EFFECT OF BREATHING PURE OXYGEN ON ERYTHROPOIESIS

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The plasma of rats breathing pure oxygen for 40 h acquires the property of inhibiting mitotic activity of erythroblasts in a bone marrow culture.

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Under hypoxic conditions an excess of erythropoietins, substances stimulating erythropoiesis, appear in the blood of man and animals [5, 11, 14]. When gas mixtures with a high partial pressure of oxygen are inhaled by man and animals, a decrease in the hemoglobin concentration and erythrocyte count in the blood are most commonly observed [2-4, 6-8, 12, 13, 15, 19, 21]. Some writers consider that these changes are due to dilution of blood as a result of displacement of tissue fluid into the lumen of the blood vessels, while others attribute them to inhibition of erythropoiesis in the bone marrow. Meanwhile, some investigators [1, 17] have described an increase in the blood erythrocyte indices or stimulation of erythrocyte development in the bone marrow under hyperoxic conditions [2]. In animals exposed to a high partial pressure of oxygen and subsequently bled, the composition of the blood is restored more slowly than in control animals [7, 18], while in tissue culture an increased oxygen concentration in the medium inhibits cell proliferation [16]. During the prolonged and continuous action of oxygen on animals, severe changes develop principally in the lung tissue, with subsequent disturbances of respiratory function [9].

It was therefore necessary to discover whether oxygen has a specific or merely a toxic action on erythropoiesis. In the present investigation we attempted to elucidate some of the mechanisms of the action of oxygen on erythropoiesis.

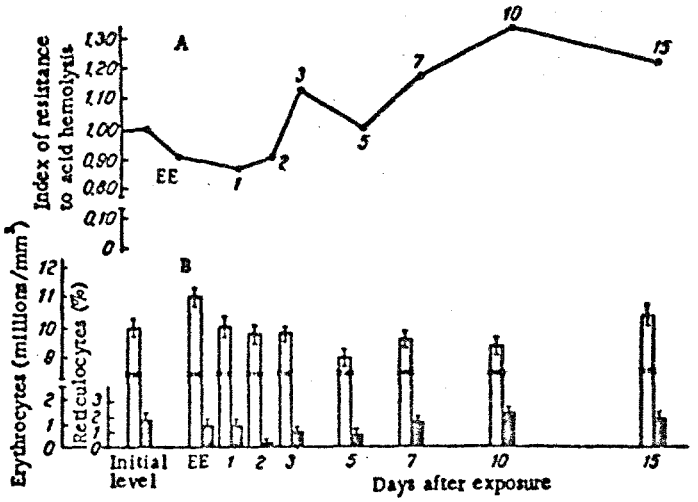

Fig. 1. Effect of exposure to pure oxygen for 40 h on resistance of erythrocytes to acid hemolysis (A) and on erythrocyte composition of rats' blood (B). EE) End of exposure; unshaded columns) erythrocyte count (millions/mm³): shaded columns) reticulocyte count (in %).

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TABLE 1. Concentration of Transport Iron in Rats Inhaling Pure Oxygen for 40 h (M±m)

Index	Control	End of exposure	Day of exposure									
			1	2	3	5	7	10	15	30
No. of animals investigated	26	3	6	6	5	6	6	5	5	6
Iron concentration (in μg %)	153±9,94	117±7,8 <0,01	220±25,3 <0,05	185±23,3 >0,05	100±14,2 <0,01	171±16,6 >0,05	201±34 >0,05	81±21,8 <0,01	148±19,1 >0,05	178±30,9 >0,05

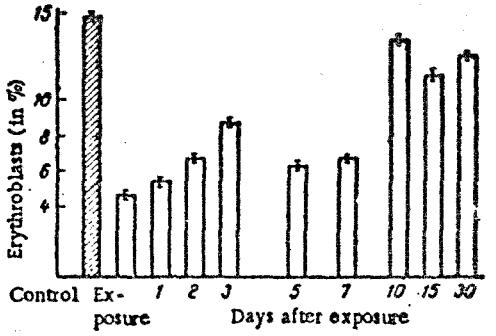

Fig. 2. Erythroblast count in bone marrow of control and experimental animals after exposure to pure oxygen. Shaded column) control animals, unshaded) experimental animals.

EXPERIMENTAL METHOD

Experiments were performed on 100 Wistar albino rats weighing 170-190 g. The rats were kept in a KNZh-2 chamber, ventilated with pure oxygen at a normal atmospheric pressure for 40 h. The control group of animals was exposed in the same environment but to ordinary atmospheric air. The erythrocyte count per mm^3, hemoglobin concentration, hematocrit index, percentage of reticulocytes, and bone marrow (obtained by puncture) were investigated by the usual methods. The acid resistance of the erythrocyte was studied by the erythrogram method of Gitel'zon and Terskov, the concentration of transport iron in the plasma by Ramsay's method, and the erythropoietic properties of the plasma by Light's liquid bone marrow culture method as modified by S. Yu. Shekhter [10]. To study the mitotic activity of the erythroblasts in the culture, colchicin solution was added to give a final concentration of 1 : 500,000.

EXPERIMENTAL RESULTS

After exposure of rats to pure oxygen for 40 h and transfer into normal atmospheric conditions, the erythrocyte count was lowered significantly for a long time. As Fig. 1 shows, toward the end of exposure the erythrocyte count per mm^3 rose (with a simultaneous increase in the hemoglobin concentration), evidently in connection with redistribution reaction, because the reticulocyte concentration did not rise, and next day the erythrocyte count had fallen. The decrease in the erythrocyte count was most marked on the 5th day after exposure. Toward the end of exposure and on the next two days the erythrocyte resistance was decreased, but in the second week after the animals had returned to normal atmospheric conditions the index of erythrocyte resistance to acid hemolysis was permanently raised. As Table 1 shows, the concentration of transport iron also was changed, falling significantly toward the end of exposure and on the 3rd and 10th day after the rats had returned to an atmospheric environment.

Some increase in the iron concentration in the blood was observed in the first 10 days after exposure, although on the 2nd day this was really no more than a tendency. The decrease in erythrocyte resistance, with accompanying disappearance of highly resistant erythrocytes, the decrease in the erythrocyte count in the peripheral blood, and the increase in transport iron concentration were evidence of hemolysis in the first days after exposure to oxygen. At the same time, the decrease in the erythrocyte resistance and increase in concentration of transport iron could indicate inhibition of erythropoiesis.

As Fig. 2 shows, toward the end of exposure to oxygen the percentage of erythroblasts in the rats' bone marrow was considerably lowered, returning to its initial level only in the 2nd week after exposure. Such a considerable decrease in the erythroblast count in the bone marrow of the animals cannot be

attributed to the toxic action of oxygen, because the general condition of the animals in the exposure period and on the subsequent days remained satisfactory and no macroscopic changes were observed in the lungs, which are most sensitive to the action of oxygen.

When the rats were transferred from the hyperoxic environment to ordinary atmospheric conditions their plasma acquired the property of inhibiting mitotic activity of the erythroblasts in bone marrow cultures. The mean percentage of mitoses (relative to the number of mitoses after addition of Hanks' solution taken as 100) was 86±8.8 for the animals of the experimental group and 139±11.3 ($P < 0.001$) for the controls. Hence, the plasma of the control animals was erythropoietically active, in agreement with data in the literature [20], while the plasma of rats exposed to an atmosphere of pure oxygen for 40 h retained this activity only until near the end of exposure. The results described suggest that the factor inhibiting proliferation of erythroblasts in the bone marrow culture is an inhibitor of erythropoiesis, which also affects the erythroblasts in the bone marrow of the animals themselves.

An excessive oxygen concentration produces reactions in the body aimed at limiting production of erythroblasts and, above all, it inhibits erythropoiesis in the bone marrow. One of the causes of inhibition of erythropoiesis may be a decrease in mitotic activity of the erythroblasts, due not to the direct action of oxygen but to the appearance of a special substance, an inhibitor of erythropoiesis, in the blood.

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