STUDY OF THE TOXIC PROPERTIES OF SERUM IN RATS AFTER BURNS

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One of the leading symptoms of thermal injury is the intoxication which arises within a few hours after trauma and continues throughout all phases of burn healing. The qualitative and quantitative characteristics of the toxemia are of great practical interest not only as indicators of the course of the burns but, more especially, as a criterion of evaluation of the efficacy of the treatment given. It is not by chance that different methods of approach to the testing of toxemia have been developed by different workers [1, 2, 8].

The object of this investigation was to compare the toxic properties of the rat serum after burns by the blood culture method and in mice with a blocked reticuloendothelial system (RES) [4, 8].

EXPERIMENTAL METHOD

Experiments were carried out on 84 Wistar rats on which a flame burn covering 15-20% of the body surface and with an exposure of 50 sec was inflicted. The sera were obtained after bleeding in separate groups of

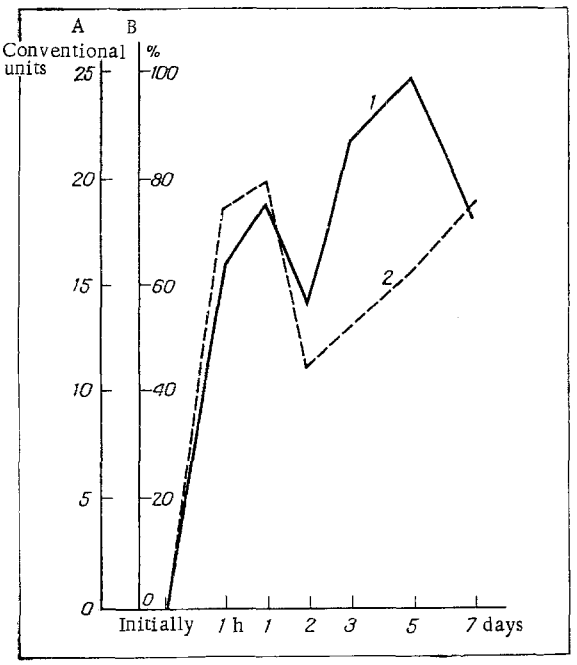

Fig. 1. Toxic properties of serum of rats after burns. 1) Toxicity in blood cultures; 2) mortality of mice. Abscissa, time after burning; ordinate: A) toxicity in blood culture (in conventional units); B) toxicity characterized by mortality of mice (in %).

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animals before burning and 1 h and 1, 2, 3, 5, and 7 days after burning. The toxic properties of the serum were determined by the blood culture method and in mice with a blocked RES.

The blood culture method is based on changes in leukocyte migration in a buffy coat culture under the influence of the test medium compared with migration of cultures after addition of Ringer's solution to the nutrient medium. The toxic action was expressed as inhibition of leukocyte migration and recorded as a percentage of migration in the control, preceded by a minus sign, whereas absence of toxic action – a stimulating effect – was recorded as a percentage of migration in the control preceded by a plus sign.

The method of biotesting in vivo is based on quantitative assessment of the mortality in mice whose resistance has been artificially reduced by blocking of their RES. We started from the well-known fact that under certain extremal conditions (burns, trauma, shock of varied origin, and so on) the function of the RES is depressed and the action of protective mechanisms is weakened. By contrast, in intact animals the RES actively neutralizes toxic products entering from outside.

The RES was blocked by a single injection of a colloidal dye (trypan blue), in the form of a freshly prepared 1% solution in a dose of 0.025 ml/g body weight, into the caudal vein. The test serum, in a volume of 1 ml, was injected intraperitoneally into the mice 30-40 min after blockade. The degree of toxicity was assessed from the percentage mortality among the mice after 72 h. All the numerical results were subjected to statistical analysis by Student's t-test.

EXPERIMENTAL RESULTS

The results are given in Fig. 1. They show that the sera of the rats before burning did not inhibit leukocyte migration and did not cause death of mice with a blocked RES. A severe toxic action of the serum of the burned rats on leukocyte migration was noted 1 h after thermal injury. At the same time a very high percentage mortality was noted among mice with a blocked RES when serum taken 1 h after burning was injected into them. The toxic action 24 h after trauma was stronger still when determined by both methods. On the 2nd day there was a marked decrease in the toxic action. A second wave of potentiation of toxicity was clearly detected on the 5th-7th days after burning. This observation confirmed yet again the biphasic character of burn toxemia described previously [2, 4].

In the modern view, burn trauma leads to the formation of new substrate, exerting a toxic action [9], in the skin. The first phase of toxemia may perhaps be due to the entry of these substances into the blood stream together with others which appear in the body as a result of the general response to the extremal situation. Weakening of the toxic action on the 2nd and 3rd days after trauma was evidently connected with absorption of toxic products from the blood stream by the tissues, leading to a marked impairment of the functional indices of the organs responsible for detoxication, namely the kidneys, liver, and RES. Previous investigations showed that it is at these times that the excretory function of the kidneys, glomerular filtration, and the secretory capacity of the tubular epithelium are sharply reduced. The renal hemodynamics is considerably impaired [6]. Functional changes in the liver also were biphasic in character. The first phase of worsening of function was observed 1-2 days after burning – the excretory-absorptive function, protein synthesis, and enzymic activity were sharply reduced. Considerable depression of the RES was found: Its phagocytic activity and ingestive function were depressed. The time of uptake of radioactive colloidal gold (^{198}Au) from the blood was lengthened, evidence of blocking of the RES after burns [6, 7].

The second wave of potentiation of the toxic action coincided in time with intensification of proteolysis in tissues remote from the pathological focus. It was shown previously that 5 days after burning activity of proteolytic enzymes in the serum and liver is significantly increased and that the toxicity of the serum and of liver extracts also is considerably increased. This increased toxicity is still present 7 days after trauma [3].

Meanwhile (5th-7th days) massive invasion of microorganisms and their toxins was observed, resulting not only in disturbance of the barrier functions of the skin and mucous membranes, but also in a marked decline of the natural defensive factors [5].

The investigation thus showed that in burned animals the appearance of toxic properties, not found in intact animals, can be detected in the serum as early as 1 h after burning, by two different methods of biological testing – by the blood culture method, and by the use of mice with a blocked RES. When tested during the period of burn shock and toxemia, the toxic action is biphasic in character: a first phase of toxicity on the first day after trauma and the second phase on the 5th-7th days after burning. The comparability of the results obtained by the two different methods suggests that the toxic effect found both in vitro and in vivo is due to the same factors; both methods accordingly can be recommended for the testing of toxemia after thermal injuries.

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ENDERPINE LEVEL IN THE MYOCARDIUM OF ANIMALS EXPOSED TO STRESS

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The writers showed previously [1-3] that animal tissues contain endogenous alkaloid-like substances which influence vascular tone and the cardiac frequency. These substances, isolated as three chromatographically homogeneous fractions, were named RP1, RP2, and RP3, or enderpines. It was suggested that they play the role of regulators of homeostasis of different forms of catecholamines and indoleamines (free, bound, and so on). The name "enderpines" is derived from the words "endogenous reserpines."

In the present investigation an attempt was made to elucidate the function of the enderpines by determining changes in the content of these substances in the myocardium of animals exposed to stress: immobilization, subcutaneous injection of water and adrenalin, and injection of α-methyldopamine.

EXPERIMENTAL METHOD

Experiments were carried out on 100 male Wistar rats weighing 250-300 g. Isolation of the enderpines from the myocardium and the first stages of their purification were carried out by the method described previously [1]. The enderpine content was determined fluorometrically on the KM-3 chromatogram-spectrophotometer ("Opton") with an excitation wavelength of 270 nm. For this purpose, in the last stages of purification the enderpines were isolated by chromatography on "Silufol" plates ("Cavalier") in a system of acteone–carbon tetrachloride–isooctane–petroleum ether–n-propanol (25:25:25:25:50). Enderpines RP1, RP2, and RP3 with electrophoretic mobilities of 0.78, 0.49, and 0.1 respectively, were eluted with a chloroform–methanol (2:1) mixture and applied as spots 5 mm in diameter to DC-Alufolien Kieselgel 66-Kieselguhr F-254 plates ("Merck"), in the same way as enderpines of known concentration to obtain calibration curves. The results were expressed in micrograms RP1, RP2, and RP3 per gram wet weight of tissue.

Rats were immobilized by stretching them by Selye's method for 3 and 24 h. Intact rats served as the control. α-Methyldopamine (dopegit, from "Egit") was given to the experimental rats by mouth through a catheter in the form of a suspension of crushed tablets in 2 ml milk in a dose of 0.5 mg/100 g body weight once daily for 3 days. Adrenalin bitartrate (from "Sigma") was injected subcutaneously into the rats in 1 ml distilled water in a dose of 0.8 ml/100 g body weight. Control rats received 1 ml of distilled water subcutaneously. The general control consisted of intact animals. The rats were killed by a blow on the head.

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