CHANGES IN TISSUE RESPIRATION AND CONTENT OF SULFHYDRYL GROUPS AND FREE RADICALS IN THE LIVER OF ANIMALS AFTER BLOOD LOSS AND HYPERBARIC OXYGENATION

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Hyperbaric oxygenation (2 atm, 40 min) prevents the decrease in intensity of oxygen absorption, the increase in content of free sulfhydryl groups, and the large increase in the level of free radicals observed in the liver of albino rats after extensive blood loss. Hyperbaric oxygenation prevents the development of an agonal state in most exsanguinated animals.

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In recent years, considerable interest has been shown in the treatment of pathological conditions by hyperbaric oxygenation [1-6, 9-11]. However, the mechanism of action of oxygen on the organism when given at an increased pressure has not yet been fully explained.

The object of the present investigation was to study the dynamics of cell metabolism (tissue respiration and content of sulfhydryl groups and free radicals) in the liver in acute posthemorrhagic anemia during hyperbaric oxygenation.

EXPERIMENTAL METHOD

The liver of 377 albino rats of both sexes, weighing 150-250 g, was investigated soon after decapitation. Bleeding was carried out from the jugular vein in a volume of 2.8% of the body weight at the rate of 1% every 10 min. Oxygenation under a pressure of 2 atm with 100% oxygen was carried out in a pressure chamber, with a volume of 90 liters, using absorbents of carbon dioxide, water vapor, and gaseous substances. Compression and decompression of the animals were applied for 5 min. The tissue respiration was studied manometrically in a Warburg apparatus: The tissue was homogenized on ice in Krebs–Ringer phosphate buffer, pH 7.7; the gaseous medium consisted of atmospheric air, and the temperature was kept constant at +37.5° [12].

Free sulfhydryl groups were estimated by amperometric titration of 1 ml supernatant in 29 ml 0.13 M tris-buffer solution, pH 7.4 [8] with 0.001 M silver nitrate solution, using a vibrating platinum electrode [7]. The active electrode was standardized relative to glutathione. The method of electron paramagnetic resonance was used to detect free radicals, the spectra being recorded on a type RÉ-1301 radio-spectrometer under standard conditions, and analyzed with respect to signal amplitude in relative units.

In the experiments of series I, tissue metabolism in the liver was studied in intact animals; in series II 30 min after the beginning of bleeding, i.e., immediately after its end (phase 1 of anemization); in series III 60 ± 10 min after the beginning of bleeding, with the appearance of agonal respiration (phase of 2 of anemization); in series IV 70 min after the beginning of bleeding, the animal receiving hyperbaric oxygenation

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TABLE 1. Changes in Tissue Respiration and Content of Free Sulfhydryl Groups and Free Radicals in the Liver of Albino Rats after Blood Loss and Hyperbaric Oxygenation (M ± m)

Animals studied		No. of animals	Tissue respiration: O_2 absorption (μliters/100 mg dry tissue)			Sulfhydryl groups		Free radicals	
			1st h	2nd h	total in 2 h	No. of animals	μmoles/100 mg lyophilized tissue	No. of animals	%
Intact		48	170±2	101±2	271±2	20	6,40±0,1	71	100
Anemization (blood loss, 2.8% of body weight)	Phase 1	20	211±1	129±2 $P<0,001$	340±3	20	7,00±0,13 $P<0,001$	20	123±2 $P<0,001$
	Phase 2 (agony)	20	155±3	70±2 $P<0,001$	225±3	22	7,60±0,15 $P<0,001$	16	149±5 $P<0,001$
	Anemization + hyperbaric oxygenation	21	200±2	127±4 $P<0,001$	327±4	20	6,60±0,1 $P>0,1$	18	117±3 $P<0,001$
Healthy (hyperbaric oxygenation; 2 atm, 40 min)		20	177±4	99±4 $P>0,1$	276±3	20	6,10±0,1 $P<0,05$	21	104±2 $P>0,1$

during the last 40 min of the period of anemization; in series V after healthy animals had been kept for 40 min at a raised oxygen pressure.

EXPERIMENTAL RESULTS

The results in Table 1 show that the intensity of cell respiration in the liver was increased only in phase 1 of anemization, to a rather greater degree in the second hour of the investigation (by 28%). However, in phase 2 of anemization (the stage of agony) the level of cell respiration was reduced by 17%. If hyperbaric oxygen therapy was given to the animals in a state of anemization, the intensity of oxygen assimilation remained high, despite the fact that the degree and duration of anemization were similar to those in the untreated animals.

The content of free sulfhydryl groups in the liver of the anemized animals was increased, more especially (by 19%) in phase 2 of anemization. Hyperbaric oxygenation of the exsanguinated animals was accompanied by restoration of the normal content of sulfhydryl groups. The content of free radicals in the liver was increased particularly sharply (by 49%) in phase 2 of general anemization. During hyperbaric oxygen therapy the level of paramagnetic particles in the liver tissue of the anemized animals was lower than in the agonal state, and remained within the characteristic limits of phase 1 of anemization.

It is important to note that more than two-thirds of the anemized animals treated with oxygen under increased pressure remained viable, and their motor activity (movement coordination, ability to walk) was restored, while all untreated animals died within 60 ± 10 min of the beginning of bleeding.

Hyperbaric oxygenation thus prevented the decrease in intensity of oxygen absorption, the increase in content of sulfhydryl groups, and the marked increase in level of free radicals observed in the liver during agony. The development of the agonal state in most anemized animals also was prevented by hyperbaric oxygen therapy.

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