CHANGES IN ATP AND 2,3-DIPHOSPHOGLYCERATE CONTENT IN ERYTHROCYTES OF RATS ADAPTED TO HYPOXIA

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UDC 612.111.1-06:612.273.2.017.1

The content of ATP and 2,3-diphosphoglycerate in the erythrocytes of rats is increased on the 30th and 60th days of adaptation of the animals to hypoxia in a pressure chamber. By modifying the affinity of hemoglobin for oxygen, these changes may play an important role in the improvement of the oxygen supply to the tissues.

KEY WORDS: hypoxia; erythrocytes; ATP; 1,3-diphosphoglycerate.

The writer previously [3] found stimulation of carbohydrate metabolism in the erythrocytes of rats during adaptation to hypoxia. In human erythrocytes the content of ATP and 2,3-diphosphoglycerate (2,3-DPG) rises during hypoxia and this correlates with the shift of the oxyhemoglobin dissociation curve to the right [5, 10].

It was decided to study the levels of these organic phosphates under the same experimental conditions and, consequently, to assess more fully the role of an increase in the rate of glycolysis in the erythrocytes of animals during their adaptation to hypoxia.

EXPERIMENTAL METHOD

Male albino rats weighing 150-200 g were adapted in a pressure chamber for 5 h daily, to a pressure equivalent to an altitude of between 2500 and 7600 m, with a daily increase of 500 m [1]. Tests were carried out on the 30th and 60th days of adaptation. Blood was taken from the rats after decapitation, washed three times with cold 0.9% NaCl solution, and the layer of leukocytes was discarded each time. The erythrocyte pellet (1 ml) was hemolyzed with 2 volumes of ice-cold distilled water. The hemoglobin concentra-

TABLE 1. Changes in ATP and 2,3-DPG in Rat Erythrocytes (M ± m)

Experimental conditions	ATP	DPG
	in μmoles/g Hb	in μmoles/g Hb
Intact animals	6,1±0,27 (n=11)	13,0±0,6 (n=16)
30th day of adaptation	8,5±0,8 (n=7) $P<0,001$	16,2±0,6 (n=12) $P<0,001$
60th day of adaptation	10,7±0,6 (n=8) $P<0,001$	17,2±0,8 (n=8) $P<0,001$

Laboratory for the Study of Resistance of the Organism, I. M. Sechenov Institute of Evolutionary Physiology and Biochemistry, Academy of Sciences of the USSR, Leningrad. (Presented by Academician of the Academy of Medical Sciences of the USSR P. N. Veselkin.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 81, No. 4, pp. 415-416, April, 1976. Original article submitted May 11, 1975.

tion (at 540 nm) was determined spectrophotometrically in 0.02 ml of the hemolysate. The proteins in the hemolysate were then precipitated by 2 ml of 1 N $HClO_4$. The ATP content was determined in one sample of supernatant by an enzymic method (using the ATP-Test combination, Boehringer, West Germany), and the 2,3-DPG content in the other [7]. The results of determination of 2,3-DPG by a nonenzymic method are in good agreement with those of the enzymic method [7, 9].

EXPERIMENTAL RESULTS AND DISCUSSION

The results are given in Table 1. They show that the ATP content in erythrocytes of rats adapted to hypoxia was increased on the 30th and 60th days by 39 and 75%, respectively, and the 2,3-DPG content was increased by 24 and 32%.

Erythrocytes of man and certain animals are known to have a high concentration of 2,3-DPG [6, 8], the functional role of which is very important In particular, it has been shown that 2,3-DPG sharply increases P_{50}. ATP has a similar but weaker action. It was shown previously that changes take place in erythropoiesis during adaptation to hypoxia [4]. Under these conditions erythrocytes with a higher rate of glycolysis than those of intact animals enter the blood stream [3]. The increase in the rate of glycolysis under such conditions, leading inevitably to an increase in the intracellular concentration of ATP and 2,3-DPG, can be assumed to facilitate the dissociation of oxyhemoglobin and thereby to increase the quantity of molecular oxygen transferred to the tissues. The reduction in the affinity of hemoglobin for oxygen under the influence of ATP and 2,3-DPG confirms Gimmerikh's view [2] that hemoglycolysis is a factor controlling the supply of oxygen by erythrocytes.

Activation of the rate of glycolysis and a consequent increase in the concentrations of ATP and 2,3-DPG in the erythrocytes of animals adapted to hypoxia is evidently one of the mechanisms of such adaptation.

LITERATURE CITED

1. Z. I. Barbashova, Acclimatization to Hypoxia and its Physiological Mechanisms [in Russian], Moscow – Leningrad (1960).
2. F. I. Gimmerikh, The Regulation of the Supply of Oxygen by the Blood [in Russian], Frunze (1960).
3. L. N. Simanovskii, Vopr. Med. Khim., No. 3, 227 (1971).
4. T. V. Tavrovskaya, O. I. Tarakanova, and Z. I. Barbashova, Byull. Éksp. Biol. Med., No. 1, 18 (1975).
5. G. J. Brewer and J. W. Eaton, Science, 171, 1205 (1971).
6. H. F. Bunn, Science, 172, 1049 (1971).
7. B. J. Dyce and S. P. Bessman, Arch. Environm. Health, 27, 112 (1973).
8. I. Greenwald, J. Biol. Chem., 63, 339 (1925).
9. M. E. Miller, C. G. Zaroulis, C. R. Valeri, et al., Blood, 43, 49 (1974).
10. D. Zaoui, E. Bursaux, C. Dubos, et al., Anesth. Analg. Reanim., 28, 223 (1971).