

CHANGES IN SOME NONSPECIFIC DEFENSIVE FACTORS  
IN EXPERIMENTAL SALMONELLA INFECTION

V. I. Pokrovskii, B. S. Nageev,  
D. I. Gabrilovich, and B. M. Ibragimov

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The study of changes in the defensive factors of the body in salmonellosis is of great importance, due both to the widespread nature of the disease, which exists in several different forms, and also the fact that development of the infectious process directly dependent on relations between the microorganisms and the host, in which a definite role is played by the degree of resistance of the host to the infectious agent.

The aim of this investigation was to study some parameters of the microbicidal system of neutrophilic granulocytes in experimental salmonellosis.

EXPERIMENTAL METHOD

A cytochemical study of the components of the intraleukocytic microbicidal system of leukocytes was carried out on two models of experimental salmonellosis in male Chinchilla rabbits weighing 1.2-1.5 kg. In the first case 14 animals were infected per os by introduction of  $5 \cdot 10^9$  bacterial cells of a strain of *Salmonella typhimurium*, isolated from a sick child and repeatedly subcultured in albino mice, after starvation for 2 days and neutralization of the gastric juice with soda solution [11]. The control consisted of 7 rabbits which received milk instead of the bacterial suspension. The animals were investigated for 35 days after infection. The second model was produced by injection of purified salmonella endotoxin into the marginal vein of the rabbit's ear in a dose of 1 mg/kg body weight [3, 8]. The experimental group consisted of 16 animals. The control consisted of 6 rabbits which received, instead of endotoxin, the same volume of physiological saline. The animals were kept under observation for 10 days after injection of the endotoxin. During the development of the disease the animals' general condition was assessed, and in the case of rabbits infected per os a bacteriological investigation of the stools and blood was carried out to verify development of the infectious process. Development of the toxidoinfection was verified clinically and by post mortem examination. Activity of myeloperoxidase (MP) was determined in the rabbit's pseudoeosinophils by the method in [13], the concentration of cationic proteins (CP) by the method in [10], glycogen as in [9], and lipids as in [4], in I. M. Raskin's modification [6]. Activity of the nitro-BT reduction test, both spontaneous and stimulated as in [14], in the writers' modification [5], also was investigated. All cytochemical parameters were expressed in conventional units [12]. The results were subjected to statistical analysis [7].

EXPERIMENTAL RESULTS

All the animals of the experimental series showed worsening of their general condition, adynamia, pyrexia of 1-1.5°C, a reduction of body weight by 5-7%, and the passage of liquid stools 24 h after infection per os. Cultures of the stools were positive for *S. typhimurium* until the 10th day. These changes reached a maximum on the 3rd-7th days after infection, and by the 14th day the animals were back to their original state. None of the infected animals died during the investigation. The clinical picture thus obtained has been assessed as one of a mild form of experimental salmonella infection. A significant decrease in CP concentration and MP activity of the pseudoeosinophils was found in all rabbits of this series, with

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Central Research Institute of Epidemiology, Ministry of Health of the USSR, Moscow.  
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TABLE 1. Cytochemical Parameters of Rabbit Pseudoeosinophils in the Course of Experimental Salmonella Infection ( $M \pm m$ )

Day of investigation in experiment	Cationic protein	Myeloperoxidase	Lipids	Glycogen	Nitro-BT test	
					spontaneous	stimulated
Control	208,1 $\pm$ 3,7	170,0 $\pm$ 3,1	182,7 $\pm$ 4,3	192,6 $\pm$ 4,1	13,2 $\pm$ 0,8	32,4 $\pm$ 1,2
1	216,0 $\pm$ 4,2	169,8 $\pm$ 4,3	182,7 $\pm$ 4,3	232,3 $\pm$ 4,0	13,8 $\pm$ 0,6	30,6 $\pm$ 0,9
3	169,0 $\pm$ 3,2*	169,0 $\pm$ 3,2	138,3 $\pm$ 2,9*	240,1 $\pm$ 3,4*	14,1 $\pm$ 1,3	38,4 $\pm$ 1,8
5	161,3 $\pm$ 3,2*	109,7 $\pm$ 4,1*	130,7 $\pm$ 3,8*	260,7 $\pm$ 4,9*	21,2 $\pm$ 1,2*	30,5 $\pm$ 1,6
7	143,2 $\pm$ 3,4*	74,3 $\pm$ 2,7*	132,4 $\pm$ 2,8*	284,3 $\pm$ 3,4*	39,7 $\pm$ 1,3*	33,7 $\pm$ 2,3
10	179,3 $\pm$ 2,8*	156,0 $\pm$ 4,1*	134,0 $\pm$ 3,7*	252,7 $\pm$ 3,8*	40,2 $\pm$ 1,6*	53,4 $\pm$ 2,4*
14	196,0 $\pm$ 4,7*	138,7 $\pm$ 4,3*	161,2 $\pm$ 4,1*	228,3 $\pm$ 4,1*	25,8 $\pm$ 1,8*	74,6 $\pm$ 2,1*
16	206,6 $\pm$ 4,1	144,4 $\pm$ 3,8*	181,3 $\pm$ 4,2	196,5 $\pm$ 3,1	20,1 $\pm$ 1,3*	58,2 $\pm$ 2,2*
20	210,1 $\pm$ 4,3	160,7 $\pm$ 4,4	188, $\pm$ 4,2	198,1 $\pm$ 4,3	12,6 $\pm$ 0,6	46,2 $\pm$ 1,2*

Legend. Here and in Table 2, \* indicates significant ( $P < 0.05$ ) results relative to control.

TABLE 2. Cytochemical Parameters of Rabbit Pseudoeosinophils during Salmonella Endotoxin Shock ( $M \pm m$ )

Time of investigation	Cationic protein	Myeloperoxidase	Lipids	Glycogen	Nitro-BT test	
					spontaneous	stimulated
Control Experiment	224,3 $\pm$ 5,7	156,7 $\pm$ 5,1	193,3 $\pm$ 3,6	193,4 $\pm$ 4,2	27,5 $\pm$ 6,4	105,5 $\pm$ 6,4
30 min	24,0 $\pm$ 3,2*	17,3 $\pm$ 2,1*	141,3 $\pm$ 5,8*	24,2 $\pm$ 5,6*	3,7 $\pm$ 0,8*	4,1 $\pm$ 0,9*
3 h	57,7 $\pm$ 6,2*	8,0 $\pm$ 2,1*	112,0 $\pm$ 9,3*	62,7 $\pm$ 4,8*	3,7 $\pm$ 0,9*	5,7 $\pm$ 1,1*
24 h	120,4 $\pm$ 5,3*	29,7 $\pm$ 3,8*	194,0 $\pm$ 2,7*	189,3 $\pm$ 4,9	28,8 $\pm$ 3,2	45,3 $\pm$ 3,6*
48 h	146,3 $\pm$ 11,2*	48,9 $\pm$ 5,4*	202,0 $\pm$ 4,9	190,0 $\pm$ 5,2	28,3 $\pm$ 4,8	101,7 $\pm$ 4,8
120 h	182,0 $\pm$ 6,7*	74,3 $\pm$ 4,2*	204,3 $\pm$ 4,2	198,3 $\pm$ 4,8	28,3 $\pm$ 5,6	112,2 $\pm$ 5,9
240 h	221,3 $\pm$ 4,8	169,8 $\pm$ 6,3	207,0 $\pm$ 5,9	196,2 $\pm$ 5,3	28,3 $\pm$ 6,9	109,7 $\pm$ 6,4

minimal values on the 3rd-5th day after infection (Table 1). The level of these parameters started to rise gradually on the 10th day and returned to normal on the 16th and 20th days after infection. Similar data were found when the lipid content in the cell was studied.

On the other hand the glycogen concentration was significantly increased with effect from the first day after infection, with a maximum on the 7th day. Activity of the nitro-BT test, both spontaneous and stimulated by *S. typhimurium*, was changed in the same direction. However, activity of the stimulated nitro-BT test was almost twice as high as they of the spontaneous test, and appeared after some delay.

Initial manifestations of endotoxic shock began to be observed in rabbits 30 min after injection of salmonella endotoxin, and the complete picture evolved after 3 h. The animals showed adynamia, a rise of temperature by 1-2°C, an increase in the respiration rate, and liquid stools. Loss of body weight by the end of the first day amounted to 12-15%. Of the 16 rabbits of the experimental series 11 died (mainly on the first day after infection, 5 of them during the first 3 h). All rabbits which died were autopsied in order to verify the diagnosis. The changes discovered confirmed that what was observed in the experiments was endotoxin shock.

A marked decrease in MP activity and also in concentrations of CP, glycogen, and lipids were observed 30 min after injection of the endotoxin. The minimal values, which were sometimes only 10% of the initial values, were observed in test carried out 30 min and 3 h after injection of the endotoxin (Table 2). The CP concentration and MP activity returned to normal 240 h after injection of the endotoxin, and the lipid and glycogen levels after 24 h.

Activity of the spontaneous nitro-BT test was reduced by almost 90% 30 min after the beginning of the experiment, and the initial level was restored after 24 h. Activity of the nitro-BT test stimulated by endotoxin was 4 times higher in the control than spontaneous activity. The minimal values of the stimulated nitro-BT test were achieved 30 min after injection of the endotoxin, and the initial level was restored after 48 h.

The results show that changes in the parameters tested were connected with the mechanisms of the models used. In the case of peroral infection a typical reaction of the blood cells to introduction of the infective agent was observed. This was expressed as increased

activity of the nitro-BT test, reflecting the metabolic "burst" observed during phagocytosis, increased phagocytic activity, and increased digestive power of the animals' leukocytes, as was demonstrated previously [2]. The decrease in MP activity and the CP concentration was linked with the outflow of these substances, which are very important microbicidal agents of blood cells. The decrease in the lipid concentration was due in all probability to the increased energy consumption of the cells.

As was expected, the use of the stimulated nitro-BT test gave no significant advantages for analysis of the processes taking place in the cells [1]. The stimulated nitro-BT test was less dynamic than the spontaneous.

Injection of purified salmonella endotoxin caused profound inhibition of activity of all the nonspecific defensive factors, and the mechanism of this inhibition was connected with the nonspecific effect of shock on the defensive functions of the blood cells, leading to their complete exhaustion.

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