient Armenian doctors [Vardanyan SA, 1976]. Renowned Armenian physician and scientist Amir-dovlat Amasiatsi pointed out the effectiveness of leeches in the presence of dandruff, "yellow" tumors and inflammation of eyelids (work published in Vienna in 1926). Ordinary people believe that the leech sucks "dirty" blood, providing anti-inflammatory and analgesic effects. This method was used for mastitis, ear diseases, inflammation of eyes, headaches, joint and bone pains, swelling of any part of the body [Isakhanyan GS, Harutyunyan VM, 1991].

ADDRESS FOR CORRESPONDENCE:

Students' Scientific Society, Yerevan State Medical University after Mkhitar Heratsi  
2 Koryun Street, Yerevan 0025, Armenia  
Tel.: (+374 55) 24-74-54  
E-mail: sssamsp99@gmail.com

According to some research data, the leech is acknowledged as a “pharmaceutical mini plant”, manufacturing a balanced complex of bioactive compounds exerting broad-spectrum effects on human organism. Its beneficial results are health improvement and body rejuvenation.

There are also a few experimental data indicating that saliva of medical leeches has burn-healing effects [Popov NS et al., 2014]. These effects of the saliva are explained by high activity of biological compounds produced in their organism [Baskova IP et al., 1984; Baskova IP, Nikonov GI, 1987; Nikonov GI et al., 1998; Seleznev KG, Nikonov GI, 1999].

Despite the widespread use of leeches (and/or drugs made from saliva of salivary glands) in clinical medicine, their side effects on organs of mammals are not almost studied. There are only a few reports that mention the ability of saliva to stimulate the degranulation of test rats' skin tissue basophils *in situ* conditions [Baskova IP et al., 2005]. However, the results obtained by the authors didn't find the required interpretation neither with the position of forming local adaptive reactions nor in terms of their pathogenic effect on specific target cells of rats. Moreover, the nature of extremely undesirable visceral manifestations, which appear in organism in a single, and furthermore, in multiple use of saliva of *H. medicinalis*, is not fully investigated.

The aim of the current research is to study the morphofunctional condition of the mesentery of experimental animals under the influence of the secretion of salivary glands of *H. medicinalis*.

#### MATERIALS AND METHODS

The experiments were performed on 60 white male rats, weighing 130-150 g. The animals were subdivided into control and test groups. Control group was comprised of intact rats. In animals of test group the leeches were placed on the shaved skin area.

The test group animals were removed from the experiment 20 minutes, 4 hours and 24 hours after the removal of leeches.

During the entire experiment, all the laboratory animal care rules including euthanasia were observed in accordance with the YSMU ethics committee requirements. Mesentery was the study objective in both groups of animals. Membranous preparations of mesentery were stained with hema-

toxylin-eosin, toluidine blue and azur II-eosin. Intravascular tissue basophils were counted in 10 fields of view of each preparation with objective 20, ocular 10.

Tissue basophils stained with toluidine blue were subdivided into two cell groups without signs of degranulation and with different degree of degranulation: tissue basophils with signs of single degranulation (I degree), tissue basophils with signs of partial degranulation (II degree), tissue basophils with signs of expressed degranulation (III degree) and tissue basophils with signs of full degranulation (IV degree).

The fluorescent microscope analysis was run on unfixed flat specimens of mesentery, processed by ortho-phthalic aldehyde, produced by "Sigma", USA, to reveal the histamine in basophils.

Quantitative analysis of histamine was conducted in 20 tissue basophiles of each specimen using luminous microscope "LUMAM I-3" and fluorescence metric nozzle "FMEL-1 A", establishing the light filter length of 445 nm which corresponded to a maximum of fluorescence of histamine; the amount of histamine was expressed in relative fluorescence units [Falck B, Owman C, 1965].

The statistical analysis was based on Student's t-test, one-way ANOVA, using Windows SPSS 13 software.

#### RESULTS

The results of morphological observations showed that catabolic processes were dominating in mesentery 20 minutes and 4 hours after placement of leeches on the skin of test animals. Structurally they were manifested by moderate diffuse edema of connective tissue matrix, focal dissociation of collagen fibers, symptoms of dystrophy of endotheliocytes, increased vascular permeability in all the integral components of the microvascular bed of the arterioles, venules and capillaries.

Processes of a plasmorrhagia were followed by the appearance of focal leuko- and erythrodiapedesis with primary location of the migrating cells in the pericapillary and perivascular spaces. Perivascular oriented tissue basophiles (of the mesentery) were mostly in a condition of moderated and expressed degranulation. (Fig. 1b, table 1). As a result, small metachromatic linearly situated granules were observed in pericapillary spaces approx-

TABLE 1.

Values of test animals' mesentery tissue basophiles degranulation after the placement of leeches

Trial periods	Without signs	Degranulation stages			
		I	II	III	IV
Control group	23.7±3.3	38.8±6.0	22.4±3.7	10.9±2.4	4.0±1.1
20 minutes	10.2±1.9 0.005>p>0.0005	14.9± 2.8 0.005>p>0.0005	20.2±2.5 0.4>p>0.25	37.4±3.3 p<0.0005	17.0±1.9 p<0.0005
4 hours	17.1±3.1 0.10>p>0.05	13.5±4.2 0.005>p>0.0005	36.2±3.21 0.005>p>0.0005	22.1±2.4 0.01>p>0.005	10.6±2.4 0.025>p>0.01
24 hours	19.0±2.4 0.25>p>0.10	35.2±2.6 0.4>p>0.25	24.3±5.6 0.4>p>0.25	15.9±1.75 0.10>p>0.05	5.2±1.3 0.4>p>0.25

Note: *p* - in relation of test group values to respective control group values.

imately along the whole length of microvessels.

As shown in Table 1, 20 minutes and 4 hours after the placement of leeches the content of tissue basophiles without degranulation signs was considerably reduced and was 2.3 and 1.4 times lower than the control level. The reduction occurred due to significant increase in the content of tissue basophiles with third and fourth stages of degranulation, the values of which were 3.4 and 2.0 times higher than the control ones. Tissue basophiles with low degree of degranulation (I and II degrees) were predominant in the mesentery of the test animals after 24 hours.

In comparison with other periods of experiment (after 20 minutes and 4 hours) after the placement of leeches the content of tissue basophiles with III and the IV degrees of degranulation noticeably decreased (their values were respectively 1.4 and 2.0 times lower than the control group's).

The results of the fluorescent microscope analysis showed that the content of histamine (table 2) in tissue basophiles of a mesentery of the test animals 20 minutes and 4 hours after placement of leeches considerably decreased.

Thus, the histamine content was respectively 1.8 and 2.1 times lower than the control level. The content of histamine considerably increased in tissue basophiles (compared to previous trial periods) 24 hours after the placement of leeches, reaching the control level.

It should be noted that processes of the expressed degranulation of tissue basophiles were followed by emission of histamine into pericapillary space. It was structurally manifested by the presence of small granules alongside the arterioles, and more precisely, venules and capillaries (Fig. 2b, 3a).

Changes of regeneration were observed in cellular

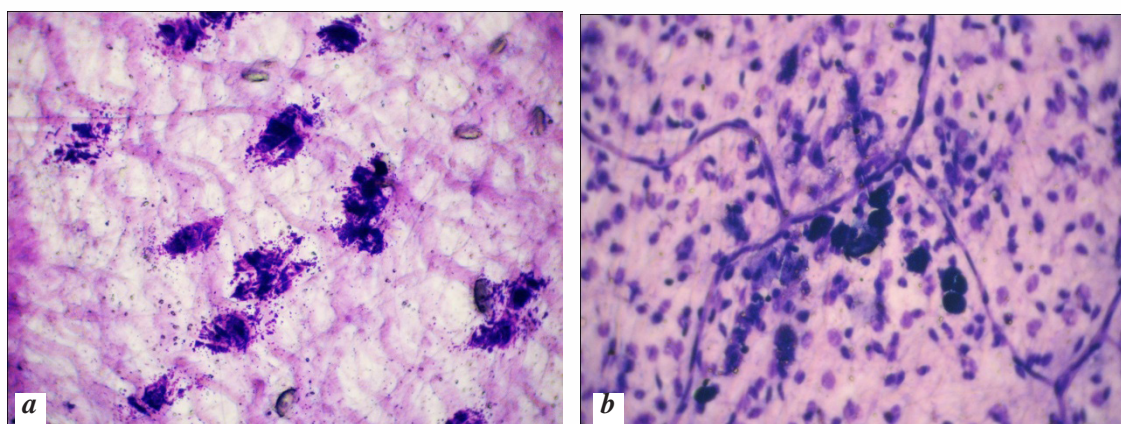


FIGURE 1. Tissue basophils of rat mesentery after placing leeches on the skin surface. Hematoxylin and eosin staining (ob. 40, oc. 10.): a) Control group rats. Tissue basophiles without sign of degranulation and cells with I stage of degranulation dominate in connective matrix of mesentery, b) Experimental group rats (on the 20th minute of observation). Expressed tissue basophiles degranulation (II to III stage).

TABLE 2.

The content of histamine in test animals' mesentery tissue basophiles after the placement of leeches

Trial periods	Indicators of histamine in relative fluorescence units
Control group	42.3±4.5
20 min	23.5±3.5
	0.005>p>0.0005
4 hours	20.4±2.45
	p<0.0005
24 hours	36.2±2.7
	0.25>p>0.10

Note: p - in relation of three test group values to control group values

and non-cellular components of connective tissue of mesentery 24 hours after the placement of leeches.

The edema of tissue considerably decreased because the increased permeability in the blood microcirculatory bed was expressed only locally and were manifested only by plasmorrhagia. The number of intercellular anastomoses considerably increased. Tissue basophiles with mostly very low degree of degranulation in the mesentery connective tissue matrix were most prevalent unlike in the previous observation periods, as shown above.

The high extent of degranulation was observed only in single fields of vision, especially in places of formation of intercapillary anastomosis. It should be noted that in these sites, in close proximity to tissue basophiles, accumulations of the eosinophils were detected (Fig. 3b). As it was shown earlier, the tendency aimed at restoring the level of histamine in

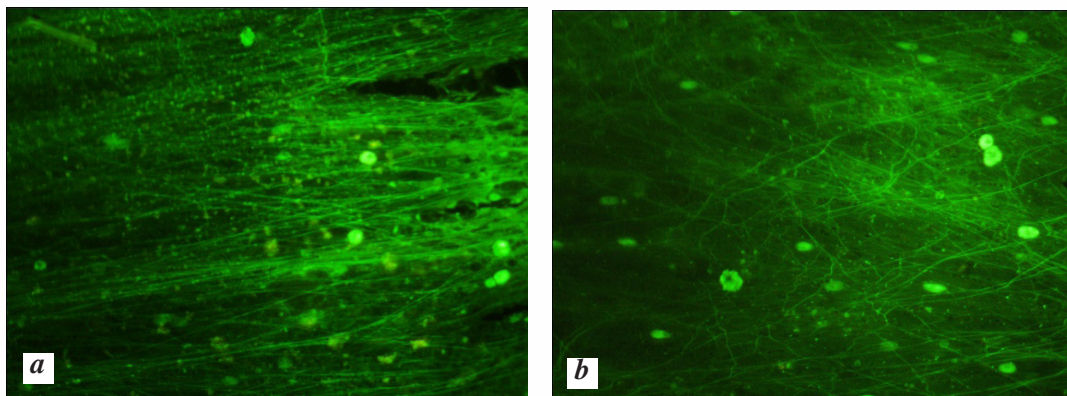


FIGURE 2. Topical features of histamine distribution in tissue basophils of rat mesentery after placing leeches on lower back skin surface. Fluorescent microscopy (ob. 40, oc. 10.): a) Control group rats. Intense specific granulation and/or homogeneous fluorescence in the cytoplasm of majority of tissue basophils of connective tissue matrix of mesentery, b) Test group rats (on 20<sup>th</sup> minute of observation). Expressed degranulation of tissue basophils with histamine-containing granules in the connective tissue matrix of mesentery.

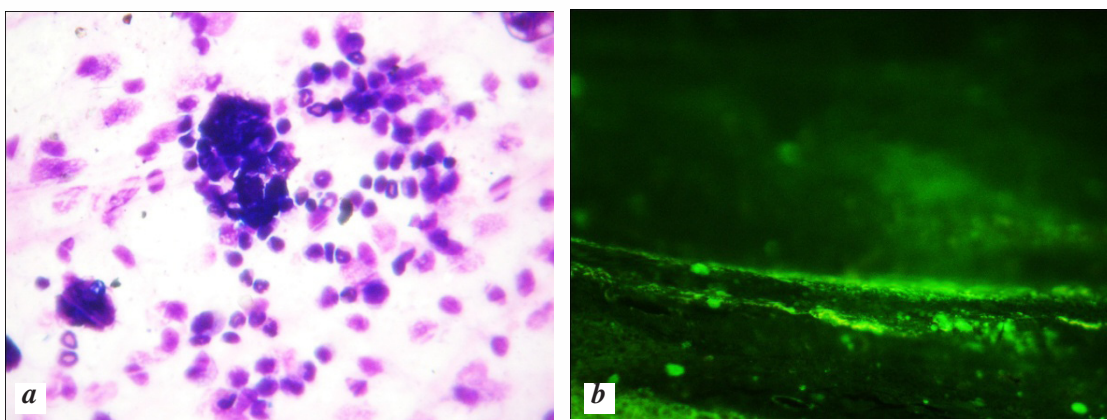


FIGURE 3. Structural alterations of rat mesentery (test group) on the 20<sup>th</sup> minute of observation and after 24 hours after the placement of leeches on the skin surface: a) Test group (on the 20<sup>th</sup> minute of observation). Presence of histamine-containing granules in capillary wall and perivascular space. Fluorescent microscopy: ob. 40, oc. 10., b) Test group (on the 24<sup>th</sup> hour of observation). Infiltration of connective tissue matrix of mesentery with inflammatory cells containing eosinophilic leucocytes among the lasts. Azur II-eosin staining: ob. 40, oc. 10.

tissue basophiles was observed. When performing the immunofluorescent assay the histamine in tissue basophiles was detected in the form of the homogeneous and/or fine-granular specific fluorescence located along the entire perimeter of a cytoplasm.

#### DISCUSSION

The morphological, morphometric and the fluorescent microscopic studies allowed us to conclude that placement of leeches on the surface of skin, within the first four hours, was followed by activation of local catabolic processes which were structurally expressed by onset of dystrophic changes in the connective tissue of mesentery. The complex of symptoms revealed by us was found in the presence of increased vascular microcirculation permeability, in the mechanism of appearance of which emphasis should be made on tissue basophiles degranulation processes in the condition of enhanced supply of histamine into pericapillary space.

Of special significance is the fact that the onset of dystrophic changes within the first four hours was of transient nature, because 24 hours later the

angioarchitecture of mesentery was restored to a considerable extent. So, in particular, permeability of microvessels considerably decreased (in comparison with that of mesentery after the 4-hour observation period). The similar tendency directed at normalization of vascular permeability in many cases was dependent on a functional condition of tissue basophiles in which signs of degranulation occurred much less often, whereas the content of histamine in the cells was higher. The migration of the eosinophilic leukocytes in pericapillary space indicates the formation of adaptive mechanisms participating in processes of normalization of earlier increased permeability. It is considered for a long time that tissue basophiles act in association with eosinophilic leukocytes in regulation of vascular permeability at the level of ways of microcirculation. The eosinophils provide the "balanced" supply of histamine to the pericapillary space, either by way of capture, or by secretion of histaminase resulting in histamine "inactivation". As a result, the high vasoactive effect of histamine of the vessels on microcirculation is neutralized.

#### REFERENCES

1. Baskova IP, Nikonov GI. [Detection of prostaglandins in the preparations of medicinal leeches (*Hirudo medicinalis*)] [Published in Russian]. Pres. In Academy of Sciences in SSUR. 1987;292(6):1492-1493.
2. Baskova IP, Yusupov GI, Nikonov GI. [Lipase and cholesterol esterase activity of salivary gland secretion of medicinal leeches *Hirudo medicinalis*] [Published in Russian]. Biokhimiya. 1984;49(4):676-678.
3. Baskova IP, Zavalova LL, Klimovich LG., et al. [Effect of secretion of the salivary glands of medicinal leeches on the state of the mast cells of rat subcutaneous tissue] [Published in Russian]. Rosiskij fiziologicheskij zhurnal imeni Sechenova. 2005;91(2):195-207.
4. Falck B, Owman C. A detailed methodological description of fluorescence method for cellular demonstration of biogenic monoamines. Acta Univ Lund. 1965;2(7):1-12.
5. Isakhanyan GS, Harutyunyan VM. [Medicinal leeches: their remedial use in medical therapy] [Published in Russian]. Ter Arch. 1991;63(8):110-112.
6. Iveson-Iveson J. Leeches in medicine. Nursing mirror. 1978;22:8-9.
7. Nikonov GI. [Biologically active substances produced by medical leeches, and their mechanisms of action] [Published in Russian]. Asklepeyon, 1998;1(4):29-41.
8. Payton BW. History of medicinal leeching and early medical references. In: Neurobiology of the leech. Ed. Muller KJ, Nicholls JG, and Stent GS. New York: Cold Spring Harbor Laboratory Press, 1981. 27-34.
9. Popov NS, Demidova MA, Shestakov VG., et al. [Influence of water extraction from the medical leech lyophilized on reparative processes in thermal skin lesions in rats] [Published in Russian]. Verkhnevolzhskiy Medicinskij zhurnal. 2014;12(2):45-50.
10. Seleznev KG, Nikonov GI. [Efficiency of biologically active compounds produced by medicinal leeches, in preparations for external use] [Published in Russian]. Arch clin and experiment. 1999;8(1):53-58.
11. Vardanyan SA. [The use of leeches in Armenian folk medicine] [Published in Armenian]. Aroghchapahutyun. 1976;2:50.