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# The Asymmetry Phenomenon in Responses of Frog Lingual Microvessels

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Qualitative evaluations and measurements of morphometric parameters (area, length, and diameter) performed in symmetrical fragments of the vascular bed of 30 frog tongues before, during, and after the production of local ischemia on the ipsi- or contralateral side reveal morphological and functional asymmetries in microvessels on the two sides of the tongue. Two groups of individuals, tentatively designated as "right-dominant" and "left-dominant," are identified.

**Key Words**: *microcirculation; morphometry; asymmetry; ischemia*

In studying postischemic disturbances of the microcirculation in paired organs [6], our attention was drawn to one object in which the right and left microcirculatory beds could be observed in a single experiment, namely the microcirculatory bed (MCB) of the tongue. It goes without saying that the tongue has an axis of symmetry - this is evidenced by anatomical data. The blood supply to the left half of the tongue and that to its right half are largely independent of one other, as was demonstrated when lingual vessels were stained *intra vitam* [7]. The property of autonomy shown by the symmetrical halves of the tongue has been utilized in chronic experiments with cleft tongues of frogs to study how the activity of the taste receptors is regulated [4,5], and also in experiments with tongues of rats to gain information on the relationship between the gustatory response and blood flow [7, 8]. In these studies no attempt was made to identify the symmetrical sides of the tongue. However, when the right and left halves of the human tongue were compared, a considerable asymmetry in the distribution density of gustatory papillae was found [3]. That the human system of blood vessels is functionally asymmetrical was known even to the school of Salerno in medieval times [2]. The present study was undertaken to identify the lingual MCB on the right and on the left.

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## MATERIALS AND METHODS

The study was conducted on 30 male frogs with destroyed spinal cord. All measurements were made

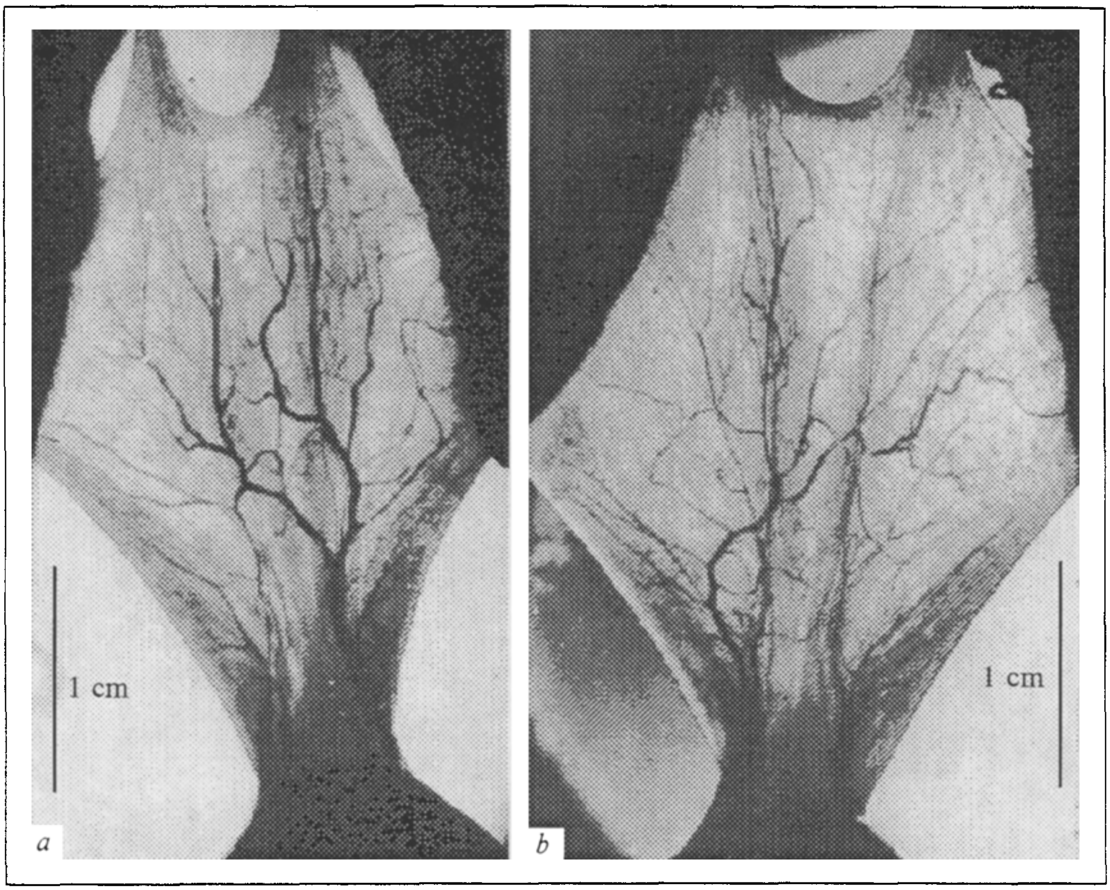


**Fig. 1.** Two structural types of frog lingual vascular bed. *a*) right – dominant; *b*) left – dominant.

at room temperature. The tongue was spread out and fixed with needles, and its ventral surface, where the blood vessels are clearly visible, was inspected. The plane of the lingual MCB and its fragments in the symmetrical halves were then photographed in transmitted light at a magnification of ×63. Quantitative analyses were carried out for a total of 85 MCB fragments before, during, and after the interruption of blood flow for 5 min on the ipsilateral or contralateral side. A total of 96 venules and 242 arterioles fell into the field of vision. Morphometric parameters of the MCB were estimated from its images using stereometric methods of analysis [1]. Areas and unit lengths of the microvessels and diameters of the venules and arterioles were determined. The significance of de-

**TABLE 1.** Parameters of Symmetrical Fragments of Frog Lingual Microcirculatory Bed before Testing ($M \pm m$)

| Parameter | Groups of frogs and sides of observation | | | | | |
|---|---|---|---|---|---|---|
| | Right-dominant | | | Left-dominant | | |
| | right side (1) | left side (2) | 1:2 ratio | right side (1) | left side (2) | 1:2 ratio |
| Mean area of microvessels, mm$^2$ | 0.966±0.158 | 1.176±0.071 | 1.217 | 1.064±0.069 | 0.904±0.113 | 0.858 |
| No. of fragments | 8 | 10 | | 13 | 9 | |
| Unit length of microvessls, mm/mm$^2$ | 0.061±0.005 | 0.087±0.008 | 1.418 | 0.071±0.008 | 0.061±0.008 | 0.869 |
| No. of fragments | 8 | 10 | | 13 | 9 | |
| Mean diameter of venules ($D_v$), μ | 124.2±8.9 | 138.6±7.7 | 1.115 | 127.3±12.4 | 120.1±12.3 | 0.943 |
| No. of venules | 10 | 9 | | 17 | 8 | |
| Mean diameter of arterioles ($D_a$), μ | 57.8±5.8 | 54.9±4.6 | 0.949 | 52.1±4.6 | 55.5±5.4 | 1.066 |
| No. of arterioles | 19 | 27 | | 44 | 22 | |
| $D_a/D_v$ ratio | 0.465 | 0.395 | | 0.409 | 0.462 | |

**Note.** All differences between the two groups in parameters on the left and right are significant at $p<0.05$.

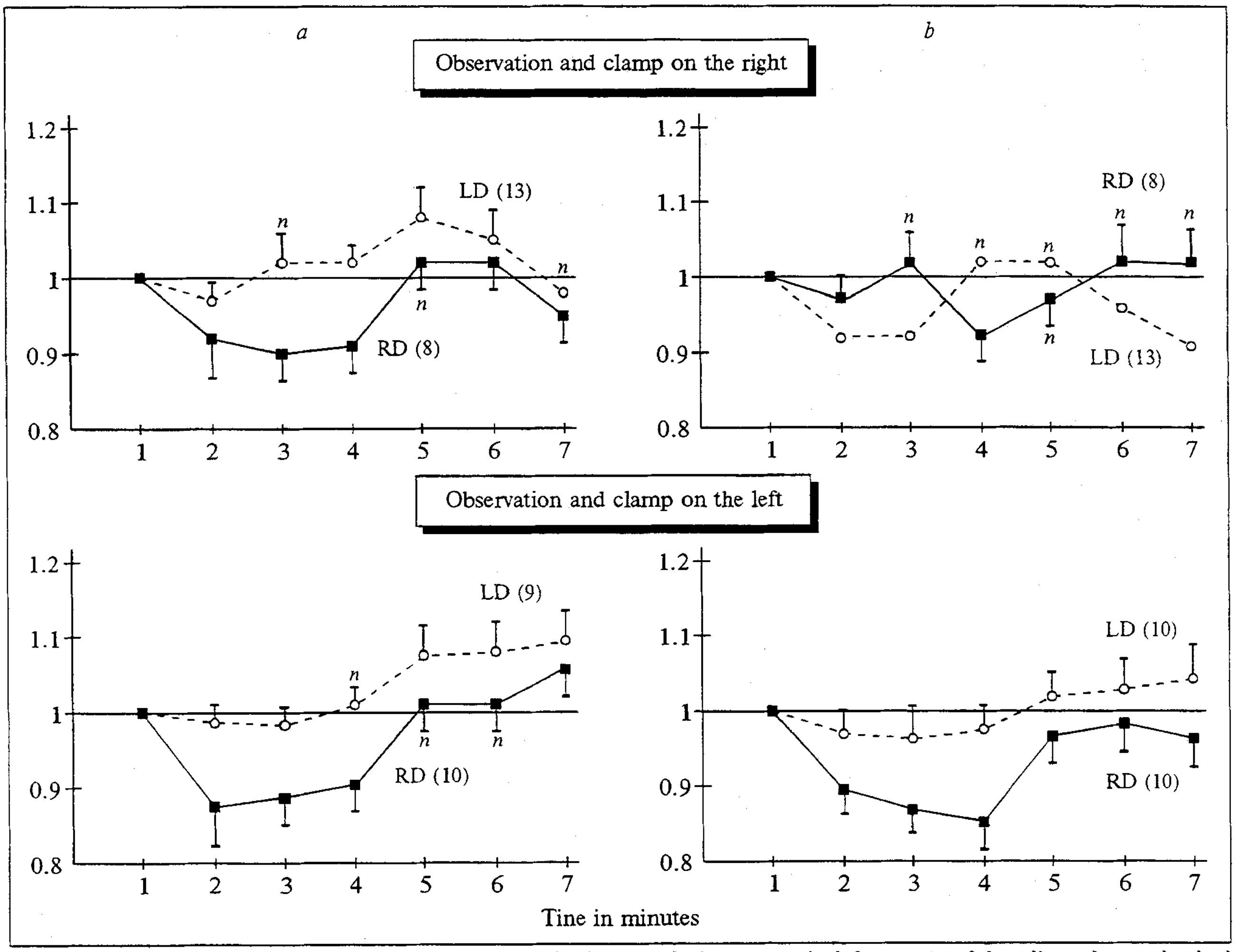


**Fig. 2.** Temporal variations in the area and length of microvessels in symmetrical fragments of frog lingual vascular bed after interruption of blood flow on the ipsilateral side. Ordinate: area (*a*) and length (*b*) of microvessels in relation to their values at the start of observation. *1*) start of observation; *2*) 1.5 min after clamp application; *3*) 3 min after application; *4*) 5 min after application; *5*) just after the clamp was removed; *6*) 1 min after resumption of blood flow; *7*) 2–3 min after blood flow resumption. The plots were constructed from mean values, and the error of the mean is shown as either + or –; figures in parentheses indicate the number of fragments for each point on the curve; *n*: nonsignificant deviation, $p>0.05$; *RD*: right–dominant; *LD*: left–dominant.

viations was evaluated by Student's test for conjugate pairs.

## RESULTS

Despite considerable individual variations in the structure of the lingual vascular bed, two types of structures were identified (Fig. 1, *a* and *b*). One type, in which the proximal part of the tongue's right half contained more powerful vessels than the distal part and which was linked up with the left half through branchlets of the vessels running from right to left, was tentatively designated as "right-dominant" (RD). The other, reverse, type was designated as "left-dominant" (LD). The number of frogs with each type was almost the same, and these two groups will be referred to below as RD and LD, respectively.

Qualitative differences between the two groups were already apparent at the beginning of the study, after destruction of the spinal cord: in RD individuals, unlike in LD frogs, the restoration of the lingual circulation proceeded faster in the right half of the tongue. On postmortem examination, the right MCB of RD individuals was filled with blood and the left MCB appeared pale, whereas the reverse was true of LD individuals.

Morphometric parameters of microvessels measured before the tests were started are shown in

**Fig. 3.** Temporal variations in the diameter of microvessels in symmetrical fragments of frog lingual vascular bed after interruption of blood flow on the ipsi– or contralateral side. Ordinate: venular (*a*) and arteriolar (*b*) diameters in relation to their values at the start of observation; figures in parentheses indicate the number of vessels for each point on the curve. Other designations as in Fig. 2.

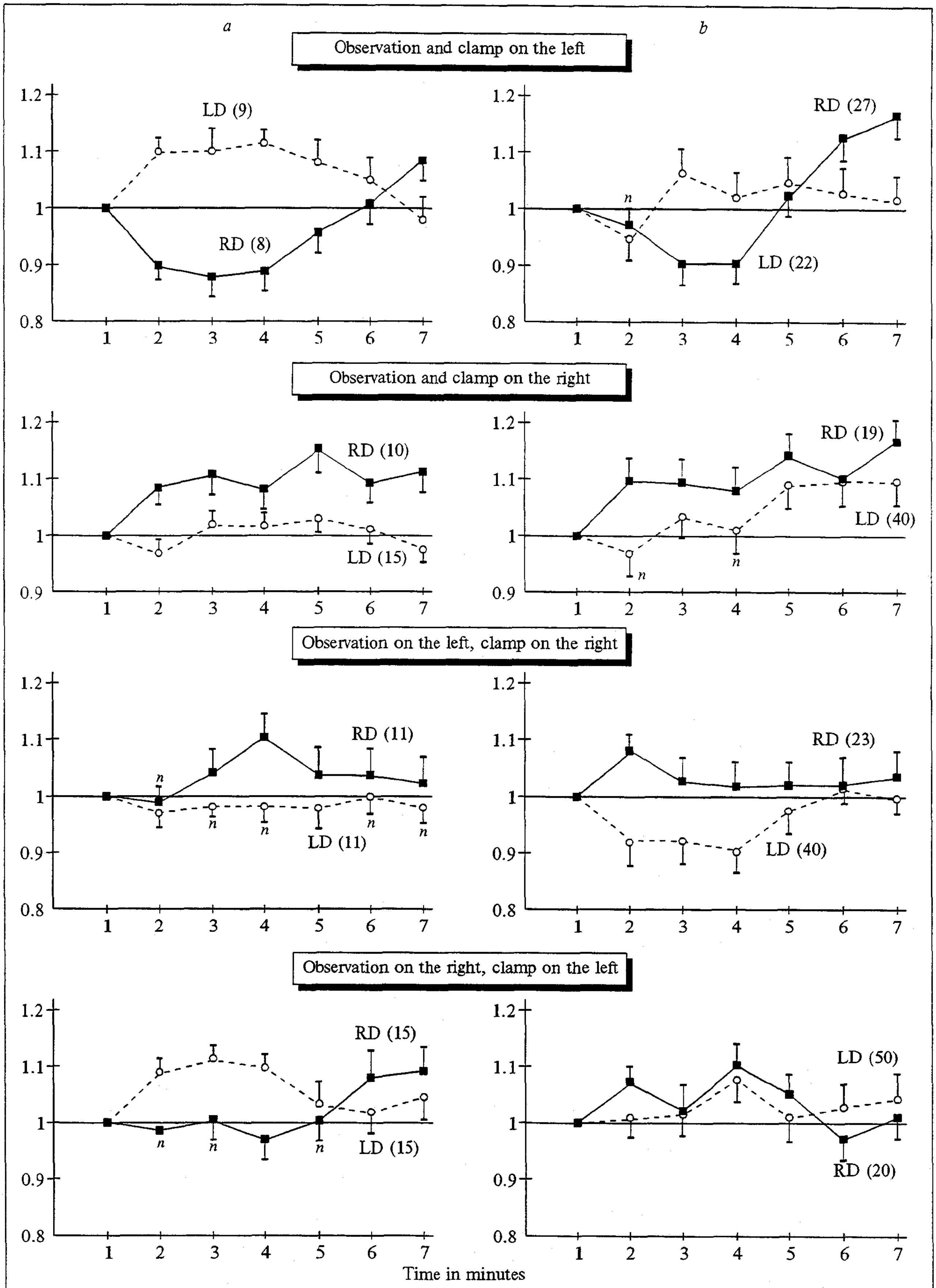
a
b
Observation and clamp on the left
LD (9)
RD (8)
RD (27)
LD (22)
Observation and clamp on the right
RD (10)
LD (15)
RD (19)
LD (40)
Observation on the left, clamp on the right
RD (11)
LD (11)
RD (23)
LD (40)
Observation on the right, clamp on the left
RD (15)
LD (15)
LD (50)
RD (20)
Time in minutes

Table 1. In the left lingual MCB of RD individuals, as compared to the right MCB, the density of microvessels was 21.7% higher, their length 41.8% greater, the mean venular diameter 11.5% greater, and the mean arteriolar diameter 5.1% smaller, but the number of arterioles was almost 50% greater. In LD individuals, such percentage differences between the right and left sides were less marked but the vascular pattern was again asymmetrical.

Figure 2 shows variations in the areas and lengths of lingual microvessels when blood flow on the ipsilateral side was stopped for 5 min. In RD individuals, the decrease in the area of microvessels on the left correlated with that in their length - similarly to what we observed previously for the MCB of rabbit ear [6]. No such correlation was noted on the right. In LD individuals, the functional asymmetry was slight or absent.

Figure 3 depicts variations in the mean diameters of lingual feeding and draining microvessels in response to blood flow interruption for 5 min on the ipsi- or contralateral side. In the right MCB of RD individuals, the prevailing response of venules to the cessation of blood supply on the ipsilateral side was that of dilation, although some heterogeneity in their reactions was noted. Persistent dilation by 12-27% was shown by most venules (7 out of 10) with a mean diameter of 121.4±10.2 μ and persistent constriction (5-15%) by those with a mean diameter of 132.0±26.9 μ. In the left MCB, by contrast, the prevailing response was constriction. Persistent constriction (6-19%) was observed for venules of 137.2±9.3 μ diameter, and only one venule, with a diameter of 150.0 μ, was seen to dilate (6-10%). In LD individuals, the prevailing response to the interruption of ipsilateral blood flow was venular dilation on the left (similar to what was observed for RD individuals on the right), but no venular constriction was noted on the right.

In tests where vessels on the contralateral side were compressed, it was found that the interruption of blood flow on the left did not lead (while the vessels remained compressed) to alteration of the venular diameter on the right in RD individuals but caused venular dilation in LD frogs. Blood flow interruption on the right resulted, after some delay, in venular dilation on the left in RD but not in LD individuals (Fig. 3, *a*).

It follows from Fig. 3, *b* that when there was no blood flow on the ipsilateral side in RD individuals, the mean arteriolar diameter did not change appreciably on the right. However, some arterioles of 50.0±5.0 μ diameter exhibited persistent dilation (11-20%) and some larger arterioles (61.1±10.9 μ), persistent constriction (12-24%). On the left, arterioles responded, on average, by constricting, but not until 3 min or so after blood flow interruption. Persistent constriction (17-24%) was shown only by arterioles of 59.7±7.0 μ diameter and persistent dilation (15-18%), by those 48.4±5.5 μ in size. In LD individuals, the prevailing arteriolar response on the right and left sides was dilation. On both sides, persistently dilated were arterioles with smaller diameters (42.7±3.2 μ on the right and 46.9±7.3 μ on the left) and persistently constricted, those with larger diameters (73.3±11.1 μ on the right and 67.3±8.1 μ on the left).

In RD individuals, arterioles on the left side responded to the interruption of contralateral blood flow predominantly by constriction (as they did in response to the interruption of ipsilateral blood flow), but the constriction was persistent only in the group of arterioles with a mean diameter of 66.1±7.9 μ; in the group with a diameter of 51.7±7.1 μ persistent dilation was noted. In LD individuals, the prevailing response was dilation. Persistent dilation (18-22%) occurred with arterioles of 47.7±4.7 μ diameter and persistent dilation (15-18%), with those 55.8±6.1 μ in size.

Finally, when blood flow was stopped on the left, arteriolar dilation on the right in RD individuals (Fig. 3, *b*) was only evident by minute 5 of ischemia. At that time, dilation was shown by arterioles 46.4±7.4 μ in diameter and construction by larger arterioles (67.9±12.1 μ). In LD individuals, the arteriolar response on the right differed little from that in LD individuals. Persistent dilation was shown by arterioles of 52.0±5.4 μ and persistent constriction by larger vessels (60.4±5.4 μ).

Thus, arteriolar constriction in RD frogs occurred in larger vessels in the left half of the tongue, irrespective of the side on which blood flow was stopped. Possibly, this can be explained by the greater density of oxygen-sensitive chemoreceptors in arterioles of the left vascular bed [8]. The predominance of constriction in the vascular system on the left and of dilation on the right may account for the differences observed in the patterns of paired vascular beds in asphyxia.

The results of this study indicate that the vascular bed of the tongue should be looked upon as a system of left and right vessels with the vessels of each side having their particular attributes and influencing those of the other side in distinct ways; the tongue, therefore, should be studied as a paired organ. The findings also show the need for separating individuals into RD and LD types, since the latter type has special features. Moreover, it should be noted that the proportions of RD and LD individuals in the frog population sample studied were almost equal, whereas the proportion of left-dominant individuals in the human populations is only between 1% and 7%.

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# Morphological Study of the Effects of Textile-Immobilized Enzymes on an Experimental Purulent Wound

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Morphological changes in an experimental purulent wound in a rat model is studied for application of surgical gauze with immobilized enzymes: trypsin, lysozyme, collitin, or co-immobilized trypsin and lysozyme. Comparison of the times of wound cleansing and healing shows that immobilized enzymes are more effective than native preparations, and the therapeutic effect of gauze with the enzyme complex is higher than that of gauze with individually immobilized enzymes. Morphological studies confirm that immobilized trypsin-lysozyme complex and collitin are the most efficient in hastening and potentiating reparative processes in a purulent wound.

**Key Words:** *purulent wound; healing; immobilized enzymes*

Proteolytic enzymes immobilized on naturally occurring materials have found a wide application in the management of purulent wounds. The therapeutic effects of trypsin immobilized on cellulose or polycaproamide (Dalcex-trypsin and Pax-trypsin, respectively) have been studied in detail [2,5]. New types of wound dressings with immobilized enzymes displaying complex therapeutic activity have been developed at the Institute of Textiles. Dalcex-trypsin-lysozyme is surgical gauze onto which the proteolytic enzyme trypsin and the bacteriolytic enzyme lysozyme are immobilized. This dressing has not only necrolytic but also bacteriolytic activity. Dalcex-collitin is gauze on which the proteolytic polyenzyme preparation collitin is immobilized. Collitin possesses trypsin-like, chymotrypsin-like, and elastase-like activities [4]. These materials have passed clinical trials; however, there is no available information regarding their effects on wound healing, and neither morphological studies nor comparison with other conventional dressings (for example, Dalcex-trypsin) have been performed.

This study is a morphological comparison of the efficiencies of different enzymes immobilized on surgical gauze in the treatment of an experimental wound.

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