KINETICS OF ERYTHROPOIESIS IN CULTURES OF DOG BONE MARROW CELLS UNDER THE INFLUENCE OF ERYTHROPOIETIN-ACTIVE SERUM

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The kinetics of erythropoiesis was investigated in cultures of dog bone marrow cells treated with erythropoietin-active serum. The response of the erythroid branch of the bone marrow to erythropoietin-active serum was manifested among mature precursors of the erythrocytes.

The fact that erythropoietin can be detected in vitro in bone marrow cultures [1, 3, 11] suggests that its action is based on direct stimulation of the hematopoietic organs. It is postulated that the point of action of erythropoietin is the ancestral hematopoietic cells, which differentiate in the presence of erythropoietin toward the erythroblastic series [4, 5, 7-9, 14]. The work of Salvatorelli et al. [13], for instance, has shown that under the influence of erythropoietin-active serum erythropoiesis is prolonged in an organ culture and the number of erythroblasts increases until the 14th day of cultivation. These results were obtained by the use of histotypical growth of organ cultures of guinea-pig embryonic liver as the model of hematopoiesis. However, as Cronkite [6] considers, erythropoietin acts not only on ancestral cells, but also on differentiated precursors of erythrocytes and it determines the rate of differentiation of erythroid cells and of hemoglobin synthesis.

The point of action of erythropoietin in bone marrow cell cultures was investigated.

EXPERIMENTAL METHOD

Dogs aged 2-3 years and weighing 18-20 kg were used. The bone marrow was cultivated by a modified Lajtha's method as described in [2].

Bone marrow for cultivation was taken (under intravenous hexobarbital anesthesia) from the femoral epiphyses and mixed with Eagle's medium in the ratio of 1:3. Heparin (1 unit/1 ml medium), streptomycin (100 units/1 ml medium), and 20% autogenous plasma were added initially to the medium. The bone marrow was suspended in the medium, carefully mixed, and the number of cells was counted in a Goryaev's chamber. The seeding dose was $5 \cdot 10^5 \pm 1.5 \cdot 10^5$ cells/ml. The bone marrow was cultivated for 10 days at 37 ± 0.3°C with a closed gaseous phase and without mixing.

All manipulations were carried out with strict observance of the rules of asepsis. Bone marrow for analysis was taken after 1, 3, 4, 5, 7, and 10 days. The cell suspension was centrifuged for 10 min at 2000 rpm. Films of bone marrow were prepared from the residue and stained by the Romanowsky—Giemsa method. The relative proportions of the cells of the erythroid and myeloid series were calculated, the differential erythroblast count determined, and the number of mitoses in the erythroid series obtained.

Erythropoietin-active serum was obtained by the method of Salvatorelli et al. [13]. For this purpose adult guinea-pigs were injected subcutaneously with phenylhydrazine solution (50 mg/kg body weight) for

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TABLE 1. Kinetics of Hematopoiesis in Cultures of Dog Bone Marrow Cells (M ± m)

Time of cultivation (in days)	Cells of myeloid series (in %)	Number of different types of cells of erythroid series (in %)				Number of mitoses (in %)
		basophilic erythroblasts	polychromatophilic erythroblasts	polychromatophilic normoblasts	orthochromic normoblasts	
1	56,0±2,0	3,7±0,6	22,0±5,0	10,0±0,5	3,2±0,4	5,0±0,5
3	81,0±3,0	1,0±0,2	3,0±0,3	5,0±0,15	6,0±1,0	4,0±0,4
4	75,0±3,0	0,5±0,1	1,5±0,3	15,0±0,4	8,0±1,5	0
5	77,5±3,0	0	0	1,0	19,0±2,5	2,5±0,2
7	92,5±3,4	0	0	0,5	5,0±0,6	2,0±0,2
10	96,5±3,6	0	0	1,0	1,0±0,1	1,5±0,15

TABLE 2. Erythropoietic Action of Blood Serum from Anemic Animals in Cultures of Dog Bone Marrow Cells (M ± m)

Bone marrow cells (in %)	Cell culture after 72 h	Cell culture after 72 h with addition of erythropoietin-active serum	
		10%	20%
Cells of myeloid series	81,0±3,0	68,0±2,4	54,5±2,5
Basophilic erythroblasts	1,0±0,2	1,0±0,2	1,5±0,3
Polychromatophilic erythroblasts	3,0±0,3	1,0±0,2	1,5±0,3
Polychromatophilic normoblasts	5,0±0,15	4,0±0,1	6,0±0,2
Orthochromic normoblasts	6,0±1,0	26,0±3,2	35,0±5,0
Number of mitoses	4,0±0,4	0	1,5±0,1

2 days. Blood for obtaining the serum was taken from the heart on the 4th day after the first injection of phenylhydrazine. Anemic serum was added in volumes of 10 and 20% of the total volume of medium. The results were subjected to statistical analysis (n = 4).

EXPERIMENTAL RESULTS

Hematopoiesis in the cell culture (Table 1) was characterized by a gradual decrease in erythropoietic activity with preservation of myelopoiesis. The cells of the erythroid series in the bone marrow ceased to differentiate depending on the duration of cultivation and probably with the associated effects of unfavorable factors in the medium. For instance, by the 5th-7th day of cultivation the population of bone marrow cells consisted mainly of large, vacuolated basophils and erythrocytes. Polymorphonuclear cells and cells resembling small lymphocytes also were found. Cells of the myeloid series underwent maturation during cultivation. This was shown by an increase in the number of granulocytes and a simultaneous decrease in the number of precursor cells. Cells with a round or horseshoe-shaped nucleus were relatively numerous under these conditions. With an increase in the period of cultivation (6-7 days) the number of fibroblast-like cells also increased. The phenomenon of transformation of bone marrow cells during cultivation in vitro has been described several times [10, 12], although somewhat different methods were used in those cases for cultivating the bone marrow.

With an increase in the period of cultivation (7-10 days) the number of degenerating cells increased. Vacuolation of the nuclei and cytoplasm, pycnosis of the nuclei, and signs of karyorrhexis, etc., were observed. However, hematopoiesis continued until the 3rd-4th day, including erythropoiesis, which means that this model can be used to investigate the bone marrow population in vitro for periods of exposure similar to the corresponding period for the response to acute anemia to develop in vivo.

Cultivation of the bone marrow with the addition of erythropoietin-active serum in concentrations of 10 and 20% of the total volume of the medium induced a statistically significant ($P < 0.01$) increase in the relative percentage of cells of the erythroid series (Table 2). The number of orthochromic normoblasts was increased (26 ± 3.2 and 35 ± 5% compared with 6 ± 1% in the control culture), indicating more rapid differentiation of the mature cells of the erythroid series. The number of mitoses among the proliferating part of the cells of the erythroid series did not increase.

These results thus indicate that the response of the erythroid part of the bone marrow to addition of erythropoietin is manifested among the mature precursors of the erythrocytes. This response of the erythroid series to erythropoietin-active serum is perhaps characteristic only of cultures of this type. This statement requires further experimental verification in histotypical and organotypical bone marrow cultures.

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