CHANGES IN HEMATOPOIESIS IN RATS UNDER STRESS

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In rats under stress a decrease in the number of cells in the lymphoid organs was accompanied by an increase in the number of lymphocytes in the bone marrow. The total number of cells in the thymus and spleen rose after an initial decrease. The number of lymphocytes in the bone marrow fell, after an initial increase, to its original level, and this was accompanied by activation of granulocytopoiesis. More prolonged exposure to stress led to a decrease in the total number of myelokaryocytes, repeated involution of the thymus, and lymphocytopenia.

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The influence of adrenocortical hormones and various stressors on the composition of the circulating blood and lymphoid tissue has frequently been investigated[1,4,9,10,12,15]. Information regarding changes in the bone marrow is less abundant.

Previous investigations were based predominantly on morphological analysis of changes in the lymphoid and myeloid tissue and on calculation of the relative percentages of different cell forms. More recently, however, the method of determination of the absolute number of cells in hematopoietic tissues has become increasingly popular in experimental hematology, for in conjunction with the morphological method it provides a more complete picture of the state of hematopoiesis during the response to stress. There are one or two references to this method in the literature. Results obtained by Gorizontov and Rudakov [2] show that hematopoiesis shows certain specific changes at various stages of the adaptation syndrome.

The object of the present investigation was to determine the course of quantitative changes in the state of hematopoiesis during development of the stress reaction.

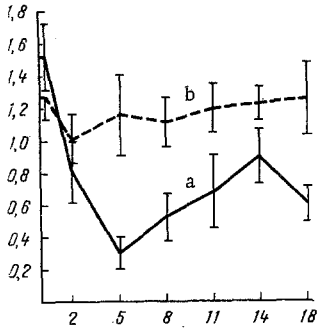

Fig. 1

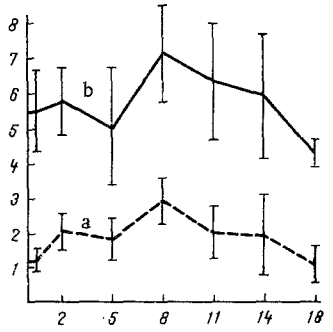

Fig. 2

Fig. 1. Total number of cells in thymus (a) and spleen (b) in the course of 18 days with electrical stimulation for 6 h daily. Abscissa, days; ordinate, number of cells $\times 10^9$.

Fig. 2. Number of granulocytes (a) and lymphocytes (b) per mm^3 of blood during 18 days of electrical stimulation for 6 h daily. Abscissa, days; ordinate, number of cells $\times 10^3$ per mm blood.

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TABLE 1. Absolute Number of Myelokaryocytes (in millions) in Rat Femur during 18 Days of Electric Stimulation for 6 h Daily (M ± m)

	Control (n=14)	2nd day (n=10)	5th day (n=10)	8th day (n=10)	11th day (n=8)	14th day (n=10)	18th day (n=8)
Total number of cells	129,0±5,5	137,8±8,3	127,1±9,4	153,3±8,6	149,2±6,8	139,1±9,7	96,7±6,1
Myeloblasts–myelocytes	5,0±0,38	10,1±0,98	8,2±1,09	12,7±1,72	10,9±1,02	8,2±1,4	3,6±1,17
Metamyelocytes	3,7±0,66	3,5±0,32	3,3±0,46	6,7±0,98	5,7±0,5	3,3±0,74	1,2±0,21
Stab cells-Polymorphonuclear neutrophils	40,0±3,21	28,6±3,32	29,1±2,18	51,2±3,42	43,3±2,47	30,1±5,47	27,0±5,20
All cells of neutrophil series	48,6±3,15	42,3±3,50	41,6±3,40	70,6±8,02	59,9±3,42	40,7±7,47	31,7±6,57
Lymphocytes	39,9±2,47	57,7±6,35	50,9±8,29	37,1±4,43	40,3±3,67	54,6±8,07	27,0±3,45

Note. Figures which differ significantly from controls ($P < 0.05$) are underlined.

EXPERIMENTAL METHOD

Experiments were carried out on male Wistar rats aged 10 weeks and weighing 160-170 g. A state of stress was reproduced by strictly measured electrical stimulation [3] under the following conditions: pulse duration 3 sec, interval 1.5 min, current 2.5 mA, frequency 2000 Hz. Stimulation was given for 6 h daily.

The number of myelokaryocytes in the femur was determined by Mantz's method [14] (without determining the volume of the bone marrow). By means of a myelogram obtained from impression films, the absolute number of cells of each type in the femur was calculated. The number of thymus and spleen cells was determined as follows: the organs were removed and placed in 5% acetic acid, the volume was made up to 5 ml, and the tissue was minced with scissors until a homogeneous mass was obtained. The suspension was passed through a syringe with needles of ever decreasing diameter until all macroscopically visible lumps had disappeared. The cell suspension was drawn up into the mixing chamber for counting of the leukocytes (dilution 1 : 10). Counts were made in 5 large squares of the Goryaev chamber.

Calculation was by the formula

$$x = a{:}4 \times 10^7,$$

where x is the number of cells in the organ and a the number of cells in the Goryaev chamber.

The number of cells per mm^2 blood taken from the cervical vessels was determined by the usual hematological method.

Experiments were carried out on 69 animals. The rats were stimulated for 2, 5, 8, 11, 14, and 18 days. So that the last electrical stimulation should not leave its mark on the animals' condition, they were sacrificed 24 h thereafter. Statistical analysis of the data was undertaken by Student's method. A graphic method was used to assess the degree of significance with the aid of confidence limits.

EXPERIMENTAL RESULTS

As a result of electrical stimulation the number of cells in the thymus fell to one-fifth by the 5th day. The response of the spleen was less marked. The number of cells in that organ fell by only 13.4% on the 2nd day (Fig. 1). In impression films of the thymus and spleen stained by Pappenheim's method, no increase in the number of disintegrating cells could be seen compared with the control. The decrease in number of cells in the thymus and spleen was accompanied by an increase in the lymphocyte population in the bone marrow, mainly on account of replacement of discharged mature cells of the myeloid series (Table 1). Morphological examination of bone marrow impressions revealed no destruction of lymphoid cells.

The lymphoid organs and populations of lymphoid cells in the bone marrow thus responded differently to stress. The decrease in the number of cells in the thymus and spleen was most probably due to the number of cells leaving these organs. The role of destruction, if present at all, was unimportant. The increase in number of lymphocytes in the bone marrow, on the other hand, could have occurred both through migration of cells from the lymphoid tissues and through proliferation of local lymphocytes.

The period from the first to the 5th day of daily electrical stimulation can be described on the whole as the initial response (the stage of mobilization). During this period, as well as disappearance of cells from the lymphoid organs, the body weight fell and the general condition of the animals worsened (untidiness of the hair, poor appetite, and so on).

The total number of cells in the spleen was restored to its initial level after 5-11 days. The number of cells in the thymus increased after 5 days, to reach half its initial value by the 14th day of daily electrical stimulation. The number of lymphocytes in the bone marrow fell, after the initial increase, to reach its initial level by the 8th-11th day, and this was accompanied by activation of granulocytopoiesis. The number of myeloblasts, promyelocytes, and myelocytes increased at the beginning of electrical stimulation and reached its maximum by the 8th-11th day (Table 1). During this period the number of mature elements of the granulocyte series in the femur and per mm^3 blood also increased (Fig. 2).

The general condition of the animals improved from the 5th to the 14th day, despite daily electrical stimulation. The rats were active and put on weight satisfactorily. This period corresponded to the stage of resistance [2].

From the 14th to the 18th day a fresh wave of loss of weight took place. The animals' condition in this period resembled that in the first days of stimulation. The number of cells in the thymus fell once again. The total number of myelokaryocytes in the femur fell because of a decrease in the numbers of all cells. The number of cells in the spleen remained unchanged. The absolute number of lymphocytes per mm^3 blood was below its initial level. The period from the 14th to the 18th day corresponded to the stage of exhaustion.

Hence, essential changes take place in hematopoiesis during repeated exposure to stress. Hyperplasia of the bone marrow affects predominantly the myeloid series. This is in agreement with the findings of Gorizontov and Rudakov [2] and it indicates that changes in the state of the blood system play a role in the mechanism of rise and fall of nonspecific resistance of the body.

In all probability the increase in number of lymphocytes in the bone marrow plays an important role in activation and hyperplasia of the myeloid series in the bone marrow. In accordance with the view developed by several authors, this may be the result of participation of the lymphocytes in hematopoiesis both as polypotent hematopoietic cells [7, 8, 11, 13] and as trephocytes [5, 6].

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