PATHOGENESIS OF INFLAMMATORY EDEMA IN RELATION TO THE INTENSITY OF THE THERMAL FACTOR

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The mechanism of development of disturbances of the permeability of the capillaries in inflammation has not yet been elucidated. Recently, in addition to histamine, considerable attention has been paid to active globulins as the mediators of the increased permeability in inflammation [1, 2, 6, 8-12, 14-17].

It has been found [8, 9] that additional β_2-globulins appear in the serum of a rabbit 24 h after the development of turpentine inflammation. T. S. Paskhina [6] found additional β_2-globulins in the blood of rabbits during inflammation and showed that the serum acquired the power of increasing the permeability of the capillaries of the skin in normal rabbits. These investigations revealed the importance of these accessory globulins as permeability factors in inflammation. V. V. Bakanskaya [1, 2] and S. M. Shchegel' [10] found an increased concentration of β-globulins and the appearance of an additional β_2-globulin fraction in the lymph flowing from the burned limb of a rabbit.

In experiments on rats, Spector and Willoughby [14, 15] showed that in thermal burns early disturbances of capillary permeability arise as a result of the liberation of histamine, and later disturbances as a result of the appearance of active β-globulins. In the experiments of V. V. Bakanskaya and S. M. Shchegel inflammation was caused by water at a temperature of 80°. Some workers consider, however, that thermal edema may develop in rats at a lower temperature (45°), and that no histamine is liberated at that temperature [13]. It is suggested that the permeability factor in these experiments was bradykinin.

We investigated the protein composition of the lymph in animals with burns of different intensity, and also the presence of permeability factors in the lymph.

EXPERIMENTAL METHOD

Two series of experiments were conducted on 25 rabbits of different sexes, weighing from 2.0 to 3.2 kg. Burns were caused by immersing the hind limb of the rabbit for 1 min in water at 80-82° (first series) or for 5 min at 50 ± 0.5° (second series). Lymph was collected by the method described previously [10] 30 min and 24 h after infliction of the burn.

The protein concentration in the clear lymph was determined by means of a type RPL-2 refractometer. The protein composition of the lymph was studied by paper electrophoresis (medinal-veronal buffer, pH 8.6, μ 0.023, time for separation of lymph into fractions 3 h at a potential gradient of 19 V/cm). The paper strips were stained with a 1% solution of bromphenol blue in a concentrated alcoholic solution of mercuric chloride. Excess dye was rinsed out with a 0.5% solution of acetic acid. Photometry of the eluted dye was performed in a type FEK-M photoelectric colorimeter with a green filter. The calculations were made, with introduction of a correction for the trace of albumin, by the method described by E. P. Smolnichev [7]. The experimental results were analyzed statistically by the difference method [4].

The effect of lymph on the capillary permeability was studied by the use of Ramsdel's principle, by injecting 0.1 ml of lymph intradermally into healthy rabbits which had previously received an injection of Evans blue in a dose of 1 ml of 1% solution per kilogram body weight into a vein of the ear [5]. Xylol, histamine, normal lymph and physiological saline (0.1 ml) were used as controls.

TABLE 1. Protein Concentration and Ratio Between Protein Fractions in Lumph Flowing from Burned Limb (Temperature 80°)

Time of investigation and significance of difference	Albumin (in g %)	Globulins					A/G ratio	Protein (in g %)
		α_1	α_2	β	β_2	γ		
Before burning	47.8	7.2	13.6	15.9	–	17.0	0.94	3.9
30 min after burning	44.8	7.5	12.3	11.6	8.5	16.3	0.82	4.1
P	< 0.1	> 0.5	> 0.5	< 0.02		> 0.5	< 0.01	< 0.02
24 h after burning	44.0	6.6	11.2	9.4	11.3	16.3	0.81	4.4
P	< 0.1	> 0.5	> 0.5	< 0.01		> 0.5	< 0.01	< 0.01

TABLE 2. Protein Concentration and Relative Proportion of Protein Fractions in Lymph Flowing from Burned Limb (Temperature 50°)

Time of investigation and significance of difference	Albumin (in g %)	Globulins				A/G ratio	Protein (in g %)
		α_1	α_2	β	γ		
Before burning	44.6	7.0	9.5	15.2	23.7	0.82	3.2
30 min after burning	46.5	7.3	9.1	14.6	22.5	0.89	4.0
P	> 0.5	> 0.5	> 0.5	> 0.5	> 0.5	> 0.5	< 0.001
24 h after burning	46.3	6.4	9.9	14.7	22.7	0.88	3.6
P	> 0.5	> 0.5	> 0.5	> 0.5	> 0.5	> 0.2	< 0.001

The intramuscular temperature in the limb muscles during burning was determined by means of a thermocouple.

Before and 24 h after burning, the perimeter of the limb was measured in the rabbits with a thread – in the proximal portion of the metatarsus, the middle, and the distal portion of the metatarsus, and linear measurements also were made – dorsal-volar and lateral-medial – at the metatarsophalangeal joints. The last two measurements were made with calipers. The criterion of the dimensions of the limbs was the arithmetical mean value (M) of all five measurements. The relative value of the standard deviation of a single determination of the limb measurements by this method was ±2%. The small value of this deviation was due to the measurement of 5 parameters.

EXPERIMENTAL RESULTS

In the first series of experiments (burning at 80°) the intramuscular temperature during the burn reached 43.8°. The burned paws of the rabbits 24 h later were edematous and showed destructive changes: the claws were fractured, the blisters had burst, the skin had sloughed down to the muscular layer. The arithmetical mean before burning was 4.5 cm, and 24 h later it was 6.7 cm ($P < 0.001$). Histological examination of sections of the skin from the dorsum of the foot, taken 24 h after burning, showed desquamation of the stratum corneum and detachment of the epidermis. The collagen fibers of the stratum papillare, and especially of the stratum reticulare, were merged into a continuous mass of granular structure. At the border between the stratum reticulare and the subcutaneous cellular tissue the lymphatics were considerably dilated and filled with a homogeneous substance staining a pale blue color. No cell infiltration was seen. The collagen fibers were separated and their nuclei poorly stained.

The results of this series of experiments confirmed the findings obtained by S. M. Shchegel' [10]. It may be seen in Table 1 that the protein concentration in the lymph flowing from the burned limb of the rabbit and collected after 30 min and 24 h had increased on the average by 0.5 g % ($P < 0.01$); the A/G ratio was lowered. Electrophoresis revealed an increase in the concentration of β-globulins ($P < 0.01$) and the appearance of additional β_2-globulins in contrast to the normal lymph, that, flowing from the burned limb of the rabbit, caused an increase in the capillary permeability of the skin of healthy rabbits.

In the second series of experiments (50°) the following results were obtained. The intramuscular temperature during burning was 38.5-38.7°. Visible edema of the burned limb developed after 2-2½ h; 24 h after burning it was severe (M before burning 4.3 cm, 24 h after burning 6.5 cm). However, no destructive changes were observed. Histological examination of skin sections from the dorsum of the foot taken 24 h after burning showed detachment of the stratum corneum of the epidermis. The cell nuclei of the epidermis were badly stained. The stratum papillare of the dermis was grossly edematous and the collagen fibers were separated by edema fluid. The edema also was marked in the stratum reticulare, but less so than in the stratum papillare. The fibers of the subcutaneous cellular tissue were thickened, and in places showed cloudy swelling. Here, too, were seen large spaces filled with a homogenous substance, staining well with eosin. Infiltration of the edematous tissue was seen, mainly with pseudoeosinophils. The distribution of these cells showed certain distinguishing features: in the stratum papillare there were few pseudoeosinophils, while in the deeper layers of the dermis and in the subcutaneous cellular tissue the infiltration was more dense; the vessels of the stratum reticulare showed signs of stasis and their walls were densely infiltrated with pseudoeosinophils.

Lymph taken 30 min after burning caused a disturbance of capillary permeability in the skin of healthy rabbits in only 7 of 15 experiments. Xylol and histamine also regularly caused a disturbance of permeability in these animals, whereas normal lymph and physiological saline did not cause these phenomena. Lymph collected 24 h after burning did not increase the capillary permeability of the skin in control rabbits in any single experiment.

To determine the role of histamine in the development of the disturbances of capillary permeability, lymph taken 30 min after burning was injected into the skin of healthy rabbits, 30 min after the animals had received a preliminary injection of diphenylhydramine hydrochloride (10mg/kg body weight). In these experiments no disturbance of capillary permeability developed.

The protein concentration in the lymph flowing from the burned limb of the rabbit 30 min and 24 h after injury was increased by 0.6 g % ($P < 0.001$). The A/G ratio was not lowered ($P < 0.2$). No additional β_2-globulin fractions were found. The relative concentration of the protein fractions was unchanged (Table 2).

The disturbance of capillary permeability following intradermal injection of lymph collected 30 min and 24 h after burning at 80° cannot be attributed to the influence of histamine alone. The appearance of β_2-globulins also must play a part. Proof of this was obtained by the experiments following preliminary administration of diphenylhydramine hydrochloride, in which lymph collected 24 h after burning at 80° led to a rapid disturbance of permeability of the skin capillaries.

In contrast to this, lymph taken 24 h after burning at 50°, despite the presence of marked edema, did not cause a disturbance of capillary permeability in the control rabbits, presumably because it did not contain peremeability factors (histamine, active β-globulins, etc.). This difference may be explained by the fact that at 50°, as histological examination showed, the tissue damage was less marked than after burning at a higher temperature (80°). During the action of a weak thermal stimulus, active permeability factors are probably formed only in the initial stages of development of inflammation, and are not therefore found in the lumph after 24 h.

Lymph obtained 30 min after burning (50°) led to a disturbance of permeability less regularly than lymph taken from rabbits after more intensive burning (80°), and this disturbance was due to the presence of histamine. This was demonstrated by the absence of effect when such lymph was injected intradermally into rabbits receiving a preliminary injection of diphenylhydramine hydrochloride.

The protein composition of the lymph collected 30 min and 24 h after burning at 50° was unchanged, by contrast with the first series of experiments (80°). The more intensive heating of the tissues evidently caused changes in the protein composition and led to the appearance of an additional globulin fraction with the electrophoretic mobility of the β_2-globulins.

The absence of changes in the protein composition of the lymph following mild burns (50°) casts doubt on the role of the globulin permeability factors in the pathogenesis of edema in rabbits exposed to this type of injury, in agreement with results in the literature in connection with the pathogenesis of edema caused by the action of a moderate temperature on rats [13]. The results obtained show that the pathogenesis of inflammatory edema differs according to the intensity of the thermal agent.

SUMMARY

In inflammation developing in rabbits as a result of thermal burn at a temperature of 80-82°C additional globulins appear in the lymph flowing from the burned extremity; by their electrophoretic mobility these gobulins are referred as β-globulins. This lymph injected intradermally to control animals disturbed the capillary permeability; this fact may be explained both by the presence of histamine in the lymph and by the appearance of additional β-globulins.

Following intradermal administration of lymph obtained 30 min after the burn at temperature of 50°, control rabbits exhibited a rise of the capillary permeability which may be attributed to the presence of histamine therein. In inflammation resulting from a burn at 50°C, no β-globulin fractions were found. Lymph obtained 24 h after the burn provoked no disturbance of the capillary permeability in rabbit skin. In burns caused by a moderate temperature (50°C) the leading role in the development of edema is evidently played by some other substances, not the additional β-globulins. The data obtained point to differences in the pathogenesis of inflammatory edema depending on the intensity of action of the thermal factor.

LITERATURE CITED

1. V. V. Bakanskaya, Byull. éksper. biol., 5, 36 (1958).
2. V. V.Bakanskaya, In Book: Pathology of the Blood Proteins and Disturbances of Vascular Permeability [in Russian], Dushanbe, p. 71 (1959).
3. I. A. Oivin, In Book: Pathology of the Blood Proteins and Disturbances of Vascular Permeability [in Russian], Dushanbe, p. 149 (1959).
4. I. A. Ovin, E. A. Venglinskaya, and S. M. Shchegel', Pat. fiziol., 3, 33 (1959).
5. I. A. Oivin and S. M. Shchegel', In Book: Pathogenesis of Inflammation and Pathology of the Blood Proteins [in Russian], No. 5, p. 167, Dushanbe (1961).
6. T. S. Paskhina, Vopr. med. khimii, 3, 159 (1955).
7. E. P. Smolichev, In Book: Pathogenesis of Inflammation and Pathology of Vascular Permeability [in Russian], No. 3, p. 237, Dushanbe (1956).
8. M. S. Surovikina, In Book: Pathogenesis of Inflammation and Pathology of Vascular Permeability [in Russian], No. 1, p. 85, Dushanbe (1954).
9. M. S. Surovikina, Byull. éksper. biol., 5, 40 (1954).
10. S. M. Shchegel', Pat. fiziol., 3, 14 (1960).
11. J. M. Elder and D. L. Wilhelm, Brit. J. exp. Path., 39, p. 335 (1958).
12. P. J. Mill, J. M. Elder, A. A. Milos, et al., Ibid., p. 343.
13. M. Rochae-Silva and A. Antonio, Med. exp. (Basel), 3, p. 371 (1960).
14. W. G. Spector, J. Path. Bact., 72, p. 367 (1956).
15. W. G. Spector and D. A. Willoughby, Nature, 182, p. 949 (1958).
16. D. L. Wilhelm, P. J. Mill, and A. A. Miles, Brit. J. exp. Path., 38, p. 446 (1957).
17. D. L. Wilheld and B. Mason, Brit. med. J., 2, p. 1141 (1958).

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