EFFECT OF HYPEROXIA ON RESPIRATORY FUNCTION OF THE BLOOD IN CATS

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The effect of isobaric hyperoxia (99% oxygen, exposure for 3 h daily for 2 weeks) on the respiratory function of the blood was investigated in adult cats. An increase in the reticulocyte count (by 170%) and a decrease in the methemoglobin concentration in the blood (by 33%), in the affinity of hemoglobin for oxygen (by 13%), and in Hill's constant (by 16%) were found. After dialysis of hemoglobin solutions the differences in affinity between the control and experimental animals disappeared. No changes could be found in the number of hemoglobin fractions or in their mobility by electrophoresis on agar. It is concluded that the reaction of cat erythrocytes to hyperoxia is typical self-regulation, on the basis of which the affinity of the hemoglobin for oxygen falls in the presence of an excess of this gas.

KEY WORDS: hyperoxia; hemoglobin; respiratory function of the blood.

The morphological picture of the blood and the hemoglobin concentration in hyperoxia has been studied in many species of animals and in man [1, 4, 6]. However, the reports so far published [4, 7, 9, 14] give no idea of the basic principles governing the change in the oxygen transport function of the blood in hyperoxia. In particular it is not clear how animals with different blood levels of 2,3-diphosphoglycerate (DPG) respond to hyperoxia. DPG is known [12, 13] to have a marked effect on the affinity of hemoglobin for oxygen, but its concentration in the blood varies in animals of different species. In rabbit erythrocytes it is about 10 μM [10], but under hyperoxic conditions the oxygen-combining properties of the hemoglobin of these animals vary to a different degree depending on the length of exposure [4]. The present investigation was carried out on cats, whose erythrocytes contain a very small amount of DPG [10].

EXPERIMENTAL METHOD

Adult noninbred cats of both sexes weighing from 1.8 to 4.6 kg were used. Oxygenation was carried out in a continuous-flow metal chamber with a capacity of 30 liters, containing 99% oxygen at atmospheric pressure, with an exposure of 3 h daily for 3 weeks. Oxyhemoglobin dissociation curves were plotted spectrophotometrically [2]. The hemoglobin and methemoglobin concentrations in the blood were measured by the cyanmethemoglobin method. The hemoglobin solutions were dialyzed in potassium phosphate buffer [15]. Hill's constant was found by drawing the tangent to the dissociation curve at the point p50. The electrophoretic properties of the hemoglobin were investigated in Difco agar.

EXPERIMENTAL RESULTS

The results are given in Table 1. Under isobaric hyperoxic conditions the reticulocyte count in the cats' blood was sharply increased (by 170%) whereas the methemoglobin concentration was reduced by 33.5%, the affinity of hemoglobin for oxygen by 13%, and Hill's constant by 16%. The changes in the other indices were not significant. The erythrocyte distribution curves by diameter were shifted toward macrocytosis (Fig. 1) because of the accumulation of a large number of reticulocytes. The reticulocytosis was evidently caused by delayed maturation of the erythrocytes and stimulation of the bone marrow. In rabbits exposed to hyperoxia, on the contrary, a sharp decrease in the reticulocyte count in the blood is observed [4].

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TABLE 1. Morphological and Functional Indices of Cats' Blood Following Exposure to Isobaric Hyperoxia

Index	Control		Experiment		P
	n	M ± m	n	M ± m	
Erythrocyte count, millions/mm³	19	7,61±0,43	14	7,40±0,53	
Reticulocyte count, %	13	6,4±1,7	13	17,2±3,0	<0,01
Hemoglobin concentration, g%	18	9,1±0,4	12	8,7±0,6	
Methemoglobin concentration, g%	19	1,55±0,03	10	1,03±0,07	<0,05
Diameter of erythrocytes, μ	6	5,50±0,07	6	5,71±0,09	
Hematocrit, %	19	35,0±1,5	14	36,4±2,3	
Relative electrophoretic mobility, mm	14	33,5±1,0	14	35,3±1,2	
p50, mm Hg	31	38,1±0,58	15	42,9±0,97	<0,001
p50 after dialysis, mm Hg	10	24,3±0,58	12	23,6±0,53	
Hill's constant	31	2,03±0,04	15	1,70±0,07	<0,001
Hill's constant after dialysis	10	2,97±0,02	12	3,06±0,09	

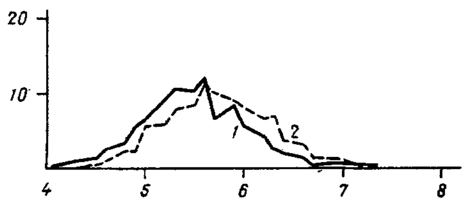

Fig. 1

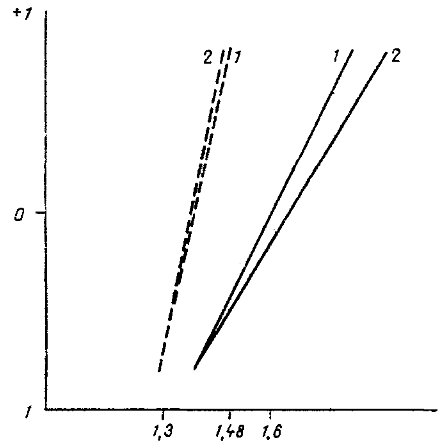

Fig. 2

Fig. 1. Distribution curves of erythrocytes by diameter in control (1) and experimental (2) cats. Abscissa, diameter of cells (in μ); ordinate, % of cells.

Fig. 2. Oxyhemoglobin dissociation curves of control (1) and experimental (2) cats. Curves after dialysis of hemoglobin solution shown as broken lines. Abscissa: log pO_2; ordinate: log $[y/(1-y)]$.

Assuming that the mean life span of the cat's erythrocytes is 77 days, in the course of 2 weeks the blood must renew itself by more than 20% of its composition. Since during exposure for 2 weeks the reticulocyte count increased almost threefold, one fifth of the blood must accumulate three times more young cells with a different internal medium and, consequently, with different functional properties, and this must be reflected in the general properties of the blood.

Previous investigations [4, 5] showed that the change in the affinity of hemoglobin for oxygen in rabbits is determined by the medium of the erythrocytes and mainly by their content of DPG. After analysis of solutions of hemoglobin from control and hyperoxic cats, the differences in the affinity of the hemoglobin for oxygen disappeared (Fig. 2). This evidently means that it is not the hemoglobin itself, but the medium of the erythrocyte which has the principal influence on the change in the oxygen-combining properties of the hemoglobin in these animals. This conclusion is confirmed by the similarity between the results of electrophoresis of the hemoglobin of the control and experimental cats.

In rabbits in similar experiments the change in the oxygen-combining properties of the hemoglobin were biphasic [4]: Initially the affinity rose, but later it fell. The whole process continued for 4 weeks. In cats no shift of the oxygen dissociation curves to the left was noted, and the shift to the right developed after 2 weeks. It has been suggested that the cause of the change in the affinity of hemoglobin for oxygen in rabbits is a fall in the DPG level during the first half of the experiment followed by a rise in its level in the erythrocytes in the second half. If the cause of the shift to the right in cats is also the accumulation of DPG, the species differences can probably be explained by a difference in the initial level of this substance in the erythrocytes. It has been shown [8] that if the DPG level in the erythrocytes is low, its further accumulation can be stimulated, whereas if it is high it can be inhibited.

Changes in the oxygen-combining properties of the hemoglobin in cats exposed to isobaric hyperoxia may be due to the fact that, when the oxygenation of the blood is increased, the oxyhemoglobin concentration in the erythrocytes rises and the ability of the hemoglobin to transport the accumulating carbon dioxide falls. This leads to acidification of the blood and to a shift of the dissociation curve to the right. The oxygen-combining properties of hemoglobin can also be reduced by the stimulation of glycolysis [3] under these conditions and by the accumulation of DPG in the cells; being an insoluble anion, DPG shifts the Donnan equilibrium toward an increase in the hydrogen ion concentration in the erythrocytes. In both cases the shift of the dissociation curves is due to a Bohr effect. The possibility cannot be ruled out that the shift to the right could be greater still in cases of a less marked reticulocytosis, because the medium in the reticulocytes is characterized by higher pH values than in mature erythrocytes [11].

The reaction of erythrocytes to hyperoxia is thus an example of self-regulation: The affinity of the hemoglobin for oxygen is reduced in the presence of an excess of this gas.

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