CHANGES IN PROLIFERATION AND DIFFERENTIATION OF ALBINO MOUSE BONE MARROW CELLS CAUSED BY HEPARIN

B. P. Lukashin and L. I. Nenarokova

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Experiments on noninbred albino mice after daily injection of heparin (250 units/kg) for 2 weeks showed that the leukocyte count in the peripheral blood increased by 30%, the number of nucleated cells in the femoral marrow was unchanged, and the leukoerythroblastic ratio rose from 4.1 to 7.6. Autoradiographic studies showed that the duration of the mitotic cycle and of its various periods were unchanged by the action of heparin: $T=13$ h, $S=8$ h, $G_2=1$ h, $G_1+M=4$ h. In heparinized animals the shape of the curve of labeled mitoses was close to the ideal theoretical curve. It is postulated that under the influence of heparin cell division is synchronized in the bone marrow and differentiation is shifted toward myelopoiesis.

KEY WORDS: heparin; bone marrow; labeled mitoses; mitotic cycle.

Experiments on small laboratory animals have shown that heparin induces hyperplasis of lymph tissue in the spleen and thymus [5], and in vitro it stimulates DNA synthesis in bone marrow cells [9]. Granulopoiesis is also known to be stimulated by administration of polysaccharides [1, 11]. On the basis of these observations it could be presumed that heparin is a regulator of hematopoiesis.

It was therefore decided to study the effect of heparin on proliferation and differentiation in the bone marrow of albino mice.

EXPERIMENTAL METHOD

Experiments were carried out on noninbred male albino mice weighing 18-20 g. The animals of the experimental group received intraperitoneal injections of heparin (Richter) in a dose of 250 units/kg daily for 2 weeks, and mice of the control group received injections of physiological saline. The animals were divided into two groups 24 h after the last injection. The leukocyte and erythrocyte counts and leukocyte formula were determined in the peripheral blood taken from the retrobulbar plexus of the mice of group 1, after which the animals were killed and the number of nucleated cells and the myelogram in the femoral marrow were counted. For investigation of the kinetics of the myeloid cells [^{3}H]thymidine was injected subcutaneously in a dose of 0.5 μCi/g body weight; the animals were killed 1-24 h after injection of the isotope and autoradiographs of the bone marrow cells were prepared [7]. At each time of the investigation 100 labeled mitoses were counted in cells of the myeloid series from four to six animals. The duration of the mitotic cycle and of its various periods was determined [3].

EXPERIMENTAL RESULTS

The results of investigation of the peripheral blood of the mice are given in Fig. 1. Under the influence of a 2-week course of heparin injections in a therapeutic dose the leukocyte count was increased by 30%, on account of a 50% increase in the absolute number of lymphocytes, whereas the number of neutrophils was unchanged. The erythrocyte count in the mice of the experimental group was indistinguishable from the control.

To study the mechanism of the increase in the lymphocyte count in the peripheral blood the morphological composition of the bone marrow was examined.

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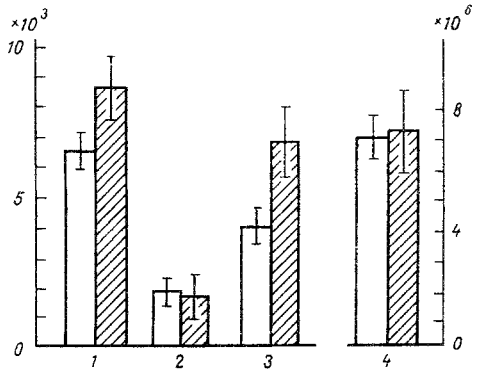

Fig. 1. Effect of heparin on peripheral blood indices of albino mice. Unshaded columns denote physiological saline; shaded columns denote heparin. 1) Leukocytes; 2) neutrophils; 3) lymphocytes; 4) erythrocytes.

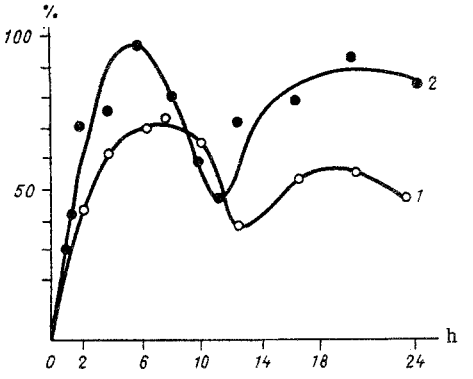

Fig. 2. Changes in percentage of labeled mitoses in myeloid cells of albino mice following prolonged administration of heparin. 1) Physiological saline; 2) heparin. Abscissa, time after injection of thymidine (in h); ordinate, percentage of labeled mitoses.

The number of myelokaryocytes was the same in the mice of both groups (Table 1). In the heparinized mice, however, the following changes were found: an increase of 1.8 times in the leukoerythroblastic ratio, a decrease in the absolute lymphocyte count and an increase in the absolute eosinophil count, and an increase in the index the maturation of cells of the erythroid series (Table 1).

The results are evidence of a shift under the influence of heparin toward myeloid hematopoiesis with reciprocal inhibition of erythropoiesis (the number of proerythroblasts in the experimental mice was only one-fifteenth of their number in the control). The increase in the maturation index in the erythroid series can be regarded as evidence of a compensatory reaction responsible for maintaining the normal erythrocyte count in the peripheral blood of the heparinized animals (Fig. 1).

The significant decrease in the lymphocyte count in the bone marrow of the heparinized animals must be emphasized; it was probably due to increased migration of these cells into the peripheral blood (Fig. 1) and lymphoid organs.

The change in the ratio between the numbers of hematopoietic cells of the myeloid and erythroid series in the bone marrow could be due both to a change in the direction of differentiation of the stem (committed) cells and also to stimulation of the precursor cells of granulopoiesis.

TABLE 1. Effects of Heparin on Number of Nucleated Cells and Their Morphological Composition in Bone Marrow of Albino Mice

Index studied	Injection of physiological saline (n = 20)	Injection of heparin (n = 19)	P
No. of myelokaryocytes, millions	22,0±0,9	22,1±1,2	>0,05
No. of cells of myeloid series, millions	15,2±0,7	17,5±0,6	<0,01
No. of cells of erythroid series, millions	3,7±0,3	2,3±0,2	<0,001
Leukoerythroblastic ratio	4,1	7,6	<0,01
Lymphocytes, millions	2,0±0,2	1,3±0,2	<0,05
Eosinophils, millions	0,6±0,04	0,9±0,08	<0,05
Maturation index in myeloid series	0,53	0,56	>0,05
Maturation index in erythroid series	0,38	0,61	<0,05

To study the proliferative activity of the myeloid cells the method of autoradiography was used. The results (Fig. 2) show that the duration of the mitotic cycle (T) and of its individual periods was practically the same in the mice of the experimental and control groups, namely: T = 12-13 h, S = 8 h, G_2 = 1 h, and G_1 + M = 3-4 h. Moreover, as a result of administration of heparin the first and second peaks of the maximum of the labeled mitoses were at a higher level (6 and 20 h after injection of the isotope the number of labeled mitoses in the mice of this group was 25 and 36% higher, respectively, than in the control). The great height of the second peak must be particularly noted, for it indicates homogeneity of the cell population in the heparinized animals. After administration of heparin the total duration of the mitotic cycle, as well as the duration of its individual periods, evidently approximated to the calculated values, i.e., cell synchronization was observed. The phenomenon of synchronization also was probably responsible for the greater height of the first peak of labeled mitoses, for the greater steepness of decline of this wave of mitoses indicates low variability of the S period, the duration of which was two-thirds of the total duration of the mitotic cycle.

The results of this investigation thus confirm the view that the direction of differentiation of most stem (committed) cells is changed by heparin toward myelopoiesis. This effect of heparin may be both indirect (through its effect on the circulation and the oxygen supply to the tissues [2, 6]), and also direct (a change in the electrokinetic potential of the cells) [4, 8, 10].

Meanwhile, whereas the mean number of cells of the erythroid series in the bone marrow of the heparinized mice was reduced by 1.4 million, the absolute number of myeloid cells was increased by 2.3 million. These calculations suggest that, besides a change in the direction of differentiation, the stimulation of myelopoiesis in the experimental group of animals could also have been due to synchronization.

The results point to the important role of heparin in medullary hematopoiesis. The changes observed in the bone marrow and lymphoid tissue of the heparinized animals explain the biological role of degranulation of the mast cells, with liberation of endogenous heparin, under the influence of unfavorable factors, for granulocytes and lymphocytes both play an active role in processes of defense and repair.

LITERATURE CITED

1. I. E. Andrianova, V. A. Razorenova, G. A. Vitovskaya, et al., Radiobiologiya, No. 5, 703 (1976).
2. A. I. Beketov and D. I. Sapegin, in: Heparin [in Russian], Leningrad (1969), pp. 42-44.
3. A. K. Dondua and G. K. Dondua, in: Investigation of Cell Cycles and Nucleic Acid Metabolism during Cell Differentiation [in Russian], Moscow–Leningrad (1964), pp. 5-36.
4. V. P. Lozovoi, S. V. Kaznacheev, V. A. Kozlov, et al., Zh. Mikrobiol., No. 5, 108 (1975).
5. B. P. Lukashin, Byull. Éksp. Biol. Med., No. 7, 120 (1974).
6. V. G. Makarova, Probl. Gematol., No. 9, 40 (1972).
7. K. N. Muksinova, L. D. Murzina, and V. S. Voronin, Med. Radiol., No. 1, 67 (1975).

8. V. F. Rusyaev, Bull. Éksp. Biol. Med., No. 8, 23 (1974).
9. L. Beneš and E. Rotreklova, Folia Biol. (Prague), 16, 196 (1970).
10. E. Viclicky, Z. Pokozna, and J. Peknicova, Folia Biol. (Prague), 21, 87 (1975).
11. J. M. Yoffey, G. S. Makin, A. K. Yates, et al., Ann. N. Y. Acad. Sci., 113, 790 (1964).

DIVISION OF STELLATE RETICULOCYTES IN THE RAT LIVER AFTER VAGOTOMY

A. M. Astakhova and B. T. Anufriev

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Diurnal fluctuations in mitoses in stellate reticuloendotheliocytes of the intralobular capillaries of the rat liver under normal conditions and after bilateral subdiaphragmatic vagotomy were investigated. The largest number of mitoses was found in all the animals during the morning, between 3 and 7 a.m. The number of mitoses in the vagotomized and laparotomized animals fell gradually until 10 p.m. After vagotomy the number of mitoses in the stellate reticuloendotheliocytes, just as in the hepatocytes also, was more than twice as high as in the control; the character of the curve reflecting the diurnal rhythm of mitosis in the denervated liver was the same as in the control.

KEY WORDS: reticuloendotheliocyte; mitotic index; hepatocyte.

Many surgeons who use the operation of selective subdiaphragmatic vagotomy for the surgical treatment of severe forms of peptic ulcer have recently become interested in the consequences of this operation and, in particular, its effect on the morphological and physiological state of the abdominal organs. Considering the practical importance of this problem, Eletskii and his colleagues [7-9] have studied the character of the morphological and physiological changes in the digestive glands of the subdiaphragmatic region after bilateral subdiaphragmatic vagotomy and, in particular, processes of compensation and proliferation arising in the denervated organs under these conditions. It has been shown, for instance [12], that vagotomy in rats does not lead to increased mitotic activity in the acinar cells of the exocrine portion of the pancreas. However, the worker concerned did not study the diurnal rhythm of mitosis in the denervated pancreas. The present writers showed previously that bilateral subdiaphragmatic vagotomy in rats leads to increased mitotic activity in the hepatocytes (low mitotic activity) and enterocytes (high mitotic activity) by roughly two to three times the control value. The character of the curve reflecting mitotic activity in the course of the 24-h period in these organs was unchanged after vagotomy (Fig. 1). A similar pattern of change in mitotic activity also was found later in the glandular cells of the exocrine portion of the pancreas [7].

Analysis of data in the literature and our own observations shows that the dynamics of diurnal rhythms of mitosis in the various organs and tissues has not only its similarities, but also considerable differences [1-3, 6]. According to certain observations [11], rhythms of mitosis in different tissues of the thyroid gland (the stroma and glandular cells) of adult rats, and also in the hepatocytes and Kupffer cells, exhibit tissue-specific features and differ from one another.

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