THE DISTURBANCE OF VASCULAR PERMEABILITY IN BURNS. AN ELECTROPHORETIC INVESTIGATION OF THE PROTEIN CONTENT OF LYMPH FLOWING FROM THE BURNED AREA

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In previous work [2] we showed that in dogs with localized thermal burns of the skin (1-3% of the body surface) of the first and second degree, sustained 24 hours previously, albumin labelled with radioactive iodine (1¹⁹¹) and injected intravenously appears more quickly in the lymph of the thoracic duct, and the concentration of labelled albumin rises in the tissues outside the burned area. On the basis of these facts, it was concluded that in thermal burns of the skin, besides local disturbance of the permeability of the capillaries in the burn itself, the permeability of the capillaries in tissues and organs remote from the burn also increases, which we [3, 4] and I.A. Mukhamedzhanov [5] observed in experimental ammonia abscesses of the skin.

In order to elucidate the pathogenesis of the general changes in capillary permeability which we observed in burns we studied the protein content of lymph flowing from the burned area and compared it with that of the blood serum.

EXPERIMENTAL METHOD

The investigation was carried out on 37 rabbits of different color and sex weighing from 1.2 to 1.8 kg.

The burn was produced by immersion of the hind paw of the rabbit for one minute in water at a temperature of 80°C.

The lymph for the electrophoretic investigation was obtained from the popliteal lymphatic gland in which a cannula was inserted against the lymph flow.

The protein composition of the lymph serum was investigated with the aid of electrophoresis on filter paper with a potential gradient of 17-18 v/cm, a veronal buffer at pH = 8.6, μ = 0.023. The strips of paper were stained with a 0.5% solution of bromphenol blue prepared in a saturated alcoholic solution of mercuric chloride. The dye was extracted with a 0.01 N solution of caustic soda and the eluted dye examined photometrically by means of a graduated photometer with an M-57 filter. Curves were drawn in accordance with the optical densities. In the calculations and correction of the albumin tracing we followed the instructions of E.P. Smolichev [9]. The composition of the serum proteins of the blood was studied by the same method.

The protein concentration in the lymph was determined by an IRF-1 immersion refractometer.

EXPERIMENTAL RESULTS

The results of the determinations of the protein composition of the lymph from the popliteal gland of healthy rabbits and of rabbits with burns of varying duration are shown in Table 1.

TABLE 1

Content of Protein and Protein Fractions in the Lymph Flowing from the Burned Limb (mean results of experiments on 37 rabbits)

Statistical Index		A lbum i n	Globulins					1/6
	Protein (g%)		٠,	4,	βι	β,	ī	A/G co- efficient
	Normal lympl	h (mean val	ues fror	n 10 e	eperime	nts)		
M	2.1	61.9	9.8	7.3	8.1		12.9	1.7
σ±	0.7	6.0	4.8	4.4	5.1		3.8	0.6
m±	0.22	61.9 6.0 1.90	1.23	1.07	1.60		1.20	0.15
60 minute	es after burning	(mean value	s from	experi	ments c	n seve	e_rabb!	(ts)
M	3.2	61.3	7.7	5,6	7.5	7,7	11.2	1.6
σ±	0.34	2.4	3.6	2.7	2.6	28	1.6	0.2
m±	0.13	61.3 2.4 0.9	1.4	1.0	1.0	1.1	0.6	0.07
90 minut	es after burning	(mean value	s from	experi	men ts c	on seve	n rabbi	lts)
M	3.8	59.9	7,9	5.7	7.2	6.1	12.3	1.5
σ±	0.5	4.9	3.0	2,3	1.7	2.1	2.1	0.2
m±	0.2	59.9 4.9 1.8	1.2	0.9	0.7	0.6	0.6	80.0
24 hours	after burning (m	ean values f	rom ex	perime	nts on	thirtee	n ra bbi	ts)
М	4.3	49.0	15.1	8.4	8.2 2.2 0.7	7.0	12.4	1.0
5±	0,6	5.3	2.5	3.4	2.2	3.4	2.9	0.2
m±	0.2	1.4	0.7	0.9	0.7	1.0	8.0	0.05

The flow of lymph in early stages after the burn had been inflicted was slow, so that the lymph was collected for about 60 minutes and it was impossible to study its composition immediately after the burn. Twenty-four hours after the burn, the paw was very hyperemic and edematous. The skin fold on this paw was from seven to eight times thicker than that on the normal paw. In all cases the lymph flow was accelerated so that it was possible to collect the quantity required for electrophoresis in five to ten minutes.

The protein concentration in the lymph was increased (P less than 0.01) in the first one to two hours after the burn. The lymph collected during the first hour after the burn was found to contain an additional β -globulin fraction (β_2 -globulin). The relative content of this fraction did not vary during 24 hours. The relative content of albumin and other globulin fractions was essentially unchanged in the lymph during the first one to two hours after the burn. After 24 hours the protein concentration in the lymph rose sharply (P less than 0.01), the relative albumin content fell (P less than 0.01) and the α_1 -globulins rose (P less than 0.01). The A/G ratio was reduced correspondingly (P less than 0.01).

The results of the investigation of the protein composition of the blood serum are shown in Table 2.

Sixty minutes after burning, a tendency of the β -globulin to increase was observed (P less than 0.1). It is possible that this was due to the appearance of the additional β_2 -globulin fraction which could not be isolated because of its low concentration in the blood.

DISCUSSION OF RESULTS

In 1955, T.S. Paskhina showed that the ability of the exudates to disturb capillary permeability does not show itself because of the inhibitory effect of albumin [6, 7, 8]. Other authors [12, 14, 15] came to the same conclusion. M.S. Surovikina [10] discovered in the blood of rabbits during experimental inflammation the appearance of an additional β_2 -globulin fraction, and this was confirmed by T.S. Paskhina [7, 8]. M.S. Surovikina began her investigation of the protein composition of the blood serum 24 hours after the application of ammonia. Accordingly, D.E. Al'pern [1] considers that active proteins are characteristic of inflammation but are not responsible for it.

TABLE 2

Content of Protein and Protein Fractions in Blood from the Auricular Vein During a Burn (mean results of experiments on seven rabbits)

Statistical index	Protein,	Albumin		A/G co-			
			æ,	e. ,	a	Y	efficient
		Befor	re burning				
M	6.4	61,9	7,5	7,3	9.2	14,0	1.6
4	1.5	4.2	2.1	4.8	3.7	3,6	0.3
m±	0.6	1.6	8.0	1,8	1.4	1.4	0.1
		After 5	minutes	•			
M	6.2	61.1	7.4	6.6	10.8	13.8	1.6
σ±	0.33	4.6	2.1	2.0	2.8	4.4	0.3
m±	0.12	1.7	0.8	0.7	1.0	1.7	0.1
		After :	20 minutes	· 5			
м	6,1	60.0	8.4	7.0	10.4	11.8	1.5
o±	0.39	3.8	3,5	1,5	5.1	3.7	0.8
m±	0.15	1.4	1,3	0.6	2.0	1.4	0,3
	• .	After 4	0 minutes	•			
М	6.1	59.8	8.5	6,9	10.2	14.5	1.5
σ±	0.33	4.7	2,8	1,6	2.5	4.4	0.8
加土	0.1	1.8	1,0	0.4	0.9	1.7	0,3
		After (60 minutes	5			
M	5,9	58.6	7.8	7.8	13.2	12.4	1.5
σ±	0.38	6.1	1.6	2,9	5.5	3.4	0.4
m±	0.14	2.3	0.6	1.1	2.1	1.3	0.1

Our experiments demonstrate the appearance of additional β_2 -globulins in the lymph in the initial phase of development of the burn. This is a convincing argument against the view of D.E. Al'pern, and one which confirms the conclusions of T.S. Paskhina and other authors, pointing out the importance of the active β -globulins as substances capable of causing the development of disturbance of capillary permeability in a focus of inflammation. Evidently, these substances are formed in the inflammatory focus and, on entering the blood, gradually lead to the appearance of the β_2 -globulins discovered in rabbits. It may be supposed that the disturbance of capillary permeability outside the inflammatory focus is caused by circulation of these active β -globulins in the blood. Increase in the capillary permeability outside the inflammatory focus can be found in rabbits in the first days of development of an ammonia abscess [5]. It is possible that the action of the active globulins is gradually inhibited on the appearance in the body of substances possessing anti-inflammatory activity [13].

In putting forward this view of the role of the B₂-globulins in the production of general disturbance of capillary permeability in inflammation we do not deny that other substances, for example proteinases, whose content in the blood is increased during inflammation, may also play a part in this process [11].

SUMMARY

Electrophoretic investigations of the lymph draining from burned rabbits' paws were performed. It was established that the concentration of protein increases within the first hour after the burn and an additional β -globulin fraction (β_2 -globulin) appears. In 24 hours, the derangement of the protein content of the lymph is even more pronounced, i.e., the concentration of protein increases, the relative content of albumin diminishes, β_2 -globulin fraction is still present and the level of α_1 -globulin is also raised. In accordance with literature data on the effect of active β -globulins on the local capillary permeability the author suggests that these proteins play

an important role in the appearance of general disturbances of capillary permeability in the areas free from inflammations. These disturbances were revealed by the author in experimentally induced inflammation in rabbits and dogs. They were described in his previous papers.

LITERATURE CITED

- [1] D.E. Al'pern, Arkh. Patol. 18, 4, 3-15 (1956).
- [2] V.V. Bakanskala, The Pathogenesis of Inflammation and the Pathology of Vascular Permeability, Stalinabad 3, 45-51 (1956).
 - [3] V.V. Bakanskala, Ibid. 2, 55-64 (1954).
 - [4] V.V. Bakanskala, Ibid. 65-73 (1954).
 - [5] L.A. Mukhamedzhanov, Ibid. 3, 53-61 (1956).
 - [6] T.S. Paskhina, Dokiady Akad. Nauk SSSR 86, 3, 609-612 (1952).
 - [7] T.S. Paskhina, Voprosy Med. Khim. 1, 3, 159-168 (1955).
 - [8] T.S. Paskhina, Clin. chim. acta 1, 24-34 (1956).
- [9] E.P. Smolichev. The Pathogenesis of Inflammation and the Pathology of Vascular Permeability. Stalinabad 3, 237-253 (1956).
 - [10] M.S. Surovikina, Biull, Eksptl. Biol, i Med. 5, 40-44 (1954).
 - [11] W. Greuer, E. Hess and J. Rostecki, Arzneimittel-Forsch 7, 128-131 (1957).
 - [12] A.A. Milles and D.L. Wilhelm, Brit. J. Exper. Path. 36, 71-81 (1955).
 - [13] T.H. Rindani, Indian J. med. res. 44, 673-676 (1956).
 - [14] W.G. Spector, J. Path, and Bact. 72, 367-380 (1956).
 - [15] W.G. Spector and D.A. Willoughby, J. pathol. and bact. 73, 133-139 (1957).

[•]In Russian.