EFFECT OF TOXIN OF BURNED SKIN ON THE STATE OF THE HEMODYNAMICS IN RATS

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Toxin for burned rat skin, purified immunochemically, possesses definite physiological activity against the cardiovascular system of homologous animals. If injected into the blood stream, the toxin lowers the systemic arterial pressure within 2 h from 125 ± 7 to 74 ± 8 mm Hg. The development of systemic hypotension is accompanied by secondary disorders of the microcirculation in the mesentery of the small intestine, characterized by constriction of the arterioles, slowing of the blood flow along the venules, and reduction of the capillary blood flow.
KEY WORDS: *burns; burn toxin; microcirculation.*

The combination of severe disturbances in the systems and organs after burns is caused by various pathogenetic factors and, in particular, by products reaching the body directly from the focus of injury [6]. The toxicity of extracts of burned skin has been confirmed by several experimental studies carried out under Fedorov's direction [2, 7]. Methods of isolating the high-molecular-weight protein toxin have been developed [3, 8].

At the present time a comprehensive study of the nature and biological properties of the toxic factor is in progress. This paper describes part of these investigations.

EXPERIMENTAL METHOD

The protein toxin was isolated from extracts of burned skin of Wistar rats 46-48 h after burning by means of a direct method based on immunochemical affinity. Monospecific antibodies against burned skin toxin were obtained by hyperimmunization of rabbits and subsequent absorption of the antisera on normal antigenic immunosorbents fixed on sepharose 4B, activated with cyanogen bromide (Pharmacia Fine Chemicals, Sweden). After blocking of the free groups by monoethanolamine, pH 8.0, and washing off the unbound protein, the resulting antibody-immunosorbents were able to bind 77 μg toxin for every 1 mg fixed antibodies. After treatment of the immunosorbent with 0.01 N HCl containing 0.14 M NaCl, the eluate was quickly neutralized with dry NaOH to pH 7.0-7.5, concentrated by ultrafiltration through a PSED-25 membrane (Millipore, USA), and used for future experiments. The resulting protein extract was homogeneous when tested by gel filtration and disk electrophoresis and it had a marked toxic action on mice, blocking the mononuclear phagocytic system (LD_{50} = 28 μg protein by intraperitoneal injection).

In this investigation the effect of the toxin on the hemodynamics indices was studied in rats. Wistar rats weighing 120-160 g were used. The experimental animals (six) received the toxin by intravenous injection in a dose of 0.9 mg protein dissolved in 0.2 ml 0.14 M NaCl solution. The five control rats received a similar injection of 0.14 M NaCl solution, pH 7.0.

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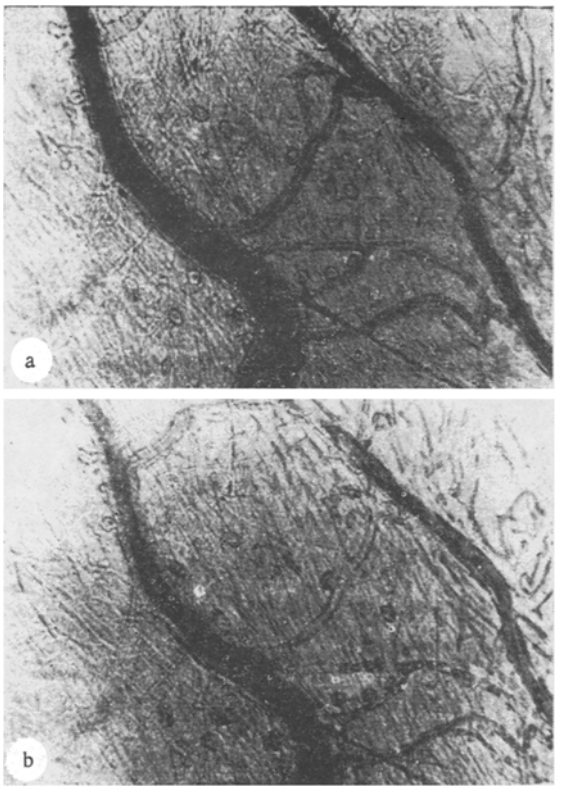

Fig. 1. Changes in microcirculation in mesentery of small intestine after injection of toxin of burned skin, objective 9, ocular 12.5: a) blood flow in mesenteric microvessels in original state; b) same region of vascular system 2 h after injection of toxin.

Changes in the systemic circulation were investigated periodically by measuring the mean arterial pressure in the common carotid artery. The carotid artery was cannulated and heparin injected into the blood stream in a dose of 0.4 mg/100 g body weight. The animals were anesthetized with pentobarbital (3 mg/100 g) and placed on a constant-temperature platform (t = 34°C). The state of local hemodynamics was estimated from the results of biomicroscopic investigation of the mesenteric microvessels. The usual methods of preparing and stabilizing the specimen were used during this investigation [4, 14, 15]. A loop of small intestine, removed from the peritoneal cavity, was placed on a constant-temperature stage (t = 37°C), wrapped in wet towels, and irrigated throughout the period of observation with buffered Ringer's solution (pH 7.2, t 37.5°C) containing 1% gelatin at the rate of 3-4 ml/min. The blood vessels were examined under the MBI-6 microscope, equipped to study the microcirculation [1]. The phenomena observed were recorded by a microfilming method.

EXPERIMENTAL RESULTS

The mean arterial pressure in animals of the control group at the beginning of the experiment was 117 ± 6 mm Hg and it remained relatively stable, falling slowly to reach 109 ± 4 mm Hg by the end of the second hour. The circulation in the terminal vascular system of the animals of this group had features characteristic of the normal physiological state of the microcirculation. Variations observed did not exceed the changes attributable to exposure of the mesentery: slight dilatation of the arterioles and moderate concentration of leukocytes in the venules [15].

In the experimental animals shortly after injection of the toxin, a tendency was observed for the mean arterial pressure to fall (from 125 ± 7 to 109 ± 8 mm Hg). After 30 min the arterial pressure had fallen to 101 ± 7 mm Hg, at which level it remained until the 60th minute. A new period of fall of pressure then began, and by the end of the second hour of observation it had fallen to 74 ± 8 mm Hg.

The blood flow in the mesenteric microvessels remained unchanged during 1.5 h of observation after injection of the toxin. Later disturbances of the microcirculation appeared: some degree of constriction of the arterioles, slowing of the blood flow along the venules, a reduction in the number of functioning capillaries (Fig. 1). The most marked changes, accompanied by considerable reduction of the capillary blood flow, developed in the experimental animals when the arterial pressure fell below 70 mm Hg.

The results indicate definite physiological activity of the toxin against the cardiovascular system. Under the influence of the toxin, soon after its injection systemic hypotension developed, and its intensity increased later. At the time of maximal severity of the hypotension, disorders of the microcirculation were added to the disturbances of the systemic hemodynamics. The disturbances of the local hemodynamics took the form of limitation of tissue perfusion and reduction of the nutritive blood flow. The sequence of circulatory changes observed suggests that the disturbances of the systemic hemodynamics were the primary process and the disorders of the microcirculation were secondary events arising in consequence of the lowering of the pressure gradient to the so-called critical zone [12, 13]. It must be pointed out that these experiments were carried out on heparinized

animals and that in the absence of heparin the pattern of changes in the microcirculation may have been more marked.

Possible mechanisms of the disturbance of hemodynamics following injection of the toxin could be a reduction of the venous return or weakening of contractile activity of the myocardium. The systolic output is considerably reduced in burns [5, 11]. The relatively stable systemic arterial pressure is maintained chiefly by contraction of the microvessels and by an increase in the peripheral resistance [5, 9, 10]. After injection of burn toxin, a fall of the systemic arterial pressure was observed in the experiments without any sign of early peripheral vasoconstriction; this result may be explained by differences in the state of the sympathicoadrenal system in burns and by injection of the purified toxin.

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