

TOXIC PROPERTIES OF EXTRACTS OF NORMAL
AND BURNED SKIN IN MICE WITH BLOCKED
RETICULO-ENDOTHELIAL SYSTEM

Academician N. A. Fedorov,*
R. V. Nedoshivina, and B. E. Movshev

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After blocking of the reticulo-endothelial system (RES) in 282 noninbred albino mice, extracts of burned skin of rats and dogs had a toxic action on the animals which depended on the absolute protein content in the extract. When the toxic factor was injected into mice after preliminary blocking of the RES it gave rise to severe signs of poisoning leading to death of almost 100% of the animals. Injection of extracts of normal skin with protein content adequate to cause toxic effects was tolerated by the animals well, despite blocking of the RES, and in most cases did not cause death. The results are evidence that burned skin contains burn toxins which are not present in the skin of healthy animals.

Investigations have shown that extracts from burned skin possess toxic properties. Injection of such extracts into intact animals and in animals whose reticulo-endothelial system (RES) has been blocked causes death in most cases, with signs of marked burn toxemia [1-8, 12, 13]. This evidence has been disputed by other investigators, who could not confirm the toxicity of burned skin [9-11, 14]. The contradictory nature of these results can largely be explained by the inadequate standardization of the preparations used and of the analysis of their toxic properties.

Toxic properties of extracts of normal and burned skin were studied in experiments on mice with blocked RES.

EXPERIMENTAL METHOD

In experiments on 282 noninbred albino mice weighing 18-20 g the RES was blocked by a single intravenous injection of a special ink (11/1431a, Gunther Wagner, West Germany) in a dose of 24 mg/100 g body weight. After 30-40 min the experimental mice received an intraperitoneal injection of 1 ml of an extract of normal or burned rat or dog skin. The toxicity of the extracts was assessed from the mortality among the mice during the next 72 h. The skin of Wistar rats was used 46-48 h after a spirit-lamp burn (20% of the body surface, exposure 30 sec). The skin of mongrel dogs was removed 24 h after a standard electrical burn (exposure 20 sec).

The area of burned skin was freed from adipose tissue, cut into pieces with scissors or passed twice through a mincer, frozen with liquid nitrogen at -196°C , and ground for 5 min in a blender with the addition

*Academy of Medical Sciences of the USSR.

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TABLE 1. Probit Analysis of Toxicity of Extracts of Normal and Burned Skin

Preparation	Dose of protein (mg)	Toxicity	Probit equation (y) as a function of log dose (x)	LD ₅₀ (mg)	95% confidence interval
Extract of normal rat skin	0,05 0,15 0,50 2,0	0/10 7/30 4/11 3/5	$y = 1,692 + 1,119x$	0,905	0,255—3,21
Extract of burned rat skin	0,05 0,07 0,12 0,15 0,30 0,50 2,00	2/10 8/10 6/10 69/63 2/2 10/11 5/5	$y = 0,886 + 2,386x$	0,053	0,032—0,087
Extract of burned dog skin	0,10 0,15 0,20 0,25 0,30 0,40	2/10 5/10 8/10 6/10 14/15 10/10	$y = 4,393x - 4,616$	0,154	0,124—0,192
Extract of normal dog skin	0,15 0,20 0,25	0/10 1/10 0/10			

Numerator gives number of mice dying within 72 h after injection of preparation; denominator gives total number of experimental mice.

of 3 volumes 0.14 M sodium chloride solution (pH 7.2). The homogenate was centrifuged at 20,000 g for 40 min and the supernatant was treated twice with ether at -15°C , dialyzed against 60 volumes physiological saline, and clarified by centrifugation under the same conditions. The protein content of the supernatant was determined by Lowry's method, and its toxic properties were then studied. Extracts were obtained in the same way from unburned rat and dog skin. All operations were carried out at a temperature of $0-4^{\circ}\text{C}$.

Statistical analysis of the results included rank correlation by the least-square method and probit analysis [1].

EXPERIMENTAL RESULTS

The toxicity of the extracts was shown to depend on their absolute protein content. When extracts of normal and burned skin with a protein content of 2 mg/ml were injected, similar results were obtained: a similar number of animals died in both groups. Reducing the protein content brought to light a difference between the toxic action of the extracts.

Extracts of burned rat skin with a protein content of 0.5 mg/ml had a marked toxic action: ten of the 11 experimental mice died. Extracts of normal skin with the same protein content were tolerated much better by the experimental animals: only four of the 11 mice died.

The clearest effect was obtained after injection of extracts with a protein content of 0.16 mg/ml. After injection of the extract of normal rat skin, a very weak toxic action was observed: seven of the 30 experimental mice died after a longer interval than usually. Conversely, injection of extract burned skin gave rise to severe toxic manifestations in the experimental animals: general depression, acute kerato-conjunctivitis, diarrhea, and paralysis of the hind limbs. The depression increased, and 59 of the 63 experimental mice died after 12–24 h.

A dose of 0.15 mg protein is thus the smallest to give rise to the maximal lethal effect. The values of LD₅₀ for the various preparations and their confidence limits are shown in Table 1.

Experiments on mice without blocking of the RES were carried out as controls; injection of extracts of normal and burned skin with the minimal toxogenic dose of protein into these animals did not cause death.

The question of whether burned skin secretes a specific substance or whether the toxic changes observed in burns are caused by the infection has been discussed for many years. In the present experiments

bacteriological investigation (N. S. Murashova) of the organs and blood of the mice which died showed no growth of microorganisms, indicating that bacteriemia was absent in the mice with experimental toxic manifestations.

Extracts of burned rat and dog skin were thus found to have a toxic action which depended on the absolute content of protein in the extract. Injection of the toxic factor into mice after preliminary blocking of the RES caused severe toxic effects and caused death of almost 100% of the animals. Conversely, injection of extracts of normal skin with adequate protein content, despite blocking of the RES, was well tolerated by the animals and in most cases did not cause death. The toxic properties of the extracts of normal skin with a protein content of 0.5-2 mg/ml can evidently be attributed to their high content of heterogeneous protein.

These results provide additional evidence that burned skin contains burn toxins which are absent in the skin of healthy animals.

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