ACTION OF TYPHOID ENDOTOXIN ON THE CIRCULATING BASOPHILS IN RABBITS' BLOOD

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The discovery of biologically active substances such as heparin [3, 13, 16], histamine [9, 10, 18, 20], and serotonin [4] in the cytoplasmic granules of the mast cells and basophilic granulocytes, and their liberation during degranulation of these cells [2, 11, 14, 15, 20] provided a basis for the study of the role of these cells in the development of allergic reactions. The more recent evidence of the participation of basophils in these reactions [21] suggests that basophilic degranulation may take place not only in truly allergic reactions, but also in other pathological processes, including infectious shock, and that it may play an important role.

In the present investigation the action of a bacterial endotoxin on the circulating basophils in the blood stream was studied in rabbits.

EXPERIMENTAL METHOD

Adult rabbits weighing 2.5-3 kg were used in the experiment. The endotoxin* (a protein-lipopolysaccharide complex obtained from Salmonella typhi strain Ty_2) was injected intravenously in doses of between 1 μg and 5 mg/kg body weight.

Blood samples were taken from the veins of the ears. Heparin solution was used as anticoagulant. Blood was taken before and 30 min, and 1, 2, 4, and 24 h after injection of the endotoxin.

The blood leukocytes were counted by the usual method in a Goryaev's chamber, and the basophils by the method described in the literature [12] with certain modifications: equal volumes (0.25 ml) of freshly taken heparinized blood and 0.25% solution of toluidine blue, made up in a 2.5% solution of acetic acid, were put into a polyethylene tube and, after exposure for 10-20 min, the normal and degranulated basophils were counted in a Goryaev's chamber. The number of degranulated cells was expressed as a percentage of the total number of basophils present in 1 mm^3 of blood.

The basophils had to be counted not later than 1 h after taking the blood because of the possibility of spontaneous degranulation.

To obtain preparations suitable for storage, 0.5 ml of heparinized blood was poured into a polyethylene tube 5 mm in diameter, sealed at one end in a flame, and centrifuged for 5 min at 4000-5000 rpm. Films were made on a glass slide from the clear layer above the erythrocytes, and stained for 10 min with 0.5% toluidine blue solution made up in 50% ethyl alcohol solution.

The circulating basophils in the bloods were counted in 70 rabbits for a period of 24 h after repeated blood samples had been taken at various time intervals and after administration of physiological saline. Together with visual observations on degranulation of the basophils, photomicrographs were taken of individual basophils.

The effect of the endotoxin on the basophils was studied in 40 rabbits in which the initial basophil count had been made before injection of the endotoxin. The subsequent changes in the number of cells were estimated from their variation from the initial number, taken as 100%. The number of degranulated cells as a percentage of the total number of basophils and the number of basophils as a percentage of the total

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Number of Leukocytes (L) and Basophils (B) in the Blood of Rabbits at Different Times after Intravenous Injection of Various Doses of Endotoxin (in % of Initial Number, Taken as 100)

Time after injection of endotoxin	Dose of endotoxin (in μ g/kg body weight)							
	1		10—15		500—1 000		5 000	
	L	B	L	B	L	B	L	B
30 min	90±16	110±17	32±11	72±11	45±8	66±19	34±7	92±12
1 h	133±30	158±41	40±10	60±16	25±4	13±5	20±5	8±3
2 h	73±13	43±12	51±20	29±11	33±6	9±4	30±10	10±5
4 h	182±88	30±5	78±12	23±10	48±6	25±6	30±10	20±8
24 h	180±24	90±24	200±80	120±30	Death		Death	

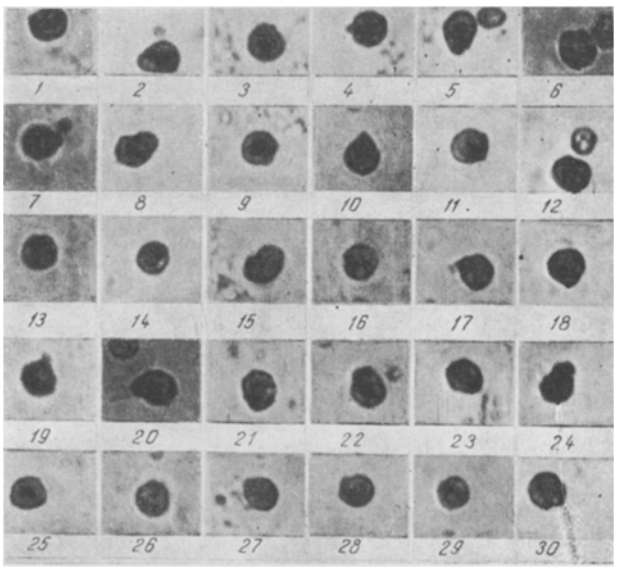

Fig. 1. Normal basophils in rabbit's blood. Toluidine blue. Objective 43×, ocular 15×.

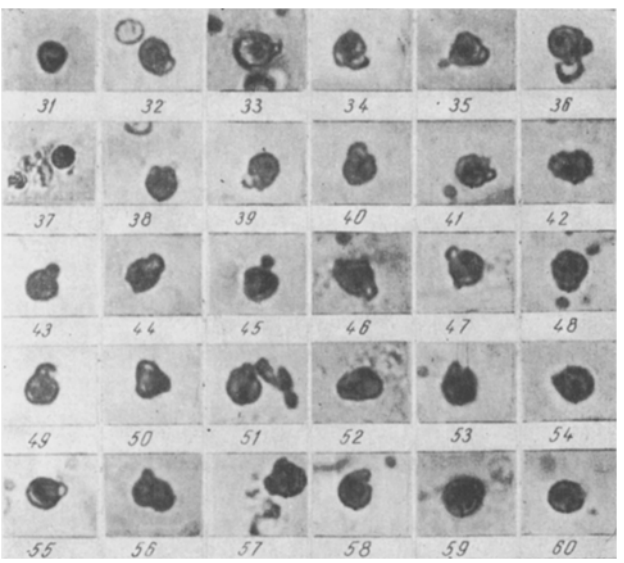

Fig. 2. Degranulated basophils in a rabbit's blood. Toluidine blue. Objective 43×, ocular 15×.

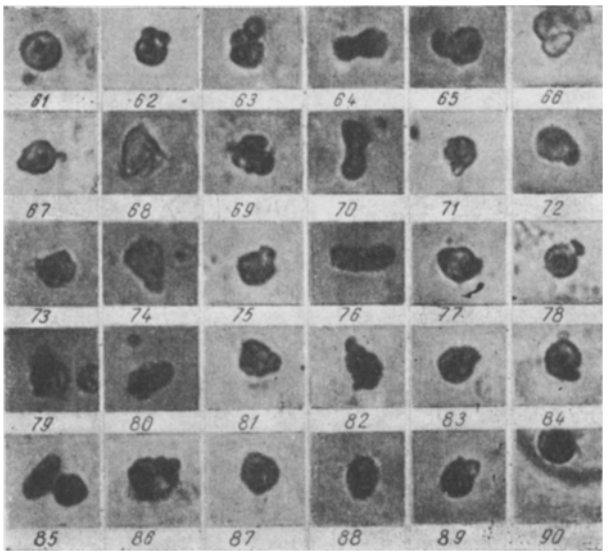

Fig. 3. Basophils in a rabbit's blood after injection of bacterial endotoxin into the animal. Toluidine blue. Objective 43×, ocular 15×.

number of leukocytes were calculated. The numerical results were analyzed by statistical methods. The differences were regarded as significant if P did not exceed 0.5% (0.005).

EXPERIMENTAL RESULTS

Visual observations on the basophils showed that side by side with normal basophils, characterized by a dark violet color of the whole cell, with a reddish tinge, due to cytoplasmic granules filling the surface of the basophils, other basophils were seen with different degrees of degranulation. The signs of degranulation were changes in the shape of the cell, a less intensive staining, partial expulsion of the granules from the cell without injury to the membrane, and accumulation of granules at the periphery of the cell in the form of a rim; sometimes expulsion of the granules was accompanied by injury to the cell.

The basophils found in the blood of normal animals and regarded as normal in the counting are shown in Fig. 1. Basophils regarded as degranulated are illustrated in Fig. 2.

The number of normal basophils amounted on the average to 56 ± 13% of the total number circulating in the peripheral blood, and the number of degranulated basophils was 44%. This evidently indicates the constant secretory activity of the basophils in physiological conditions.

In the course of the day the total number of basophils showed considerable fluctuations, corresponding to the fluctuations in the total number of leukocytes. In the morning the number of basophils in the blood was 236 ± 22 per mm^3 and in the evening 342 ± 146 per mm^3, while the corresponding leukocyte counts were 11,600 ± 600 and 18,500 ± 4275 cells. This difference between morning and evening number of cells was not statistically significant. According to some authors [7], the leukocyte count varied from 10,000 to 12,000 per m^3 blood depending on the time of day, and the basophil count is 400 ± 234; according to other data the mean number of basophils is 187 ± 67, and of leukocytes 7900 per mm^3 [19].

The result of the study of variations in the numbers of basophils and leukocytes during repeated blood sampling at different time intervals with or without injection of physiological saline (1-3 ml intravenously) showed that the changes observed in the number of cells compared with the morning sample were not significant (P > 0.5%). The number of normal and degranulated basophils after injection of physiological saline likewise showed no significant change. The observed changes in the number and quality of the cells were within the limits of physiological variation or were random in character.

The spontaneous increase in the number of basophils after midday [7] and their increase 2 h after injection of an isotonic solution [17] has been reported.

The next series of experiments was carried out on rabbits, which received injections of various doses of endotoxin (from 1 μg/kg to 5 mg/kg intravenously). Each dose was injected into 8-10 rabbits. The basophils were counted after 30 min and 1, 2, 4, and 24 h.

It was found that after injection of nontoxic doses of endotoxin (1-10 μg/kg) dissociation of the cell reaction to the injected preparation took place. Whereas after intravenous injection of endotoxin in a dose of 100 μg/kg or more, a significant decrease took place in the number of leukocytes and basophils after 10-30 min, progressing until the animal's death, after injection of 1 μg/kg, only the basophil count showed a significant decrease by 30-50% (P = 0.4%) 4 h after injection of the preparation, with a return to the initial level after 24 h without a decrease in the total leukocyte count.

When endotoxin was injected in a dose of 10-15 μg/kg, the leukocyte count fell after a time varying from 30 min to 2 h, with a return to the initial values after 4 h, whereas at this time a basopenia developed (P = 0.2%) (see the table).

After injection of lethal doses, in particular before death of the animals, against the background of a marked basopenia a relative increase took place in the number of normal basophils, presumably on account of destruction of the cells. The degranulated cells were more severely injured (Fig. 3). This demonstrates the injurious action of the endotoxin. Evidently the basopenia in this case developed as a result of destruction of the cells rather than true degranulation because of exhaustion of the granules. At the same time, it may be postulated that the selective basopenia following administration of small doses of endotoxin, without a total leukopenia denoted degranulation rather than injury. These observations do not provide the answer to the question whether this degranulation was primary in character or whether it was secondary as a result of the reaction to the endotoxin, because under the influence of the endotoxin the liberation of corticosteroids may take place from the adrenal cortex, producing basopenia both in man [6] and in rabbits [5-7].

In the present experiments, conducted on 8 rabbits, a significant decrease in the basophil count by 30-50% without a total leukopenia was also observed 4 h after the intramuscular injection of cortisone (10 mg/kg body weight). These findings may indicate the secondary character of the degranulation of the basophils, but they cannot rule out the possibility of a direct action of the endotoxin on the basophils.

The results of these experiments thus demonstrate quantitative and qualitative changes in the basophils in response to the action of bacterial endotoxin and the possibility that in these circumstances active substances such as histamine and heparin (and other active substances also, possibly) may be liberated during the period of bacterial poisoning. It is known that histamine takes part in the development of bacterial poisoning, although the question of its role in the development of irreversible shock has not yet been settled. The liberation of heparin concurrently with degranulation of the basophils may play a role in preventing the initial intravascular coagulation, because some of the symptoms of infectious shock may be due to intravascular coagulation with emboli and thrombi, leading to blocking of the circulation.

The liberation of these substances from the granules of the basophils as a result of the action of endotoxin show that they may be concerned in the general chain of reactions developing in bacterial poisoning.

The question of whether the liberation of these substances is compensatory or pathological in character will be the subject of a further study.

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All abbreviations of periodicals in the above bibliography are letter-by-letter transliterations of the abbreviations as given in the original Russian journal. *Some or all of this periodical literature may well be available in English translation.* A complete list of the cover-to-cover English translations appears at the back of the first issue of this year.
