EFFECT OF ERYTHROPOIETIC SUBSTANCES ON LIPOLYTIC ACTIVITY OF BONE MARROW

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Experiments on rabbits showed that erythropoietically active sera can stimulate the lipolytic activity of bone marrow in vitro. Injection of exogenous erythropoietin and hemolyzed red cells into rabbits increased the lipolytic activity of the bone marrow. The rate of oxygen consumption by the bone marrow was considerably increased under these circumstances. The increased lipolytic activity of the bone marrow accompanying intensified erythropoiesis can presumably be explained by the action of the erythropoietically active substances on the blood-forming tissue.

Lipids are the largest component of the bone marrow, for on the average they account for 50% of the wet weight of this tissue [4, 5]. However, the role of the bone marrow lipids in erythropoiesis has not been explained. Their possible participation as sources of energy or building materials for this tissue during hematopoiesis has been postulated [6, 12, 14]. The writers' previous investigations showed that under conditions accompanied by increased regeneration of red cells there is a marked decrease in the content of total lipids in the bone marrow with a simultaneous increase in the lipolytic activity of that tissue [11].

The investigation described below was carried out to study the possible mechanisms of these changes.

EXPERIMENTAL METHOD

Sixty rabbits of both sexes weighing 2.5-3.6 kg were used. Bone marrow for the investigations was taken from the long bones. A fat-free homogenate of bone marrow prepared in Krebs-Ringer solution, pH 7.4, was used to determine lipolytic activity. For this purpose, in a medium containing erythropoietically active sera 1 ml of the resulting bone marrow homogenate was mixed with 1.7 ml of substrate prepared from cows' milk with the addition of 3% albumin, and 0.3 ml of the test serum was added. Blood sera were obtained from intact animals, from rabbits with phenylhydrazine and posthemorrhagic anemias, and from rabbits kept in a pressure chamber at an "altitude" of 6000 m for 6 h daily on 6 consecutive days. The erythropoietic activity of the test sera and of the erythropoietic substances was estimated from the changes in reticulocytes in recipient animals, for which purpose hypoxic polycythemic mice were used [1, 13]. After inhibition of bone marrow homogenate in a Warburg apparatus at 37°C for 1 h the increase in concentration of nonesterified fatty acids was determined. In the experiments in which erythropoietic substances were injected in vivo into rabbits the esterase activity was estimated from the liberation of fatty acids from the substrate (3 ml of a 2.5% emulsion of Tween-80 with 4% serum albumin in Krebs-Ringer-bicarbonate buffer solutions was used) as the result of incubation for 1 h at 37°C with 100 mg bone marrow. Esterase activity was calculated in microequivalents of palmitic acid per 10 mg tissue protein per hour. Erythropoietin was obtained from the plasma of rabbits with phenylhydrazine anemia [16]. The plasma was freeze-dried, dissolved in physiological saline, and then injected intravenously in a dose of 100 mg/kg daily for 4-5 days. The hemolyzed red cells were obtained by the addition of bidistilled water to rabbits'

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TABLE 1. Reticulocyte Count in Peripheral Blood, Lipolytic Activity, and Velocity Constant of Respiration of Bone Marrow after Administration of Erythropoietin and Hemolyzed Red Cells to Rabbits

Experimental conditions	Reticulocytes			Lipolytic activity			Velocity constants of respiration of bone marrow		
	$M \pm m$	% change	P	$M \pm m$	% change	P	$M \pm m$	% change	P
Control	1,5±0,13			0,220±0,017			0,0115±0,00128		
Administration of hemolyzed red cells	3,3±0,35	+120	<0,001	0,311±0,023	+41	<0,02	0,0179±0,0026	+60	<0,05
Administration of erythropoietin	4,5±0,76	+200	<0,001	0,886±0,163	+302	<0,01	0,0236±0,00059	+123	<0,01

red cells (up to the volume of whole blood), followed by freezing and thawing. The hemolyzed red cells were injected intraperitoneally in a dose of 3 ml/kg daily for 5 days. The partial oxygen pressure (pO_2) in the bone marrow was determined polarographically [7]. The rate of oxygen utilization by the bone marrow was determined in vivo by the decrease in PO_2 after cessation of the circulation in the bone marrow [8, 9].

EXPERIMENTAL RESULTS

The experiments in vitro showed that in the presence of sera obtained from rabbits with posthemorrhagic anemia and exposed to chronic hypoxic hypoxia the lipolytic activity of the bone marrow was increased by 62% ($P < 0.001$) and 56% ($P < 0.01$) respectively compared with the lipolytic activity of bone marrow incubated with the serum of intact rabbits. The ability of the test sera to activate lipolysis was probably connected with their increased content of erythropoietic substances. It is easy to explain on this basis the fact that the serum of animals with hemolytic anemia had the greatest action on lipolysis (the lipolytic activity of the bone marrow was increased in the presence of the serum by 112%; $P < 0.001$), for the erythropoietic activity of this serum was the highest. The protein-free filtrates of the test sera clearly remained sufficiently capable of activating lipolysis.

However, these results could also be explained by the presence of hormones capable of activating lipolysis in the test sera [2, 3], for all the sera tested were obtained from animals in a state of hypoxia, in which the formation of such substances and their liberation into the blood stream are increased [15]. To solve this problem, changes in the lipolytic activity of the bone marrow were investigated after direct injection of exogenous erythropoietin and hemolyzed red cells into rabbits. The values of pO_2 and the rate of oxygen consumption by the bone marrow were determined at the same time.

Administration of erythropoietic substances to the animals considerably increased the formation of reticulocytes and their liberation into the peripheral blood. The well marked stimulation of erythropoiesis after injection of erythropoietin was accompanied by higher lipolytic activity of the bone marrow (Table 1). The partial oxygen pressure in the bone marrow fell a little after injection of hemolyzed red cells and erythropoietin (by 17.1 and 17.5% respectively), but the velocity constant of oxygen utilization by the bone marrow rose considerably. Since the rate of oxygen consumption by the tissue is directly proportional to pO_2 in it and to the velocity constant of oxygen consumption [9, 10], the rate of consumption of oxygen by the bone marrow was considerably higher than initially after administration both of erythropoietin and of hemolyzed red cells (by 84 and 33.3% respectively).

These results show that after administration of erythropoietin and hemolyzed red cells the bone marrow receives an adequate supply of oxygen. The increase in the lipolytic activity of the bone marrow after administration of the erythropoietic substances was not the result of the developing hypoxia, but was probably connected with the action of these substances on hematopoietic tissue. The products of lipolysis thus formed could be used as energy-supplying materials by the actively regenerating bone marrow. This is confirmed by investigations of the intensity of oxygen utilization carried out on the bone marrow mitochondria of rabbits injected with erythropoietic substances. The rate of oxidation of β-hydroxybutyric acid by the bone marrow mitochondria of these animals was 29% higher ($P < 0.05$) than the corresponding rate in intact rabbits.

LITERATURE CITED

1. T. I. Koretskaya, G. P. Moskaleva, V. I. Gudim, et al., Pat. Fiziol., No. 3, 77 (1969).
2. S. M. Leites and N. K. Davtyan, Byull. Éksperim. Biol. i Med., No. 5, 60 (1963).
3. S. M. Leites and Chou-Hsu, Vopr. Med. Khimii, No. 3, 289 (1962).
4. E. N. Morozova, Vopr. Med. Khimii, No. 1, 73 (1960).
5. É. Ya. Shenina, in: Ya. G. Uzhanskii (editor), Problems in the Pathophysiology of Regeneration of Blood [in Russian], Sverdlovsk (1968), p. 104.
6. Ya. G. Uzhanskii, Pat. Fiziol., No. 3, 3 (1970).
7. I. M. Épshtein, Byull. Éksperim. Biol. i Med., No. 12, 104 (1960).
8. I. M. Épshtein, in: Polarographic Determination of Oxygen in Biological Objects [in Russian], Kiev (1968), p. 246.
9. A. P. Yastrebov, Byull. Éksperim. Biol. i Med., No. 2, 18 (1971).
10. A. P. Yastrebov, in: Ya. G. Uzhanskii (editor), Experimental Investigations of the Mechanisms of Hematopoiesis [in Russian], Sverdlovsk (1971), p. 13.
11. E. A. Yatsenko and A. P. Yastrebov, Pat. Fiziol., No. 2, 77 (1971).
12. G. Acerman, Lab. Invest., 9, 298 (1960).
13. U. De Gowin, D. Hafstra, and C. Gurney, J. Lab. Clin. Med., 60, 846 (1962).
14. G. Evans, Am. J. Physiol., 171, 48 (1952).
15. G. Fowler, R. Shabetai, and J. Holmes, Circulat. Res., 9, 427 (1961).
16. P. Lowy and H. Borsook, in: Erythropoiesis, London (1962), p. 33.