CONNECTION BETWEEN ERYTHROPOIESIS AND OXIDATIVE METABOLISM OF THE BONE MARROW

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The increase in regeneration of erythrocytes in rabbits with hemolytic anemia or exposed to chronic hypoxia is accompanied by an increase in the respiration rate of the bone marrow and an increase in the intensity of oxidative phosphorylation. In animals with depression of erythropoiesis resulting from blood transfusion, a decrease in oxidative phosphorylation is found in the bone marrow.

The widely held view that hypoxia is a direct stimulus for erythropoiesis requires reexamination [2]. It has been shown that hypoxia of the bone marrow does not stimulate, but depresses regeneration of erythrocytes [1, 9, 10]. Investigations of the oxygen concentration in the bone marrow after blood loss have not confirmed the role of hypoxia as a stimulator of erythropoiesis [7, 8, 9]. The writer has previously shown that increased regeneration of erythrocytes in hypoxia is connected with the increased rate of recovery of the oxygen tension (pO_2) in the bone marrow [5].

The object of the present investigation was to study the oxygen demand of bone marrow during increased blood regeneration and to examine the relationship between respiration of the bone marrow and oxidative phosphorylation.

EXPERIMENTAL METHOD

Experiments were carried out on 73 rabbits of both sexes weighing 2-3.2 kg. The oxygen tension in the bone marrow was determined polarographically. The electrodes were a pair recommended by I. M. Épshtein: copper amalgam and cadmium [3]. The cathode, which was amalgamated before the investigation, was introduced into the bone marrow through a special hole drilled in the upper third of the tibia. The second electrode (the anode), consisting of a cadmium-coated needle, was introduced beneath the skin of the thigh. The oxygen tension was recorded on a type Lp-60 polarograph. The rate of oxygen utilization by the bone marrow was determined in vivo from the decrease in pO_2 after stopping the circulation in the bone marrow by applying an arterial tourniquet to the upper third of the femoral artery, and the velocity constant of oxygen utilization by the bone marrow was calculated [4, 6]. The following methods were used to increase the rate of regeneration of blood in the animals: placing the rabbits in a push-pull pressure chamber with a degree of rarefaction corresponding to an altitude of 6000 m, for a period of 6-8 h daily for 6 days; injection of 2.5% phenylhydrazine hydrochloride solution in a dose of 0.6 ml/kg daily for 3 days; subcutaneous injection of a 2.47% solution of cobalt nitrate in a dose of 3 mg/kg. Transfusion polycythemia was produced by intravenous injection of 30-40 ml blood into the rabbits, and the animals were sacrificed for investigation 3 days after blood transfusion. The bone marrow was removed from the long bones. Mitochondria were isolated by differential centrifugation in 0.25 M sucrose solution made up in 0.02 M tris buffer (pH 7.4) and 0.001 M EDTA. The mitochondria were incubated at 37° in a chamber with a rotating open platinum electrode, about 1 ml in volume. The incubation medium contained (in 1 ml) 150 μmoles sucrose, 20 μmoles $MgCl_2$, 15 μmoles KCl, 20 μmoles K-phosphate buffer (pH 7.4), and 1 μmole EDTA (pH 7.4). The oxidation substrate was succinate (5 μmoles) and the phosphate acceptor was ADP (200 μmoles). The mitochondria were added to the sample in an amount corresponding to 1-1.7 mg protein.

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TABLE 1. Oxidative Phosphorylation of Bone Marrow Mitochondria of Rabbits with Experimental Changes in Erythropoiesis (M ± m)

Experimental conditions	No. of animals	Oxygen assimilation (in μg/mg protein/min)	Changes	P
Hypoxia:				
without ADP	8	92,0 ± 7,46	+34	< 0,003
with ADP	8	119,0 ± 7,67	+50	< 0,01
Hemolytic anemia:				
without ADP	17	102,2 ± 7,05	+49	< 0,002
with ADP	17	160,6 ± 11,11	+102	< 0,001
Transfusion polycythemia:				
without ADP	6	72,2 ± 5,88	+5,6	> 0,1
with ADP	6	77,7 ± 5,56	—2,3	> 0,1
Control:				
without ADP	8	68,3 ± 5,8		
with ADP	8	79,6 ± 4,88	—	—

Note. Values given in percentages of oxygen absorption with and without ADP by bone marrow mitochondria of intact rabbits.

EXPERIMENTAL RESULTS

In the rabbits exposed to chronic hypoxia, an increase in the velocity constant of respiration of the bone marrow by 43 ± 7.2% was observed ($P < 0.001$). However, the greatest increase in the velocity constant of respiration of the bone marrow (by 161 ± 10.4%, $P < 0.001$) was found in the animals with hemolytic anemia, in which the most intensive regeneration of erythrocytes occurred.

To determine the efficiency of oxygen in the bone marrow, respiration and phosphorylation of the bone marrow mitochondria were investigated during experimental changes in erythropoiesis. No reference could be found in the accessible literature to investigations of oxidative phosphorylation of bone marrow. The experiments carried out showed that an increase in the intensity of erythropoiesis in the bone marrow following exposure to hypoxic hypoxia is accompanied by activation of respiration of the bone marrow mitochondria and by an increase in the rate of their oxidative phosphorylation (Table 1).

The changes in the rate of oxidative phosphorylation correlated closely with the changes in erythropoiesis in the various series of tests. For example, the highest rate of phosphorylation was observed in tests on the bone marrow mitochondria of rabbits with hemolytic anemia, in which the most intensive regeneration of erythrocytes was observed at that time. In animals with transfusion polycythemia, with depression of erythropoiesis, the intensity of oxidative phosphorylation was reduced in the bone marrow mitochondria.

It can be concluded from the results of these experiments that an increase in the intensity of erythrocyte regeneration in bone marrow is associated with an increase in the respiration rate of this tissue and an increase in the intensity of oxidative phosphorylation, aimed at supplying an adequate amount of energy for the actively regenerating bone marrow.

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