

# FORMATION OF ACTIVE ANTITOXIC IMMUNITY IN GERM-FREE RATS AFTER BURNS

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The mechanism of appearance of the antitoxic properties of convalescent serum after burns was investigated in rats. A flame burn covering 20-25% of the body surface with an exposure of 20 sec was inflicted on 10 germ-free and 12 ordinary contaminated rats. Convalescent serum was obtained 2 months after burning and was tested by the blood culture method for the presence of a neutralization effect against toxic serum taken 24 h after burning. The sera of five unburned rats were used as the control. The sera of the germ-free and ordinary contaminated rats had an equally strong antitoxic action. The sera of the control unburned rats gave no antitoxic effect. The results suggest that the formation of antitoxic immunity after burning takes place not only on account of the bacterial flora, but also on account of antigens of tissue origin.

KEY WORDS: burns; germ-free animals; bactericidal action of blood serum.

In 1955 a method of immunotherapy of burns was developed by N. A. Fedorov and S. V. Skurkovich. The advantages of this type of therapy were demonstrated in experimental and also in clinical investigations [2-5, 7]. Nevertheless, the mechanism of the therapeutic action of the serum from convalescents from burns still remains unexplained. It is not known whether the antitoxic antibodies appearing in burns are produced as a result of extensive infectious invasion characteristic of burns or whether these antibodies are the result of immunization by products of histogenic origin [1].

The most adequate model with which to study the mechanism of formation of antitoxic immunity in burns is provided by gnotobiotic animals — either germ-free or with a limited microbial flora. A previous investigation [6] showed that toxemia after burns developed in gnotobiotic rats with no pathogenic flora in exactly the same way as in ordinary contaminated rats. Serum and saline extracts of the internal organs possessed a definite toxic action both in experimental (gnotobiotic) and control (ordinary) burned rats.

The object of the present investigation was to study the possibility of obtaining active antitoxic serum in burned gnotobiotic rats with no pathogenic microbial flora.

## EXPERIMENTAL METHOD

Experiments were carried out on 27 Wistar rats of line AF/HAN: 10 gnotobiotic and 17 contaminated. The gnotobiotic rats had only three species of nonpathogenic microorganisms: Bacillus subtilis, Clostridium putrificum, and one species of Saccharomyces. A flame burn covering 20-25% of the body surface with an exposure of 20 sec was inflicted on 22 rats. Five unburned rats were used as the control. The burned rats 2 months after burning and the unburned rats were exsanguinated.

The antitoxic effect of the serum was investigated by the blood culture method. As was shown previously [2], the serum of an animal convalescent after burns neutralizes the toxic action of serum taken 24 h after burning (T-24) on the migration of leukocytes. Serum taken from a dog 24 h after an electrical

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burn of 20% of the body surface with an exposure of 20 sec was used as T-24 in all the experiments. The sera of the convalescent and unburned rats were mixed with the toxic serum in equal volumes and incubated for 2 h at 37°C. The neutralizing effect was assessed with respect to complete abolition of the inhibitory action of the T-24 on migration of the leukocytes in blood cultures on the addition of the test serum.

#### EXPERIMENTAL RESULTS

The sera of convalescent gnotobiotic rats in nine of 10 cases had an antitoxic action. A neutralization effect was found in 10 of 12 cases in the sera of the ordinary contaminated burned rats. Abolition of the toxic effect was not observed in any of the five cases in which T-24 was incubated with normal serum obtained from unburned animals.

The absence of pathogenic microorganisms characteristic of burns (Escherichia coli, Staphylococcus, Streptococcus, etc.,) in the gnotobiotic rats suggests that the formation of antitoxic immunity in burned animals takes place not only on account of the bacterial flora, but also on account of antigens of histogenic, nonbacterial origin.

The therapeutic action of serum of an animal convalescent after burns is thus directed not only against microorganisms and their toxins, but also against toxic products of protein origin appearing in the tissues of the body under the influence of burn trauma.

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