MECHANISM OF CHANGE IN BONE MARROW CELL COMPOSITION IN EXPERIMENTAL TOXIC DIPHTHERIA

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In toxic diphtheria the influence of the sympathetic nerves on activity of the heart, blood vessels, gastrointestinal tract, and other organs is disturbed [1, 3, 4, 6]. However, the role of injury to the sympathico-adrenal system in the mechanism of changes in the cell composition in the bone marrow and peripheral blood in this disease has not yet been explained. The reason why this problem is raised is the fact that cells of the erythroid series of bone marrow possess β-adrenoreceptors [7, 10].

It was accordingly decided to study the cell composition of the myeloid and erythroid branch of the bone marrow at different stages of toxic diphtheria in rats and to determine whether correlation exists with changes in the cell composition of the peripheral blood.

EXPERIMENTAL METHOD

Experiments were carried out on female Wistar rats weighing 100-120 g. Diphtheria toxin was injected subcutaneously (1 MLD for guinea pigs, 0.003 mg) into the animals 3-4 days before the experiment began. In the course of this period the rats developed a clinical picture of toxic diphtheria: absence of fever yet a comparatively serious general condition, bradycardia, fall of blood pressure, diarrhea. The experimental rats were divided into three groups: The animals of group 1 received only an injection of diphtheria toxin, the animals of group 2 were subjected to bleeding (2% of the body weight) when they had developed a clinical picture of toxic diphtheria, and the animals of group 3 were bled and also received an intraperitoneal injection of ephedrine in a dose of 10 mg/100 g body weight. In the animals of all groups blood was taken for hematologic investigations from the caudal vein, after which the rats were decapitated and blood was collected for investigation of the erythropoietic activity of the serum by two methods on polycythemic mice exposed to hypoxic hypoxia [2, 9], after which the femoral bone marrow was removed, films were prepared, and stained with azure-eosin.

The experimental results were subjected to statistical analysis by Student's t-test.

EXPERIMENTAL RESULTS

To begin with, the cell composition of the myeloid and erythroid series of the bone marrow was studied in rats with toxic diphtheria. It will be clear from Fig. 1 that during development of the picture of toxic diphtheria, a marked increase was observed in the myeloid branch in the number of granulocytes, especially of immature forms, whereas the number of lymphoid cells and of immature cells of the erythroid series fell appreciably. An increase in the reticulocyte count was observed in the circulating blood (Fig. 2).

The next series of experiments consisted of similar tests on animals poisoned with diphtheria toxin and bled, i.e., under conditions of hypoxia leading to stimulation of the sympathico-adrenal system. Bleeding led to a marked decrease in the number of immature granulocytes and a simultaneous increase in the number of mature cells in the bone marrow (Fig. 1). The number of lymphocytes fell under these conditions but the response of the erythroid branch was absent altogether: Bleeding did not change the composition of cells of the erythroid series, but examination of the peripheral blood revealed a decrease in the erythrocyte count and hemoglobin concentration and a low degree of reticulocytosis. Against this background the ability of the serum of the experimental rats to increase the reticulocyte count in the peripheral blood of polycythemic mice

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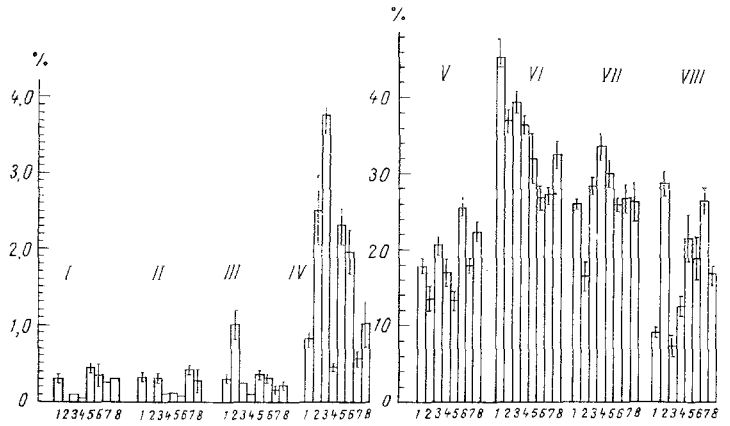

Fig. 1. Changes in composition of bone marrow after bleeding, injection of ephedrine, and injection of diphtheria toxin. 1) Control, 2) 24 h after bleeding, 3) 48 h after bleeding, 4) injection of diphtheria toxin, 5) injection of ephedrine, 6) injection of diphtheria toxin and ephedrine, 7) injection of diphtheria toxin and investigation 24 h after bleeding, 8) injection of diphtheria toxin and investigation 48 h after bleeding; I) plasma cells, II) other cells, III) undifferentiated cells, IV) immature cells of erythroid series, V) mature cells of erythroid series, VI) lymphocytes, VII) immature granulocytes, VIII) mature granulocytes. Ordinate, number of cells (in % of total number of cells).

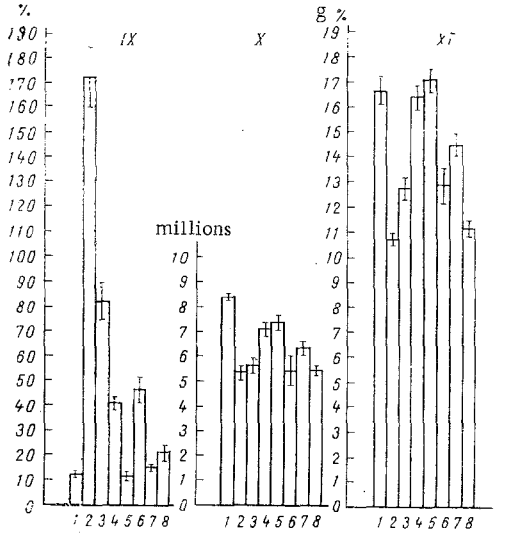

Fig. 2. Changes in peripheral blood indices after bleeding and injection of ephedrine and diphtheria toxin. IX) Reticulocytes, X) erythrocytes, XI) hemoglobin. Ordinate, number of cells. Remainder of legend as to Fig. 1.

was reduced, although at the same time incorporation of ^{59}Fe into the erythrocytes of these mice was increased,* indicating an increase in the erythropoietin concentration in the blood serum.

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TABLE 1. Erythropoietin Concentration in Blood Serum of Rats Receiving Diphtheria Toxin (M ± m)

Experimental conditions	Reticulocytes, % (noninbred mice)	Incorporation of ^{59}Fe, %
1. Control	3,5±0,88 (2)	2,31±0,58 (4)
2. Bleeding	18,1±1,8(16) $P_{1-2}<0,001$	7,24±1,5(5) $P_{1-2}<0,02$
3. Diphtheria toxin + bleeding	8,0±1,26(5) $P_{2-3}<0,01$	11,77±1,99 (5)
4. Diphtheria toxin + bleedings + ephedrine	22,5±4,2(4)	18,08±1,42(5)

Legend. Number of mice given in parentheses.

On the second day after bleeding the poisoned rats showed a similar tendency toward a change in the cell composition of the granulocytic series, whereas in the erythroid branch there was a small increase in the number of mature cells. The blood serum of the experimental rats could no longer increase the reticulocyte count in the peripheral blood of the polycythemic mice, despite an increase in ^{59}Fe incorporation into their erythrocytes (Table 1). Analysis of the peripheral blood showed that on the 2nd day the erythrocyte count and hemoglobin concentration were reduced still more, whereas the reticulocyte count was increased a little.

Since adrenergic neurons and chromaffin cells are the point of application of the toxin in vivo [1, 4, 6, 8] it was decided to determine whether the composition of the bone marrow cells is changed in rats receiving diphtheria toxin after injections of ephedrine. Injection of ephedrine into control and poisoned animals stimulated cell proliferation, chiefly in the erythroid series, but examination of the peripheral blood of such animals showed an increase in the reticulocyte count and a decrease in the erythrocyte count and hemoglobin concentration (Fig. 1).

A parallel increase was observed in the incorporation of ^{59}Fe into the cells.

It can thus be concluded that in animals poisoned with diphtheria toxin and in which hematopoiesis is stimulated by bleeding, the cell composition of the bone marrow does not change significantly despite a higher increase in the blood erythropoietin concentration than in the control. Rejuvenation of cells, primarily of the erythroid series, during artificial stimulation of adrenergic processes by ephedrine indicates that this probably takes place as a result of the simultaneous action of erythropoietin and adrenergic mediators on the bone marrow cells.

LITERATURE CITED

1. A. A. Artishevskii and Yu. A. Él'kina, Zdravookhr. Beloruss., No. 4, 38 (1968).
2. T. I. Koretskaya, V. I. Gudim, G. P. Moskaleva, et al., Patol. Fiziol., No. 6, 49 (1969).
3. I. K. Musabaev and F. Z. Abubakirova, Diphtheria [in Russian], Tashkent (1967).
4. K. F. Samsonova-Firsova, "Date on the pathogenesis of diphtheria intoxication," Doctoral Dissertation, Kazan' (1960).
5. M. N. Cheboksarov, Russ. Vrach., 10, No. 23, 966 (1911).
6. S. C. Agarwall and P. U. Nasmyth, J. Physiol. (London), 147, 511 (1959).
7. J. P. Bilezikian, A. M. Spiegl, E. M. Brown, et al., Mol. Pharmacol., 13, 755 (1977).
8. D. M. Brahmaknar, S. N. Umathe, and C. T. Chopde, Mater. Med. Pol., 8, 392 (1976).
9. P. M. Cotes, in: Kidney Hormones, (J. W. Fisher, ed.), New York, Vol. 1 (1971), p. 243.
10. G. D. Fink and J. W. Fisher, in: Erythropoietin, (J. W. Fisher, ed.), London (1977), p. 387.