GLOBULIN TRANSPORT BY THE MICROCIRCULATORY SYSTEM UNDER NORMAL CONDITIONS AND IN STRESS

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KEY WORDS: microcirculation; vascular permeability; lymphatic microvessels; stress.

Despite the importance of the study of transport of materials in the microcirculatory system of blood vessels–interstitial tissue–lymphatic microvessel, under normal and pathological conditions [8], there have been few such investigations [1, 6, 8, 12, 13]. Different types of stress are known to cause an increase in venular permeability [3-5]. It has been shown that various physiologically active substances (PAS), synthesized in large quantities during stress, stimulate contractile activity of lymphatic microvessels [10, 11, 14]. It has also been found that heat stress increases lymphatic drainage because of an increase in the inflow of lymph from the cutaneous and visceral lymphatic vessels [15]. However, the specific character of the processes involved in transport of macromolecules at the level of the microcirculatory system is not clear.

The aim of this investigation was to study globulin transport in the system of venule–interstitial tissue–lymphatic microvessel in normal animals and during immobilization stress.

EXPERIMENTAL METHOD

Experiments were carried out on 80 male Wistar rats weighing 200-250 g. Immobilization of the animals in the supine position for 1, 5, 9, and 24 h was used as the extraordinary stimulus. Permeability of the venules and lymphatic microvessels was studied by quantitative contact luminescence biomicroscopy on a system mounted on the base of the LYUMAM KF-1 (LOMO, Leningrad) microscope. Rabbit globulin (60 mg), labeled with fluorescein isothiocyanate (FITC), injected intravenously in 1 ml of physiological saline, was used as a marker of disturbances of vascular permeability. Dried luminescent serum against horse globulins was used for this purpose. The intensity of luminescence was estimated quantitatively by means of an FMÉL-1 photometric attachment of the microscope. Contact objective 10 and ocular 7 were used. The test objects were mesenteric microvessels. The measurements were made as follows. After the area of observation had been found, in which there were a contracting lymphatic microvessel 80-100 μ in diameter and a venule 20-30 μ in diameter, the intensity of the background fluorescence of the tissue was measured. For this purpose, a 0.5 mm probe was located consecutively at 3 points near the wall of the venule, after which it was moved into the lumen of the lymphatic microvessel (Fig 1, points 1, 2, 3, and 4). The diameter of the area for photometry with the magnification mentioned above was 50 μ. Measurements were then made at these points 1, 3, 5, 7, 10, 15, 20, and 30 min after intravenous injection of the luminescent serum. The increase in the intensity of luminescence was expressed as a percentage of the initial background level, taken as 100%. The number of contractions of the lymphatic vessel studied was counted in the course of 30 min.

The types of the lymphatic microvessels were not determined histologically. However, it could be concluded from the decrease in the distances between the valves to 90-100 μ that the microvessels studied belonged to the initial lymphatic microvessels and not to the lymphatic postcapillaries. Additionally, in a special series of experiments, on a system mounted on the base of a "Docuval" microscope ("Carl Zeiss," East Germany) the effect

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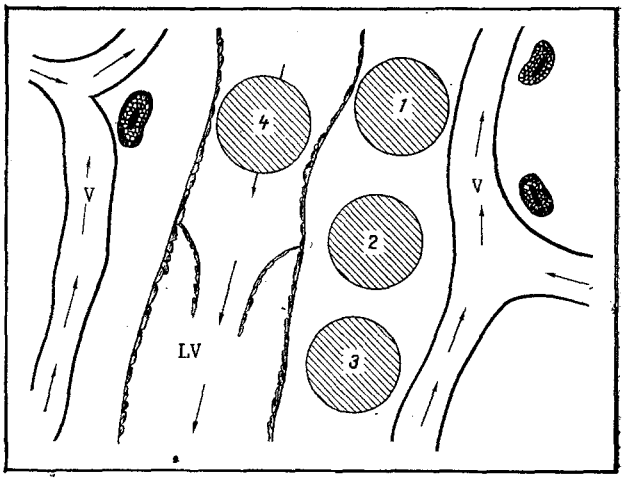

Fig. 1. Diagram of area of microcirculatory bed studied experimentally by contact luminescence biomicroscopy. LV) Lymphatic microvessel, V) venule; 1, 2, 3, 4) areas in which photometry was carried out in the order stipulated.

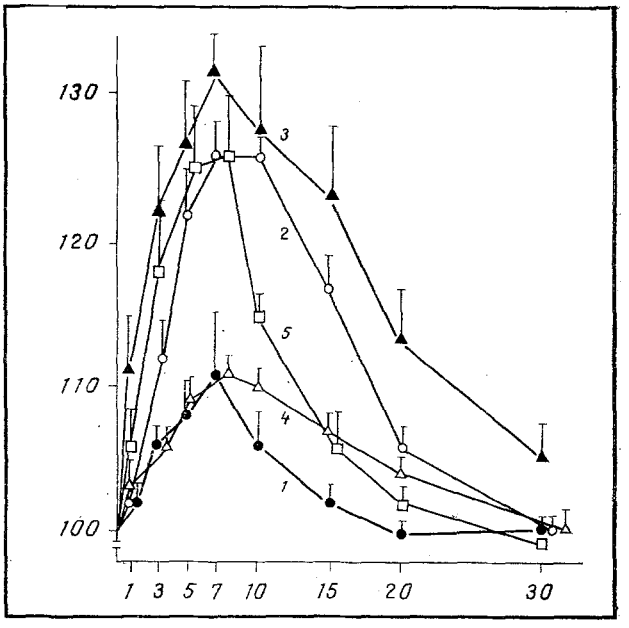

Fig. 2. Effect of immobilization on venular permeability for FITC-globulin. Abscissa, time after injection of serum (in min); ordinate, increase in intensity of fluorescence (in %, initial background fluorescence 100%); 1) control; 2-5) immobilization for 1, 5, 9, and 24 h, respectively.

of injection of the serum and of different periods of immobilization on the contractile activity of the lymphatic microvessels were assessed separately.

EXPERIMENTAL RESULTS

Investigation of venular permeability after immobilization for 1 h revealed a significant (compared with the control) increase in the intensity of fluorescence in the perivenular tissue 3-20 min after injection of the serum, 1-30 min after immobilization for 5 h, and 1-15 min after immobilization for 24 h (Fig. 2).

Investigation of the concentration of FITC-globulin in the lymphatic microvessels showed a significantly greater increase in the intensity of fluorescence from the 3rd to the 30th minute after injection of the serum into animals immobilized for 1, 5, and 24 h.

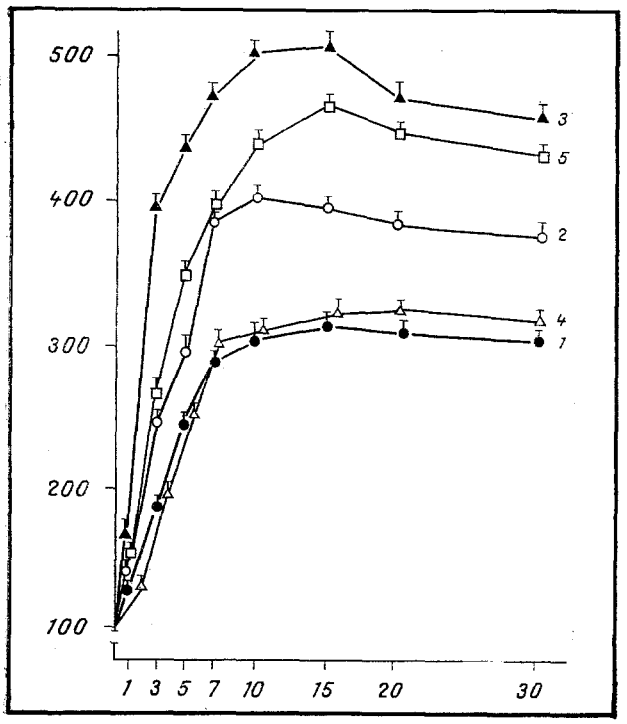

Fig. 3. Effect of immobilization on FITC-globulin concentration in lymphatic vessel. Legend as in Fig. 2.

The increase in the labeled globulin concentration may have been due not only to an increase in its entry through the wall of the test lymphatic microvessel, but also to an increase in protein transport from the lymphatic capillaries, especially if the vessel studied was a postcapillary. After immobilization for 9 h the concentration of FITC-globulin in the lumen of the lymphatic microvessels did not differ from the control (Fig. 3).

During the biomicroscopic experiments no significant effect of intravenous injection of serum on the frequency of contractions of the lymphatic microvessels could be found, whether initially they were at rest or in the contracted state.

At the same time, the immobilization process was found to have a significant effect on the frequency of contractions of the lymphatic microvessels. For instance, immobilization for 1, 5, and 9 h led to an increase in the contractile activity of these microvessels, but immobilization for 24 h led to a decrease (Table 1).

In the course of the experiments described above correlation was thus found between the phasic process of disturbance of venular permeability and the concentration of macromolecules in the lymphatic microvessels. For instance, the increase in venular permeability after immobilization for 1, 5, and 24 h was accompanied by an increase in the FITC-globulin concentration in the lymphatic microvessels, which was evidently due to its high concentration in the interstitial tissue, as a result of the increased venular permeability and its increased transport into the lymphatic microvessels (directly through the wall and from lymphatic capillaries). Under these circumstances the increase in the globulin concentration in the lymphatic microvessels took place despite enhancement of their drainage function, as shown by a fourfold increase in the number of contractions after immobilization for 1 h, whereas after immobilization for 5 h, when venular permeability was not increased, the concentration of the marker in the lymphatic microvessels likewise was not increased.

This correlation can perhaps be explained by the fact that the same physiologically active substances which are activated during stress participate in the regulation of permeability of the vascular walls. It was interesting to determine, in particular, the morphological and physiological state of the mast cells during immobilization stress. In a previous publication [5] the writers showed that stress leads to increased secretory activity of mast cells, accompanied by an increase in the release of histamine, serotonin,

TABLE 1. Effect of Immobilization on Contractile Activity of Lymphatic Microvessels

Expt. No.	Experimental conditions	Number of contractions per minute	p
1	Control (n = 6)	3,0±0,7	
2	Immobilization for 1 h (n = 7)	12,0±0,4	$p_{1-2}<0,001$
3	Immobilization for 5 h (n = 7)	6,2±0,4	$p_{1-3}<0,001$
4	Immobilization for 9 h (n = 10)	5,7±1,1	$p_{1-4}<0,01$
5	Immobilization for 24 h (n = 8)	1,8±0,5	$p_{1-5}\leqslant 0,05$

Legend. n) Number of observations.

heparin, and other PAS involved in the increase of permeability, from them. Increased contractile activity, of the lymphatic microvessels during immobilization (1-9 h) was a compensatory process, directed at eliminating foreign macromolecules, which have accumulated in large amounts in the interstial tissue. An increase in the frequency of rhythmic contractions of the walls of the lymphatic microvessels in the initial period of immobilization may be due to the activation of secretory activity of the mast cells, for histamine, serotonin, and small doses of heparin have been shown to increase the frequency and amplitude of contractions of lymphatic microvessels [2, 9, 14]. Activation of the sympathicoadrenal and adrenergic systems, and an increase in the concentrations of catecholamines and corticosteroids in the blood plasma, accompanying stress, may also be among the causes of increased contractile activity of the lymphatic microvessels [9]. It must be emphasized that during immobilization for 9 h, not accompanied by increased venular permeability, the increase in contractile activity was preserved. During immobilization for 24 h, when venular permeability was again increased, contractile activity of the lymphatic microvessels was depressed, and this correlated with the development of severe microcirculatory disorders, in the form of slowing of the venular blood flow, aggregation of the blood cells, and stasis and plasmatization [5], leading to the onset of tissue hypoxia, which depresses contractile activity of the lymphatic microvessels [9]. A combination of increased venular permeability with reduced contractile activity of the lymphatic microvessels, i.e., reduction of drainage, may lead to the development of tissue edema.

Phasic changes in transport of FITC-globulin through the wall of the venules and lymphatic microvessels were thus demonstrated biomicroscopically in the course of immobilization stress. The early periods of immobilization are characterized by increased drainage of macromolecules through the lymphatic section of the microcirculatory system, but the later stages are characterized by reduced drainage.

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CHANGES IN THE LUNGS IN THE EARLY PERIOD OF CLOSED CHEST INJURY IN RATS

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The CO_2 concentration in arterial blood depends mainly on the state of the external respiratory system [4, 8]. The high oxygen consumption in the lungs found in the early post-traumatic period of closed chest injury and also the fact that the value of p_aO_2 can be influenced by activation of free-radical lipid oxidation in the lungs [2, 3] suggest that metabolic processes in the lungs may be involved in regulation of the arterial blood CO_2 concentration.

The aim of this investigation was to study the time course of metabolic processes and their morphological expression in the lungs in the early period after closed chest trauma, in the injured and uninjured lung separately, in order to discover the importance of these processes in the changes in the arterial blood CO_2 concentration.

EXPERIMENTAL METHOD

Experiments were carried out on 130 male Wistar rats weighing 200-250 g. A contusion of the lung was inflicted by means of a spring-operated pistol, in the right half of the chest [2, 3]. The CO_2 concentration in the end-portion of the expired air (f_ACO_2) was recorded in rats, fixed in the prone position, on an MKh-6202 gas analyzer. Blood was taken by puncture from the left and right ventricles for determination of pO_2 and pCO_2 on a "Corning" gas analyzer (England). Values of p_ACO_2, the CO_2 concentration in arterial and mixed venous blood (C_aCO_2, C_vCO_2) [4], the difference between p_vCO_2 and p_ACO_2 ($p_{v-a}CO_2$), and the difference between C_vCO_2 and C_aCO_2 ($C_{v-a}CO_2$) were calculated. The rats were decapitated and the concentrations of protein [5], total lipids [10], and glucose [1] were determined in the right and left lung (RL and LL), and morphologic and electron-microscopic investigations also were undertaken. Sections through RL and LL were stained with azure-eosin, orcein, and picrofuchsine. Material for electron microscopy was fixed in paraffin and 1% osmic acid solution by Palade's method and embedded in Araldite. Semithin sections were stained with toluidine blue. Ultrathin sections were cut on an LKB 8-8800 III Ultrotome (Sweden) and stained with uranyl acetate and lead citrate. The material was examined in the ÉVM-100L electron microscope. To analyze the state of the lung over a period of time, parameters obtained in intact rats and 1 and 2 h and 1 and 7 days after trauma were studied. The results were subjected to statistical analysis by Student's test.

EXPERIMENTAL RESULTS

The upper and middle lobes of RL were injured as a result of trauma. No mechanical injuries were found in LL. Arterial hypoxemia developed in the rats during the first hours

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