# EFFECT OF BURN TOXIN ON PHAGOCYTIC ACTIVITY OF THE RETICULOENDOTHELIAL SYSTEM

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

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An important place in the group of pathological changes arising in the body after burn trauma is occupied by the body's defenses and, in particular, the phagocytic activity and ingestive power of the reticuloendothelial system (RES). Depression of the reticuloendothelial cells of the liver in dogs after severe thermal burns, as the writers showed previously, persists for a long time and lowers the body's resistance to infection [4]. The mechanism of the pathogenetic effects of heat-damaged skin on RES function is evidently mainly connected with the action of toxemia factors and, in particular, the high-molecular-weight toxin of burned skin [1, 5]. The object of the present investigation was to study functional activity of the RES during burn toxemia due to thermal trauma or injection of a preparation of burn toxin.

## EXPERIMENTAL METHOD

Experiments were carried out on 85 Wistar rats weighing 180-200 g. The animals were divided into four groups: 1) burned rats, 2) rats into which extracts of burned skin were injected in a dose of 1.0 mg protein/100 g body weight, 3) rats in which extracts of normal skin were injected. A 4th-degree burn was inflicted by exposure for 40 sec to the flame of a spirit lamp. The area of the burn was equivalent to 20-25% of the total body surface.

Tests were carried out on the 1st, 2nd, 3rd, 5th, and 7th days, on 5-7 rats at each time. Extracts of normal and burned skin were prepared by the method developed and described by the writers previously [2, 6]. Burn toxin was isolated by an immunochemical method directly from extracts of burned skin on a column with immunosorbent containing pure antibodies against the toxin but not contaminated with antibodies against antigens of normal tissues and serum. The immunosorbent used for the work was based on CNBr Sepharose 4B (from Pharmacia, Sweden). After application of extract of burned skin to the column and washing with buffer solution (0.01 M phosphate, pH 7.0) to remove unbound proteins, the toxin was eluted with 0.01 N HCl, and immediately neutralized as it flowed from the column with a 1 M solution of sodium bicarbonate. To reduce nonspecific sorption of proteins, 0.5 M sodium chloride was added to the washing and eluting solutions. The eluate was desalted by ultrafiltration. The functional state of the RES was assessed from the blood clearance after intravenous injection of $^{198}$Au. The percentage retention of $^{198}$Au in the blood stream and phagocytic activity were determined by the formula described in [3].

## EXPERIMENTAL RESULTS

Under the influence of burn trauma (group 1) considerable inhibition of phagocytic activity of the liver macrophages took place (Fig. 1). In all experimental animals 2 h after burning the rate of elimination of radioactive colloidal gold injected into the blood stream was reduced and its retention in the blood after 15 min amounted to 69.8%, compared with the normal level of under 1%. On the 1st, 2nd, 3rd, and 5th days the ingestive function of the RES still remained depressed. On the 7th day it began to rise, as shown by an increase in the rate of elimination of $^{198}$Au from the blood stream (the radioactivity of the sample was 42.0%).

The phagocytic index, which reflects the intensity of activity of the Kupffer cells, fell to very low values (0.02 compared with the normal 0.12). As early as on the 1st day after burning blockage of the RES took place (Fig. 2). This may signify that the phagocytic cell was no longer able to ingest the injected material or that the rate at which it could ingest additional particles was sharply reduced.

The mechanism of RES blockage has recently been linked with exhaustion of plasma factors (opsonins) as the principal physiological regulators of phagocytic activity of the RES [8-10]. The importance of this mechanism is confirmed by the presence of severe $\alpha_2$-globulin hypo-opsoninemia in various extremal states. The change in the opsonin level may be

*Academician of the Academy of Medical Sciences of the USSR.

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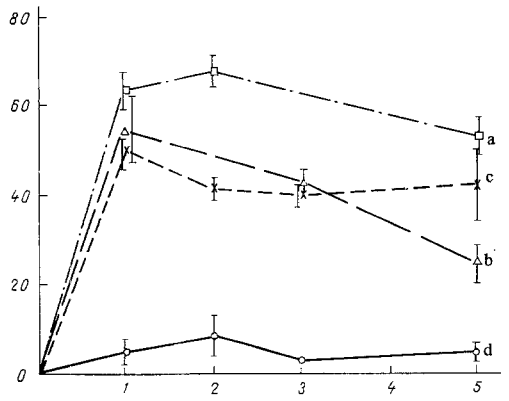


Fig. 1

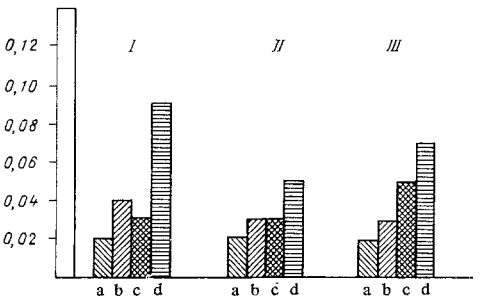


Fig. 2

Fig. 1. Dynamics of elimination of $^{198}$Au from rat's blood. a) Thermal trauma, b) injection of burn toxin, c) injection of extract of burned skin, d) injection of extract of normal skin. Abscissa, time of investigation (in days); ordinate, % of retention of $^{198}$Au.

Fig. 2. Changes in phagocytic index on 1st (I), 2nd (II), and 3rd (III) days. a) Thermal trauma, b) injection of extract of burned skin, c) injection of burn toxin, d) injection of extract of normal skin. Ordinate, phagocytic index (in min).

due to loss of plasma proteins, a particularly characteristic feature of burn trauma. The hypo-opsoninemia correlates with depression of the phagocytic activity of the RES [11]. The phagocytic index still remained within limits of its minimal values (0.02) on the 2nd and 5th days after burning.

In the experiments on the animals of group 2 extracts of burned skin injected intraperitoneally into intact rats were found to have a similar action on ingestive function of the RES to that of burn trauma. The blocking effect of the extracts was detected from the first day after injection. Investigation of blood samples showed the development of considerable depression of the ingestive capacity of the teticuloendothelial cells of the liver. Blood clearance from $^{198}$Au was delayed and its retention in the blood amounted to 53.3%. Disturbance of the function of the liver RES after injection of the extracts continued for 5-7 days. The phagocytic index fell in the same way as after natural burn trauma to the lowest limits (0.02), and not until the 5th day did it rise again to 0.05.

In the animals of group 3 phagocytic activity and the ingestive power of the macrophages showed similar changes after injection of extracts of burned skin to those produced by thermal burns (Fig. 1b). The rate of elimination of colloidal gold decreased and its retention in the blood amounted to 54.0%. Depression of macrophage function persisted for 5-7 days. The similarity of the action of the toxin preparations will be noted, although the dose which was injected was only one-sixtieth of that of extracts of burned and normal skin. The phagocytic index on the 1st day reached its minimal level (0.03). On the 5th day it had increased again to 0.05.

In the control animals (group 4) into which extracts of skin of intact rats were injected, phagocytic activity was virtually unchanged. The rate of elimination of $^{198}$Au from the blood stream likewise was substantially unchanged: Its retention in the blood of all the animals of this group was 5% (Fig. 1). The phagocytic index varied from 0.09 to 0.10.

The experiments thus showed that stimulation of burn toxemia by injection of extracts of burned skin or of purified high-molecular-weight burn toxin depresses the phagocytic activity of the liver macrophages in the same way as the action of a natural burn. A particular feature of the toxic preparations is their ability to block the RES of the liver. The results confirm the previous hypothesis that the starting point for burn toxemia is evidently the focus of thermal injury [5]. Accumulation of burn toxic products in the primary burn focus and their subsequent circulation in the blood stream are factors which shape the clinical picture of burn toxemia. Lowering of the resistance of the body facilitates the development of local and generalized infection, complicating the course of burns.

Depression of the phagocytic activity of the liver macrophages during burn toxemia caused by injection of extracts of burned skin and burn toxin may provide a sensitive indicator of the state of the nonspecific immune resistance of the body in burn trauma, and burn toxin can be regarded as the initiator of the changes in the early stage of burn toxemia.

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