THE EFFECT OF CORTISONE ON THE BACTERICIDAL ACTIVITY OF THE SERUM OF INFECTED ANIMALS

V. N. Solov'ev

From the Division of Chemotherapy (Head – Dr. Med. Sci. A. M. Chernukh) of the Institute of Pharmacology and Chemotherapy (Director – Active Member of the AMN SSSR V. V. Zakusov) of the AMN SSSR, Moscow

(Received June 13, 1958. Presented by Active Member of the AMN SSSR V. V. Zakusov)

Cortisone depresses the defensive reactions in experimental animals and lowers their resistance to the majority of infections which have been studied. Nevertheless favorable results have been obtained clinically from the combined application of cortisone and antibiotics in the treatment of various forms of tuberculosis and other infectious diseases [4, 6, 8, 11]. In individual cases in experimental conditions also the administration of cortisone has increased the resistance to infection [1, 5, 11]. By the combined use of cortisone, adrenocorticotropic hormone (ACTH) and butadione with tetracycline in mice infected with the pneumonia bacillus, we observed an increase in the therapeutic activity of tetracycline. These findings suggest that the action of cortisone on the process of infection and on the defensive reactions of the body is complex and depends on the state of the animal and on the conditions of application of the cortisone.

In a previous communication [3], in agreement with the contemporary experiments of Oravec [8], it was shown that during the administration of cortisone to white mice and rats the bactericidal activity of the serum of these animals against Bacillus dysenteriae flexneri, strain 4833 W, was reduced; this was evidently due to a fall in the titer of properdine. The importance of these changes for the resistance of the animal was still not clear, however, for until now the action of bactericidal factors in the serum within the system of defensive reactions has not been adequately studied.

In order to investigate this problem we carried out experiments on white mice and rats and compared the changes in the bactericidal activity of the serum and the inflammatory exudate in infected animals during administration of cortisone with the changes in resistance to the same infection. In order to permit a fuller comparison to be made of the results with those obtained in previous experiments in which the effect of cortisone was studied on an infection caused by Bacillus pneumoniae, in the present work we also devoted particular attention to this infection.

EXPERIMENTAL METHOD

In the experiments we used white mice of the strain "Pure Czechoslovak Albino AK", weighing 14-16 g, and white rats weighing 100-110 and 150-170 g. The rats in different categories of weight were distributed equally among the groups in the experiments. No peculiarities were observed in the reactions of rats of different weight to the procedures carried out.

Blood was taken from the rats from the caudal veins by means of an aspirating device or from the heart under anesthesia. In the first case investigations were carried out repeatedly on the same animals, and blood was taken twice before the start of the experiment. Blood was taken from the heart in the case of the mice, under anesthesia, in a volume of 0.6-0.8 ml, and pooled by groups. For the production of an inflammatory exudate the rats were injected in the right pleural cavity with 0.1 ml of turpentine. The exudate was collected when the chest was opened 5 hours after the injection. In all cases the pH of the exudates was 7.6 ± 0.1.

TABLE 1

The Effect of Cortisone on the Resistance of Mice to B. pneumoniae

Dose of cortisone mg/kg	Number of animals	Percentage of animals surviving 4 days	Total duration of life in days
20	15	60	83
4	15	40	65
1,3	15	80	102
None given	15	93,3	116
Confidence limit by Prigg's method for the control group			
1,3	45	28,9	57
None given	45	35,6	53
Confidence limit by Prigg's method for the control group			

TABLE 2

The Effect of Cortisone on the Resistance of Rats to B. pneumoniae

Dose of cortisone mg/kg	Number of animals	Percentage of animals surviving 4 days	Total duration of life in days
20	30	43,3	124
None given	30	43,3	138

In order to determine the bactericidal activity, to 0.48 ml of serum or exudate was added 0.02 ml of a suspension of B. dysenteriae flexneri No. 4833 W or of B. pneumoniae in 0.85% NaCl solution, in a concentration of 4000-20,000 organisms per ml. The same volume of bacterial suspension was added to 0.48 ml of 0.85% NaCl solution. All the tubes were incubated for 2 hours at 37°C, and then 0.1 ml of fluid from each tube was used to inoculate 3 dishes with meat-peptone agar or Endo's medium. After incubation for 20 hours, the colonies on each dish were counted. The bactericidal index was determined by dividing the number of colonies on the dishes inoculated from the tubes containing this particular reagent by the number of colonies on the dishes inoculated from the control tubes. It was shown that after incubation for 2 hours of a suspension of B. pneumoniae in physiological saline there was no change in their number.

Animals were infected with a 24-hour culture of B. pneumoniae on meat-peptone agar suspended in 0.85% NaCl solution: rats subcutaneously, and mice subcutaneously or intraperitoneally. In case of subcutaneous inoculation, blood was taken for examination after 24 hours; in case of intraperitoneal inoculation 5 hours afterwards.

EXPERIMENTAL RESULTS

1. Changes in the resistance to infection under the influence of cortisone. As can be seen from Table 1, the resistance of the mice to B. pneumoniae shows a tendency to fall even after injection of small doses of cortisone – 1.3 mg/kg. In contrast to this, in rats even 20 mg/kg of cortisone does not perceptibly lower the resistance (Table 2). The natural resistance of rats to this infection is many times higher than that of mice. On infection of mice with 400 organisms, for instance, all the animals died. In rats weighing 110-120 g, a similar result was observed only after inoculation with 20 million organisms.

TABLE 3

Changes in the Bactericidal Activity of Rat Serum Against B. pneumoniae After Administration of Cortisone

Number of animals	Bactericidal index before injection of cortisone	Change in the bactericidal index after injection of cortisone	
		20 mg/kg on two occasions	20 mg/kg on three occasions
10	1,7259	—0,3854±0,33	—1,2882±0,2627

TABLE 4

The Effect of Cortisone on the Bactericidal Activity of Mouse Serum Against B. pneumoniae

Number of animals	Dose of cortisone, mg/kg	Bactericidal index
12	None given	0,373
12	15; once	0,348
12	15; twice	0,347

2. Changes in the bactericidal activity of the serum against B. pneumoniae during administration of cortisone. The activity of the serum and inflammatory exudate against B. dysenteriae falls sharply after treatment on two occasions with zymosan and heating to 56°C for 1 hour. This is in accordance with the known properties of the properdine system [9], and it may be considered that the bactericidal activity of the serum and exudate against B. dysenteriae is due primarily to properdine. The activity of the serum and exudate against B. pneumoniae after adsorption with zymosan is either unchanged or reduced only slightly. This strongly suggests that the activity of the serum and exudate against this microorganism is due to a factor, different in nature from properdine. In support of this view also there are the following findings: the difference between the bactericidal activity of the serum of rats and mice against B. dysenteriae is very large, but the activity of these sera against B. pneumoniae is reasonably close (compare Tables 5 and 8). Neither is there any regular relationship between the activity of the sera of the different animals against the microorganisms named.

The factor from serum and exudate which are active against B. pneumoniae is inactivated by heating to 56°C for 1 hour. Attempts to adsorb it on bentonite and animal charcoal were unsuccessful.

It was pointed out earlier that the activity of the mouse serum against B. dysenteriae falls after injection of cortisone [2]. The bactericidal activity of the serum of these animals against B. pneumoniae also falls as a result of administration of cortisone (Tables 3 and 4) but more slowly, and after injection of larger doses of the drug. Evidently this factor is more resistant to the action of cortisone than is properdine.

3. Changes in the bactericidal activity of the serum and exudate in case of infection with B. pneumoniae. On infection of rats and mice with B. pneumoniae the bactericidal activity of the serum and exudate against this microorganism fell. This fall was seen more clearly during infection with a culture of higher virulence (Table 5) than in the experiment with a culture the virulence of which had been considerably reduced by prolonged subculture through nutrient media without passage through animals (Table 6).

In rats, which before inoculation were given injections of cortisone, the fall in the bactericidal activity of the serum and exudate after inoculation took place to a lesser degree (see Tables 5 and 7) or instead of a fall in the bactericidal activity it was observed to rise (see Table 6). Thus cortisone has a different effect in infected animals from that in noninfected animals, and it permits the bactericidal activity of the serum and of the inflammatory exudate to be maintained at a higher level.

In experiments on mice (Table 8) the degree of lowering of the bactericidal activity of the serum of infected animals and the effect of cortisone on this process depended on the dose of culture inoculated. During the trial of a virulent culture at this period it was noticed that all the animals inoculated subcutaneously with 20,000

TABLE 5

Changes in the Bactericidal Activity of Rat Serum After Infection and Administration of Cortisone

Group	Experimental conditions	Number of animals	Bactericidal index	Significance of variation, p
1	Noninfected rats	39	1,955±0,28	0,0001
2	Infected rats	11	0,7061±0,1034	
3	Rats infected after injection of 20 mg/kg of cortisone	11	1,232±0,2927	0,0891 > 0,072

TABLE 6

Changes in the Bactericidal Activity of Rat Serum After Infection and Administration of Cortisone (Attenuated Culture)

Dose of cortisone, mg/kg	Number of animals	Bactericidal index before infection	Change in the bactericidal index after infection	Significance of variation	
				t	p
20	15	1,69	+0,922±0,355	2,66	0,0093>0,0069
None given	14		−0,529±0,417		

TABLE 7

Changes in the Bactericidal Activity of Inflammatory Exudates in Rats After Infection and Administration of Cortisone

Group	Experimental conditions	No. of animals	Bactericidal index	Significance of variation P	
				1st group in relation to 3rd	3rd group in relation to 4th
1	Noninfected rats	19	1,655±0,3937		
2	Noninfected rats after administration of 20 mg/kg of cortisone	15	1,101±0,1503	0,05743>0,0455	
3	Infected rats	9	0,832±0,1556		
4	Rats infected after administration of 20 mg/kg of cortisone	8	1,216±0,095		0,053>0,044

organisms or more died within the first 72 hours after infection, but only those mice inoculated with not less than 10 million organisms died within the first 24 hours. Animals which, in accordance with these findings, were injected with more than 1 C L D* of culture, showed a considerable lowering of bactericidal activity of the serum, and cortisone intensified this process. In these mice bacteriemia was observed. After inoculation with nonlethal doses of culture, injection of cortisone led to maintenance of the bactericidal activity of the serum at a higher level.

* Critical Lethal Dose — a dose not lethal to all the mice in a given period of time. — Translator.

TABLE 8

Changes in the Bactericidal Activity of Serum in Mice After Infection and Administration of Cortisone

No. of animals	Number of organisms inoculated	Method of inoculation	Dose of cortisone, mg/kg	Bactericidal index
12	—		—	0,373
12	100	Intraperitoneally	—	0,411
10	100	»	15	0,505
10	1 000	Subcutaneously	—	0,237
10	1 000	»	15	0,268
10	100×10^6	»	—	0,172
6	100×10^6	»	15	0,01

At the same time the changes in the bactericidal activity of the serum are evidently not the deciding factor among the group of changes brought about in the defensive systems of the body by administration of cortisone. Otherwise the higher level of bactericidal activity of the serum in rats infected after administration of cortisone would be accompanied also by a higher level of resistance, which however was not found to be the case.

Summing up the results of the investigation it may be stated that after inoculation of white rats and mice with a culture of B. pneumoniae, the bactericidal activity of the serum and the inflammatory exudate against this species of microorganism is lowered. The bactericidal activity of the serum of rats and mice is also lowered after administration of cortisone. However, in case of preliminary administration of cortisone to the inoculated animals the bactericidal activity of the serum and exudate remains at the higher level or even rises. The bactericidal activity of the serum is not of decisive importance in the mechanism of lowering of the resistance of mice to B. pneumonia after administration of cortisone.

SUMMARY

Serum bacterial activity and that of the inflammatory exudate is decreased to B. pneumoniae in infection of white rats and mice with this culture. Bactericidal activity of rats and mice's serum is also reduced after the administration of cortisone. However, in case of preliminary cortisone administration to infected animals the bactericidal activity of the serum and of the exudate remains high or even rises. The change of the serum bactericidal activity is not of decisive significance in the mechanism of decreased resistance of mice to B. pneumoniae under the effect of cortisone.

LITERATURE CITED

[1] V. I. Goncharova, Byull. Eksptl. Biol. i Med. 40, No. 11, 14-16 (1955).

[2] V. N. Solov'ev, Antibiotiki No. 2, 63-67 (1958).

[3] N. A. Shmelev, Problemy Tuberk. No. 3, 63-67 (1957).

[4] F. Boyer and L. Chedid, Ann. Inst. Pasteur (1953), v. 84, p. 453.

[5] L. E. Houghton, Lancet (1954), v. 1, p. 595.

[6] E. H. Kass, M. M. Lundgren and M. Finnland, J. Exper Med. (1954), v. 99, p. 89.

[7] L. W. Kinsell and J. P. Jahn, Ann. New York Acad. Sc. (1955), v. 61, p. 397.

[8] C. Oravec et al. Neoplasma, (1957), v. 41, p. 7.

[9] L. Pillemer et al. Science, (1954), v. 20, p. 279.

[10] H. J. Robinson et al. Proc. Soc. exper. Biol. a. Med. (1953), v. 84, p. 312.

[11] J. E. Smadel et al, Ann. Intern. Med. (1951), v. 34, p. 1.