

EXPERIMENTAL STUDY OF HEMATOPOIESIS
IN RATS WITH EXCHANGE REPLACEMENT
OF BLOOD BY PERFLUOROTRIBUTYLAMINE
EMULSION

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Exchange blood replacement with perfluorotributylamine (PFTBA) emulsion was carried out in experiments on unanesthetized rats. During the period of blood exchange and for the next 5 days the rats were maintained on an increased oxygen concentration. On the sixth day the animals were returned to ordinary atmospheric conditions, and systematic observation commenced on that day and continued for 1 year. On the sixth day after exchange blood replacement, marked activation of erythropoiesis was found in the bone marrow of the rats and reticulocytosis in their peripheral blood. Meanwhile a decrease in the hemoglobin concentration and an increase in the number of lymphocytes and mature and immature neutrophils were found in the blood; immature erythropoietic cells - polychromatophilic erythroblasts and normoblasts - were present. The normal peripheral blood indices were completely restored by the 13th day and the morphological composition of the bone marrow by the 28th day; for the next 12 months hematopoiesis in the rats was maintained without appreciable deviations. It can be concluded from these results that the PFTBA emulsion has no adverse effect on hematopoiesis in animals and does not disturb the normal process of regulation of hematopoiesis under these experimental conditions.

KEY WORDS: hematopoiesis; blood substitute; perfluorotributylamine emulsion.

In the last decade an important place in the problem of creation of artificial blood substitutes has been occupied by the investigation of certain organofluorine compounds as media with high ability to dissolve gases, including oxygen, physically [1-3]. Since fluorohydrocarbons are biologically inert substances insoluble in water, a specially prepared emulsion of fluorohydrocarbons and low-molecular-weight polymers is used for experimental research. Particles of an emulsion not exceeding 0.25μ in diameter are regarded as a possible model of an erythrocyte, in which the role of hemoglobin is played by the organofluorine compound and that of the erythrocyte membrane by the emulsifier. The Soviet preparation perfluorotributylamine (PFTBA) has been obtained as an artificial blood substitute solution in the Laboratory of New Blood Substitutes, Central Research Institute of Hemoglobin and Blood Transfusion. Experiments with almost total blood replacement have been carried out in rats. One of the aims of investigation of the physiological state of PFTBA was to discover how the recipient rat's own blood regenerates in these experiments. Hematopoiesis was accordingly studied in rats after replacement of their blood with PFTBA emulsion.

EXPERIMENTAL METHOD

Unanesthetized Wistar rats weighing 100-120 g were used. The operation of blood exchange was carried out by injecting PFTBA emulsion (from a syringe with a needle) into the femoral vein and withdrawing blood from the jugular vein. On average 10 ml of PFTBA emulsion was injected per 100 g body weight, and in the course of this procedure the erythrocyte count fell to $800,000 \pm 5400$. During the period of blood exchange the rats inhaled oxygen, after which they were placed in special chambers with an increased oxygen concentration for 5 days; they were not examined during this period. On the sixth day the rats were transferred to inhalation of atmospheric air, and from that time their systematic observations commenced. If the rats were not placed

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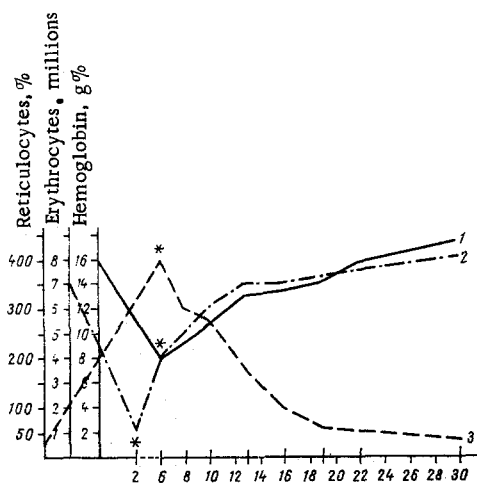


Fig. 1. Peripheral blood indices of rats at various times after exchange replacement of blood with PFTBA emulsion. Abscissa, time of examination of rats (in days); ordinate, hemoglobin concentration (1) and erythrocyte (2) and reticulocyte (3) counts from 6th to 30th days after exchange replacement of blood by PFTBA emulsion. Results differing significantly from normal values indicated by asterisk.

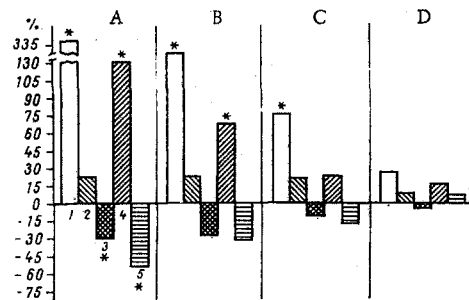


Fig. 2. Morphological composition of rats' bone marrow at various times after replacement of blood with PFTBA emulsion. A) After 6 days, B) after 13 days, C) after 28 days, D) after 58 days. Ordinate, indices of individual groups of rat bone marrow cells as percentages of control indices, taken as 100%. 1) Undifferentiated cells, 2) immature granulocytes; 3) mature granulocytes, 4) total number of erythropoietic cells, 5) lymphocytes. Remainder of legend as in Fig. 1.

initially in an increased oxygen concentration they died. Considering the limited opportunities presented by the oxygen chambers and the need for round-the-clock observations on the animals, experiments were carried out on five rats which survived for 12 months after replacement of their blood with PFTBA emulsion, after which they were decapitated for histological examination. Hematopoiesis in the rats at different times after the blood replacement operation was studied in these experiments.

The peripheral blood was removed from the caudal vein of the rats on the 6th, 8th, 10th, 13th, 22nd, and 30th days, and thereafter once a month until the end of the experiment. The hemoglobin concentration and the total number of the leukocytes, erythrocytes, and reticulocytes in the blood were studied and the leukocyte formula was determined. The bone marrow was obtained by femoral puncture, the morphological composition was studied, and myelogram determined. The results of investigation of the rats' blood and bone marrow were compared with indices of hematopoiesis of intact rats investigated at the same time.

EXPERIMENTAL RESULTS

The results of the study of the peripheral blood indices are given in Fig. 1. They show that on the 6th day after exchange blood replacement the hemoglobin concentration in the experimental rats was 8.2 ± 0.7 g%, compared with 17 ± 1.1 g% in the intact rats. The erythrocyte count by this time had increased to $4,200,000 \pm 2600$ ($P < 0.05$) compared with $800,000 \pm 5400$ at the end of blood replacement, but it was only half the level in intact rats ($7,740,000 \pm 260,000$). The reticulocyte count also was sharply increased in the blood during this period, to $400 \pm 15.2\%$ (normal $25 \pm 1.7\%$; $P < 0.001$), and immature erythropoietic cells also were found ($4.2 \pm 0.75\%$), namely polychromatophilic erythroblasts and normoblasts. Later, during maturation and a reduction in the number of reticulocytes, intensive restoration of the hemoglobin concentration took place in the blood and erythrocytes. By the 13th day these indices were completely back to normal, and they remained within normal limits for 12 months. Besides the observations described above, on the 6th day an increase in the total leukocyte count to $20,000 \pm 1800$ (9000 ± 680 in the control; $P < 0.05$) was observed in the blood, mainly on account of an increase in the number of lymphocytes, and also of juvenile, stab cell, and polymorphonuclear neutrophils. By the 13th day the leukocyte composition of the peripheral blood was back to normal and it remained without appreciable changes until the end of the experiment.

The study of the morphological composition of the rats' bone marrow (Fig. 2) showed a considerable increase in the number of cells of the erythroid series, mainly basophilic and polychromatophilic erythroblasts, on the sixth day after exchange replacement of the blood. The total number of cells of the erythroid series was increased by $130 \pm 8.2\%$ ($P < 0.01$) compared with the control. Meanwhile, the number of undifferentiated cells was increased fourfold. In the myeloid series of the bone marrow the number of mature granulocytes and lymphocytes was reduced. On the 13th day the bone marrow as before contained many cells of the erythroid series, significantly more than in the control. By the 28th day the normal morphological composition of the bone marrow was restored; subsequently, the indices for the bone marrow of the rats remained within normal limits.

The investigation thus showed that on the 6th day after exchange replacement of blood in rats with PFTBA emulsion marked activation of erythropoiesis, combined with definite reticulocytosis, were observed in the peripheral blood. These changes in hematopoiesis during the first period of observation are evidence of increased regeneration of cells of the erythroid series. This was evidently a natural response of hematopoiesis to the acute anemia developing on the first days after exchange transfusion, and which was still partly present on the 6th day. The results agree with observations made by American workers [2] who observed rapid regeneration of blood, activation of erythropoiesis, and absence of toxic effects of organofluorine compounds on hematopoietic tissue.

It can be concluded from these experiments that the emulsion of organofluorine compounds tested has no adverse effect on hematopoiesis in animals and does not disturb the normal process of regulation of hematopoiesis under the experimental conditions used.

LITERATURE CITED

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