ERYTHROPOIESIS AND ERYTHROPOIETIC ACTIVITY OF THE BLOOD PLASMA IN LEAD ANEMIA

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Rabbits aged three months were poisoned by mouth with 2.5% lead acetate solution in a dose of 1 ml/kg body weight. Examination of the bone marrow showed an increase in the number of reticulocytes and a marked normoblastic response. Reliculocytosis was observed in the peripheral blood. However, an investigation of bone marrow function showed that the efficiency of erythropoiesis was reduced. A shift of the acid erythrogram to the left also reflected the extinction of red cell production. Plasma of animals poisoned with lead had a low erythropoietin content, and it therefore slowed the rate of maturation of reticulocytes of intact rabbits, inhibited their hemoglobinization, and inhibited migration of the leukocytes in blood cultures. The decrease in the erythropoietic activity of the blood plasma is evidently the cause of the limitation on the compensatory power of the bone marrow in lead poisoning.

Activation of erythropoiesis is considered [6-9] to take place in lead anemia. This view is based on the peripheral blood reticulocytosis and the increase in the number of cells of the erythroblastic series in the bone marrow. However, no data could be found in the accessible literature to show whether there is a true increase in red cell production in lead poisoning.

In the investigation described below the character of the change in erythropoiesis and the erythropoietic activity of the plasma were studied in lead poisoning.

EXPERIMENTAL METHOD

For three months 36 rabbits were poisoned by mouth with 2.5% lead acetate solution in a dose of 1 ml/kg body weight. Bone marrow was removed before and after poisoning from the ilium. In each film from 500 to 1000 cells were counted, and the ratio between the myeloid and erythroid series and the maturation index of the erythroid series were calculated. The reticulocyte count was determined in vitally stained bone marrow films. The hemoglobin concentration and the red cell and reticulocyte counts were investigated in the peripheral blood. The 24-hourly production of red cells [5] and the acid resistance of the red cells [1] also were studied. The erythropoietic activity of the blood plasma also was studied by the blood culture method [4]. The erythropoietic or inhibitory properties of the blood plasma of animals poisoned with lead were judged from the ability of the plasma in vitro to influence maturation and hemoglobinization of the reticulocytes of intact rabbits. For this purpose, 0.5 ml blood of intact animals was incubated at 37°C with 0.5 ml of the test plasma. Maturation of the reticulocytes was determined after incubation for 4 h. The hemoglobin concentration was investigated before and after incubation for 5 h [2].

EXPERIMENTAL RESULTS

A marked normoblastic reaction was observed in the bone marrow of the rabbits. After poisoning with lead for three months the ratio between cells of the myeloid and erythroid series fell from 1.8 ± 0.1 to 0.61 ± 0.15. Mature erythroblasts were predominant in the partial erythroblast count in the bone marrow was increased from $57 \pm 7‰$ (initial) to $125 \pm 20‰$ (three months after lead poisoning).

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TABLE 1. Changes in Erythropoietic Activity of the Blood Plasma in Lead Poisoning

Index of erythropoietic activity	Test object		
	physiological saline	plasma of animals before poisoning	plasma of animals poisoned with lead
Zone of migration of leukocytes in blood culture (conventional units)	0	+15±2,3	−57±6,8
Number of reticulocytes of intact rabbits maturing during incubation for 4 h (in %)	25,9±1,7	25,2±1,5	12,9±2,7
Increase in hemoglobin concentration in blood of intact rabbits during incubation for 5 h (in mg %)	0,8±0,11	0,81±0,14	0,44±0,12

Reticulocytosis was observed in the peripheral blood of the animals with lead poisoning (63 ± 5.4‰ reticulocytes compared with an initial figure of 21.0 ± 4.04‰ reticulocytes compared with an initial figure of 21.0 ±4.04‰). Despite the active bone marrow and reticulocytosis, progressive anemia developed in lead poisoning. The hemoglobin concentration fell from 12.7 ± 0.4 to 8.3 ± 0.5 g%. The red cell count fell from 5,700,000 ± 300,000 to 4,300,000 ± 360,000.

The 24-hourly red cell production fell appreciably during lead poisoning. Before poisoning the 24-hourly red cell production was 172,600 ± 7,100 cells/mm^3 blood. One month after the beginning of the experiment the number of red cells produced had fallen to 163,600 ± 3,600/mm^3. By the end of the third month of lead poisoning the 24-hourly red cell production was only 88,900 ± 5,400/mm^3; i.e., it was reduced by almost a half below the original level. Despite the morphologically active picture of the bone marrow, the efficiency of erythropoiesis was thus reduced in lead poisoning, and liberation of young cells of the erythroid series from the bone marrow was inhibited.

Meanwhile in lead anemia the rate of maturation of the reticulocytes in the peripheral blood was sharply reduced. In the intact animals during incubation of the blood for 4 h in vitro 24 ± 4.32% of the reticulocytes matured, but three months after the beginning of lead poisoning the number was only 5.2 ± 0.87%. Consequently, the reticulocytosis in lead poisoning does not reflect an increase in the intensity of regeneration. It arises as a result of the accumulation of reticulocytes in the peripheral blood because of a sharp decrease in the rate of their maturation.

This hypothesis is supported by results obtained during periodic investigation of the acid erythrogram. A shift of these erythrograms to the right, which would correspond to increased liberation of red cells from the bone marrow, was never observed. On the contrary, after lead poisoning for three months the time of acid hemolysis was shortened to 5 min from an original 7.5 min. The maximum was shifted to 2.5 min (from 3.5-4 min initially). The shift of the acid erythrograms to the left, combined with the decrease in the red cell count and hemoglobin concentration, are evidence of extinction of red cell production [1].

An interesting contribution to the elucidation of the pathogenesis of the anemia associated with lead poisoning was made by Gajdos and Gajdos-Torök [11], who showed that the radioactive isotope of iron is assimilated more slowly in lead poisoning, on the basis of which they explain the development of lead anemia by the inhibition of hemoglobin synthesis. However, the facts discovered by these workers can also be interpreted as a manifestation of the inhibition of erythropoiesis.

The decrease in red cell production by the bone marrow during the development of lead poisoning was accompanied by a decrease in the erythropoietic activity of the plasma, in some cases to negative values. Figures showing the erythropoietic activity of the rabbit blood plasma before and after lead poisoning for three months together with control figures obtained from animals receiving physiological saline instead of lead acetate solution are given in Table 1.

These results show that the blood plasma in lead poisoning inhibits and reduces the zone of migration of leucocytes in blood culture. On addition of the plasma of rabbits poisoned with lead in vitro to the blood of intact animals the rate of maturation of the reticulocytes is slowed, and hemoglobin synthesis is inhibited in the immature red cells. In lead poisoning the equilibrium between erythropoietin and the inhibitor of erythropoiesis is evidently disturbed. The deficiency of erythropoietins affects the differentiation of the

stem cells of the bone marrow [10], maturation of erythroblasts which have already differentiated [12], and the liberation of young cells from the bone marrow [3]. The results of the present experiment show that in lead poisoning the increase in the number of erythroblasts and reticulocytes in the bone marrow is not brought about by activation of bone marrow activity but is explained by delay in the maturation of the bone marrow cells so that they remain longer at each successive stage of their development.

The increase in the reticulocyte count in the peripheral blood is explained by the fact that they undergo no further maturation for a long time. The reticulocytes in lead anemia, while retaining their morphological distinction, are essentially cells in old age, and they therefore have low resistance to acid hemolysis.

Consequently, in lead poisoning the production of mature red cells by the bone marrow is reduced. This decrease in the 24-hour production of erythrocytes is also evidence that in lead anemia the compensatory powers of the bone marrow are limited.

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