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EFFECT OF HYPEROXIA AND THE PROTECTIVE ACTION OF UREA ON SERUM HEMOGLOBIN, TRANSFERRIN, AND TOTAL IRON CONCENTRATIONS

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During exposure of albino rats to oxygen under a pressure of 4 atm for 1 h (the preconvulsive state) and of 6 atm (convulsive state) an increase in the serum hemoglobin concentration was found. The total ion concentration increased at the same time. By disc electrophoresis in 7.5% polyacrylamide gel, changes in the ratio between the hemoglobin fractions in the serum were found in both stages of oxygen poisoning. An increase in the concentration of transferrins was found during hyperoxia under these conditions. If urea was administered to the animals before the session of hyperbaric oxygenation, the changes observed were less marked.

KEY WORDS: hyperoxia; blood serum; hemoglobin; total iron; transferrins.

Oxygen under increased pressure (hyperbaric oxygenation) is widely used in medicine and in various types of occupation. One of the complications encountered during the use of hyperbaric oxygenation in clinical practice is oxygen poisoning.

The first link in the chain of reactions in oxygen poisoning is the accumulation of free-radical and peroxide compounds. Many factors considerably potentiate the primary hyperoxic effect. In the writers' view, the escape of hemoglobin from the erythrocytes into the blood serum and its penetration into the tissues is one such factor. This is because of the ability of hemin iron to catalyze peroxidation of lipids [1].

In the investigation described below the hemoglobin concentration, its fractional composition, and the total concentrations of iron and transferrins were studied in the blood serum of rats exposed to the action of

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TABLE 1. Concentrations of Hemoglobin, Iron, and Transferrin in Rat Blood Serum (in mg %)

	Hemoglobin	Iron	Transferrin
1. Control	26,11±1,26 (20)	1,22±0,06 (20)	1,54±0,06 (20)
2. Administration of urea	29,10±1,32 (20)	1,05±0,04 (20)	1,47±0,07 (20)
P_{1-2}	>0,1	<0,05	>0,1
3. Exposure to 4 atm oxygen for 1 h	38,10±2,25 (10)	1,93±0,11 (10)	2,06±0,15 (10)
P_{1-3}	<0,001	<0,001	<0,01
4. Protection by urea against hyperoxia (4 atm oxygen for 1 h)	31,20±2,67 (10)	1,21±0,11 (10)	1,99±0,09 (10)
P_{1-4}	<0,1	>0,1	<0,001
P_{3-4}	<0,1	<0,001	>0,1
5. Hyperbaric convulsions (6 atm oxygen)	47,60±4,39 (10)	2,35±0,26 (10)	1,92±0,12 (10)
P_{1-5}	<0,001	<0,001	<0,01
6. Protection by urea against hyperoxia (6 atm oxygen)	37,50±5,60 (10)	1,28±0,30 (10)	2,14±0,17 (10)
P_{1-5}	<0,1	>0,1	<0,01
P_{5-6}	>0,1	<0,02	>0,1

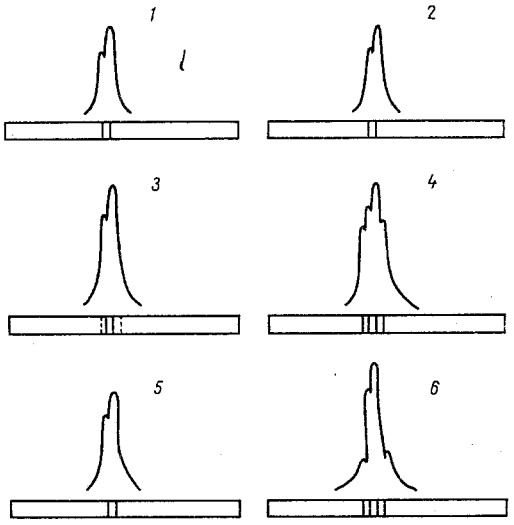

Fig. 1. Densitograms of hemoglobin fractions from rats' blood serum after exposure to hyperbaric oxygen and protection with urea: 1) control; 2) injection of urea; 3) oxygen 4 atm; 4) oxygen 6 atm; 5) protection by urea during exposure to 4 atm oxygen; 6) protection by urea during exposure to 6 atm oxygen.

TABLE 2. Content of Hemoglobin Fractions in Blood Serum of Rats during Hyperoxia (in % of total hemoglobin)

	I	II	III	IV
Control	—	27,0	73,0	—
Administration of urea	—	21,3	78,7	—
Hyperoxia (4 atm, 1 h)	Traces	26,7	69,1	Traces
Protection by urea against hyperoxia (4 atm, 1 h)	—	30,7	69,3	—
Hyperoxia, 6 atm, convulsions	13,6	16,8	45,4	24,2
Protection by urea against hyperoxia (6 atm)	Traces	32,0	67,8	Traces

oxygen under pressures of 4 and 6 atm. In a special series of experiments the animals were treated with a solution of urea, an antihyperoxic protector [4].

EXPERIMENTAL METHOD

Albino rats of both sexes, weighing 150-200 g, were used. The parameters of hyperbaric oxygenation were: oxygen pressure in the chamber 4 or 6 atm, time of compression and decompression 3 min. Duration of the animal's stay in the pressure chamber 1 h at 4 atm (the nonconvulsive form of oxygen poisoning), and at 6 atm until the development of convulsions. Urea was injected intraperitoneally (200 mg/100 g body weight) 30 min before exposure to 6 atm oxygen and immediately before compression in the case of exposure to 4 atm oxygen. Two rats, one intact and the other receiving urea, were placed at the same time in the pressure chamber. In the case of exposure to 4 atm oxygen the experiment ended after 1 h. The experiment with 6 atm oxygen ended after the unprotected animal had developed strong convulsions (under these conditions this was on average after 25 min). Animals protected with urea did not develop convulsions [3, 4].

The control group consisted of animals kept under normal atmospheric pressure – one intact, the other receiving urea. After decompression four animals were decapitated simultaneously – two from the pressure chamber and two controls; their blood was collected and the serum obtained. Hemoglobin in the blood serum was determined spectrophotometrically [2]. The hemoglobin fractions were determined by disc electrophoresis with staining for hemoglobins by Ornstein's method [6]. Densitometry of the gels was carried out at 540 nm. The relative quantity of hemoglobin in the fractions was determined from the areas of the peaks on the densitogram. The total iron concentration was determined by the thiocyanate method [8] and the iron-binding capacity of the blood serum by Ramsey's method [7].

EXPERIMENTAL RESULTS

The serum hemoglobin concentration of the rats after exposure to 4 atm oxygen for 1 h was increased by 45.9%. During oxygen convulsions (exposure to 6 atm oxygen) the serum hemoglobin concentration increased to 82.3% (Table 1). The increase in the serum hemoglobin concentration could be due to partial hemolysis of the erythrocytes under hyperoxic conditions [10]. During oxygen poisoning changes also take place in the fractional composition of hemoglobin. The results of electrophoresis in 7.5% polyacrylamide gel showed that the hemoglobin of the erythrocytes was divided into four fractions, in agreement with data in the literature [12]. On electrophoresis of the blood serum proteins of the rats, only two hemoglobin fractions could be identified among them.

After exposure to 4 atm oxygen for 1 h an increase in the hemoglobin concentration in two serum fractions was observed in half of the animals. Traces of another two hemoglobin fractions appeared in half of the cases. This result indicates differences in the resistance of the erythrocytes of individual rats to hyperoxia. During convulsions (6 atm oxygen) four hemoglobin fractions were always found in the serum (Fig. 1, Table 2). This is evidence of progressive damage to the erythrocyte membranes with increasing severity of oxygen poisoning. In animals which received urea before exposure to 4 and 6 atm oxygen the serum hemoglobin concentration was 18.1 and 21.2% lower respectively than in the unprotected animals. Less marked changes also took place in the hemoglobin fractional composition. Traces of fractions I and IV of hemoglobin, which appeared in the blood serum of rats after exposure to 4 atm oxygen for 1 h without protection by urea, were absent in the protected animals. During exposure to 6 atm oxygen, hemoglobin fraction I fell to the trace level, and only in 70% of experiments could traces of fraction IV be observed (Fig. 1, Table 2). The decrease in the serum hemoglobin concentration and restoration of its normal fractional composition are the result of the protective action of urea on the erythrocyte membranes. It was shown previously that administration of urea to the animals under the same conditions of hyperoxia reduced peroxidation of lipids in the brain and lung tissues [3].

An important role in the mechanism of oxygen poisoning may be played by "activated oxygen" (superoxides, hydrogen peroxide, the hydroxyl radical, oxygen in a singlet state), which arises in the erythrocytes in reactions with hemoglobin [11] and at the same time reacts with heme as far as opening of the porphyrin ring. We found an increase in the total serum iron concentration of 58.2 and 91.8% respectively after exposure to 4 and 6 atm oxygen (Table 1). The increase in the iron concentration exceeded its level in the liberated hemoglobin, the reason possibly being partial destruction of heme.

Administration of urea to the animals before the session of hyperoxygenation reduced the serum iron concentration to the control level (Table 1). The serum transferrin concentration of the rats exposed to 4 and 6 atm oxygen was increased by 33.7 and 24.6% respectively. This can be regarded as a compensatory reaction to an increase in the iron concentration (Table 1). Administration of urea did not change the transferrin concentration.

To summarize these results, it can be postulated that hemoglobin and its iron-containing destruction products, liberated from the erythrocytes, are carried by the blood flow into the tissues, where they initiate further peroxidation [5, 9]. Urea, by preventing an increase in the intensity of peroxidation reactions in the membranes, inhibits the liberation of hemoglobin from the erythrocytes and thereby reduces the effect of one of the most important components of potentiation of the primary toxic effect of hyperoxia. This is in agreement with the considerable postponement of hyperoxic convulsions and normalization of many stages of metabolism in animals receiving urea prophylactically before a session of hyperoxia, described by the writers previously and confirmed in the present investigation.

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