THE PHOSPHORUS FRACTIONS OF MUSCLE DURING HYPOXIA

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The explanation of the biochemical changes taking place in an animal during oxygen lack is of great importance. In the greater part of the research undertaken on this subject, the changes occurring in the blood and urine have been investigated, and it is only comparatively recently that the study has begun of the biochemical changes in the organs and tissues of the animal deficient in oxygen.

We studied the changes in the phosphorus fractions of skeletal muscle during oxygen lack induced by keeping the animal in a low barometric pressure of air.

EXPERIMENTAL METHOD

Experiments were carried out on male white rats weighing 220-260 g kept on an ordinary diet and in approximately the same conditions. The animals were taken for the experiments in the morning, in a fasting state. The rat was placed in a pressure chamber with influx-extraction ventilation. In the course of 5 minutes the barometric pressure in the chamber fell from its normal level to 190 mm Hg, corresponding to a "height" of approximately 10,000 m. The rat was kept in such a rarefied atmosphere for one hour; the barometric pressure was then restored to its original level in the course of 2 minutes, the animal was quickly taken from the pressure chamber and killed by exsanguination under ether anesthesia. Pieces of the thigh muscles were taken and immediately frozen in liquid oxygen. Control rats were killed in precisely the same way and at the same time.

The frozen muscle was ground in a mortar with the addition of liquid oxygen and weighed samples of the frozen muscle powder were taken for estimation of the following fractions:

1) total phosphorus (after mineralization with H_2SO_4 with addition of H_2O_2);

2) lipoid phosphorus (after mineralization of lipids extracted with Bloor's mixture);

3) creatine phosphate by A. M. Alekseeva's method (estimation as creatine; result calculated in terms of creatine phosphate phosphorus);

4) acid-soluble phosphorus; for this test the muscle powder was added to a 4% solution of CCl_3COOH and, after extraction for 24 hours, a certain proportion of the extract was mineralized with H_2SO_4 and H_2O_2. The inorganic phosphorus content of another sample of this extract was estimated, representing the total preformed inorganic phosphorus and the creatine phosphate phosphorus.

In every case (apart from the estimation of creatine phosphate as creatine) the final step was the colorimetric estimation of the inorganic phosphorus by the Fiske-Subbarow method.

The results were calculated as mg% per known weight of frozen muscle powder. In practice, this was identical with the concentration in fresh muscle; although the mean value of the dry residue of frozen muscle powder was slightly greater than the mean value of the dry residue of the fresh muscle, this difference was not statistically significant.

Phosphorus Fractions of Skeletal Muscle of White Rats, Normal (Control) and Exposed for One Hour to the Action of a Low Barometric Pressure (Experiment)

Phosphorus fractions	Control		Experiment		Dif. between expt. and control	$t = \frac{M_1 - M_2}{\sqrt{m_1^2 + m_2^2}}$ evaluation of significance
	M	m	M	m		
	mg %					
Total phosphorus	244.5	±1.07	236.0	±1.8	−8.5	4,1
Lipoid phosphorus	46.4	±0.48	48.6	±0.57	+2.2	2,96
Acid-soluble phosphorus	184.2	±1.11	177.1	±1.15	−7.1	4,4
Inorganic phosphorus + creatine phosphate phosphorus	90.0	±0.84	80.5	±0.93	−9.5	7,6
Creating phosphate phosphorus	57.0	±0.6	47.9	±0.88	−9.1	8,6
Dry residue of muscle (in %)	23.63	±0.12	23.74	±0.16	+0.11	0,56
Dry residue of muscle powder (in %)	24.19	±0.37	24.34	±0.37	+0.15	0,28

Altogether 42 control rats and 42 rats exposed for one hour to the action of a lowered barometric pressure were investigated. The degree of rarefaction in the experiments was almost at the limit beyond which rats would die (according to data in the literature rats begin to die at rarefactions corresponding to a height of roughly 10,000 to 11,000 m [4]). No rat died in the pressure chamber in our experiments. During their stay in the pressure chamber at 190 mm Hg for one hour the animals were usually quiet, sitting or lying down and only occasionally changing their position; marked acrocyanosis was observed; respiration was usually deep and rhythmic, at a rate of 120-160 per minute (in some rats the respiration fell to 70-80 per minute).

All analysis were made separately for the muscles of the right and left thighs (no regular difference was found between them under these circumstances). Both results were discarded if the difference between them exceeded 10%; in the remaining cases the mean value of the results of the parallel experiments was calculated and the mean values thus obtained were analyzed statistically.

EXPERIMENTAL RESULTS

The results of our experiments are shown in the table.

It can be seen from the table that after the rat had remained for one hour in the pressure chamber at a "height" of about 10,000 m, the creatine phosphate, acid-soluble and total phosphorus contents of the skeletal muscle of the animal fell; the lipoid phosphorus of the skeletal muscle rose slightly, and as statistical treatment showed, this increase was not absolutely significant but very probably so.

It was interesting that both the creatine phosphate phosphorus and the sum of the creatine phosphate phosphorus and the preformed inorganic phosphorus fell in our experimental conditions by the same amount; hence that the content of preformed inorganic phosphorus in the muscle did not change during the anoxia. In fact the concentration of preformed inorganic phosphorus (calculated by the difference between the inorganic phosphorus of a 24-hour trichloroacetic acid filtrate and the creatine phosphate phosphorus) in the skeletal muscle of normal rats was on the average 33 mg%, with a mean error of ± 0.73 mg%, and in the experimental group of rats it was on the average 32.6 mg% with a mean error of ± 0.72 mg%, i.e., it did not differ from the controls. The value obtained for the preformed inorganic phosphorus was high, since in the course of extraction of the muscle tissue with trichloroacetic acid solution for 24 hours a part of the labile phosphoric esters was hydrolyzed, with the formation of inorganic phosphate. In order, therefore, to provide stricter confirmation of the correctness of the conclusion that the true inorganic phosphorus content of the muscles in the conditions of our experiment was unchanged, we carried out further analogous experiments on 5 normal rats and on 5 rats kept for one hour in the pressure chamber at a "height" of about 10,000 m. In these experiments the inorganic phosphorus of the trichloroacetic acid extract of the muscle was determined after extraction for one and for 24 hours. The difference was

very constant and amounted for normal rats to 7.4 mg% (mean value of 10 determinations), and for the rats of the experimental group, 7.2 mg% (mean of 9 determinations).

It may thus be concluded that the content of creatine phosphate, acid-soluble and total phosphorus in the skeletal muscle of rats fell during anoxia, whereas the concentration of true inorganic phosphorus was unchanged. These changes were not the result of the redistribution of water in the body, since the percentage content of water in the muscle in our experimental conditions was unchanged.

We believe that the results obtained maybe explained in the following way. A fall in the total and acid-soluble phosphorus in skeletal muscle during anoxia evidently took place in consequence of a fall in the level of creatine phosphate, which itself arose as the result of the predominance of decomposition of high-energy phosphorus compounds over their resynthesis. The liberated inorganic phosphate could not be fully utilized for rephosphorylation, since oxidation phosphorylation in conditions of hypoxia is limited. No increase took place, however, in the content of inorganic phosphate in the muscle, and presumably the excess phosphate which develcped was transferred to the blood stream and, possibly, excreted from the body.

There are, in fact, indications in the literature of an increase in the inorganic phosphate in the blood in anoxia, and of the increased excretion of phosphate in the urine of an animal kept under hypoxic conditions [1-3].

I regard it my duty to express my gratitude to Prof. Yu. M. Gefter for his guidance and help in the execution of this work.

SUMMARY

The author studied 42 normal and 42 rats subjected to lowered barometric air pressure (190 mm Hg.) for the period of one hour. Under these conditions the content of creatinphosphate, acid-soluble and total phosphorus is decreased in the skeletal muscle of rats, while concentration of preformed inorganic phosphorus remains unchanged. The lipoidal phosphorus content somewhat increases, but this rise is not statistically confirmed, though quite probable.

LITERATURE CITED

[1] G. E. Vladimirov, I. M. Dedyulin, N. A. Kudryavtsev et al., Transactions of the S. M. Kirov Military Medical Academy, vol. 16, pp. 71-92, Leningrad, 1938 [In Russian].

[2] I. I. Gorelov, Fiziol. Zhur. SSSR, 27, 4, 490-498 (1939).

[3] I. M. Dedyulin, The Acid-Base Balance and the Respiratory Function of the Blood in Man in Rarefied Atmospheric Conditions, Leningrad, 1941 [In Russian].

[4] N. N. Sirotinin, cited by N. V. Lauer, Hypoxia, pp. 84-86, Kiev, 1949 [In Russian].

[5] T. B. Fetisova, Problems of the Biochemistry of Muscle, pp. 97-112, Kiev, 1954 [In Russian].

[6] G. Marcotte-Boy, J. Cheymol., Compt. rend. soc. biol., 1950, v. 144, p. 630-632.