EFFECT OF LONG-TERM EXPOSURE TO WEIGHTLESSNESS ON SKELETAL MUSCLE DEHYDROGENASE ACTIVITY IN RATS

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The study of tissue respiration of rat skeletal muscle preparations during the first day after 18-20-day flights on manned satellites of the "Kosmos" series revealed inhibition of oxidative and phosphorylating activity and of the final stage of the oxidative chain, namely cytochrome oxidase [3]. However, the involvement of earlier stages of substrate oxidation in the development of neuromuscular atrophy in response to exposure to weightlessness remains an unsolved problem [10].

The aim of this investigation was to study activity of dehydrogenases involved in the Krebs' cycle: NAD-dependent malate dehydrogenase (MDH) and NADP-dependent isocitrate dehydrogenase (ICDH) in mitochondrial and cytoplasmic fractions, and also lactate dehydrogenase in the skeletal muscles of rats after an 18.5-day space flight on the "Kosmos-1129" manned satellite, and also during stimulation of the effect of weightlessness in rats exposed to hypokinesia for 20 days.

EXPERIMENTAL METHOD

The conditions under which the animals were kept, the method of treatment of the skeletal muscles and isolation of tissue preparations during in-flight experiments, and the characteristics of the groups were all fully described previously [4]. Hypokinesia of the rats was created by the standard method [2]. In each group of the investigations 6-10 rats took part. Enzyme activity was determined spectrophotometrically [7, 11] and protein by Lowry's method [8]. The results were subjected to statistical analysis by Student's t test.

EXPERIMENTAL RESULTS

The results obtained with rats after space flight on the "Kosmos-1129" manned satellites are given in Fig. 1. Clearly during the first day after the flight (F_1) activity of the various enzymes studied in the mitochondria was reduced by 1.5-2 times compared with the control (K_1), suggesting an inadequate supply of reducing equivalents from the Krebs' cycle to the mitochondrial respiratory chain. MDH and ICDH activity in the cytoplasm also was reduced, but by a lesser degree (by 50-70%). The intensity of glycolysis, to judge from LDH, was lower than in the control group. There are two possible explanations of this phenomenon: either changes taking place during the flight and still present at the time of landing, or changes arising as a result of relative hypergravitation after landing.

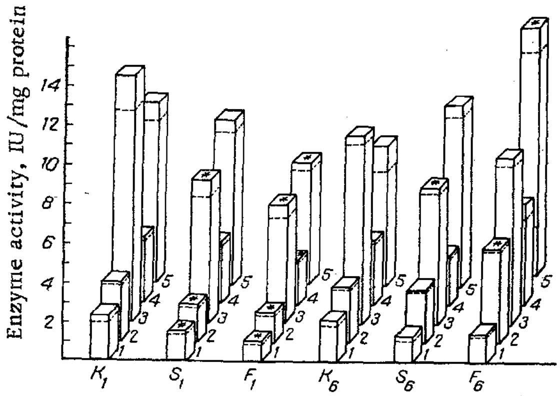

Fig. 1. Dehydrogenase activity in skeletal muscles of rats 1 and 6 days after space flight. Horizontal axis — experimental groups (explanation in text). Broken line — standard error of mean. Asterisk indicates significant difference from control group; $p < 0.05$. 1) Mitochondrial ICDH; 2) cytoplasmic ICDH; 3) mitochondrial MDH; 4) cytoplasmic MDH; 5) LDH.

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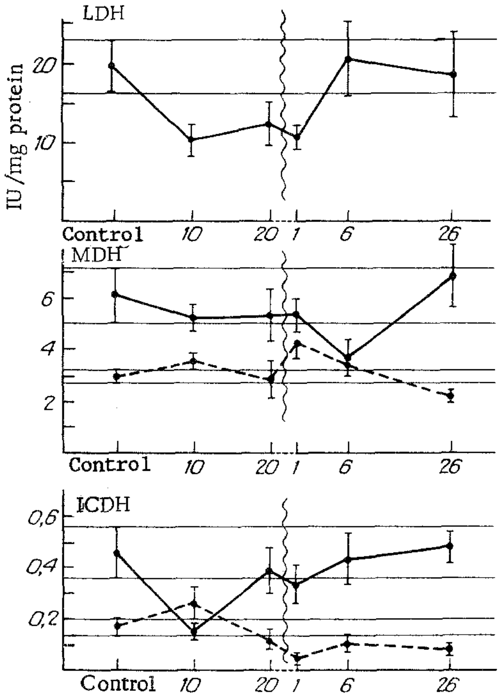

Fig. 2. Dehydrogenase activity in rat skeletal muscles during hypokinesia and subsequent readaptation. Abscissa, days of hypokinesia (10, 20) and of adaptation periods (1, 6, 26). Continuous line — enzyme activity in cytoplasm, broken line — in mitochondria, continuous thin line — standard error of the mean for control group.

In a synchronized experiment (S_1), in which the animals were exposed to the action of all factors of space flight except weightlessness, mitochondrial MDH and ICDH activity also was significantly depressed, although not so considerably as in the flight series. In the cytoplasm, however, MDH, ICDH, and LDH activity has only a tendency to fall. This indicates that reduction of dehydrogenase activity on the first day is not a specific response of the muscle cells to weightlessness, but the latter aggrevates the influence of space flight factors.

Investigations conducted on the 6th day of the readaptation period shows that MDH and ICDH activity in the mitochondria of the skeletal muscles of the flight group (F_6) was restored up to the control level. Activity of the test enzymes including LDH in the cytoplasm was increased by 1.5-2 times. A synchronous experiment (S_6) revealed no significant differences from the control (K_6), suggesting that weightlessness has a specific action on cytoplasmic dehydrogenases, which is detectable on the 6th day of the recovery period.

Lowering of activity of mitochondrial MDH and ICDH immediately after landing indicates a lowered level of mitochondrial function, expressed as a decrease in the rate of turnover of oxidation substrates and of NADH formation in the Krebs' cycle. This may be one of the causes of reduced O_2 consumption by tissue preparations found in the same rats in the skeletal muscles of the posterior femoral group [4]. Subsequent restoration of enzyme activity in the mitochondria on the 6th day is evidence that the inhibition of substrate oxidation at the stage of the tricarboxylic acid cycle is reversible. However, as has been shown, the necessary level of intensity of tissue respiration and of oxidative phosphorylation does not take place at these times [4]. Evidently the intensity of substrate oxidation through glycolysis increased, as shown by increased LDH activity.

Cytoplasmic forms of MDH and ICDH in the cell take part of operation of the so-called shuttle mechanism, by which reducing equivalents are transferred from the intramitochondrial NAD pool into the cytoplasmic pool without transfer of NADH molecules, which cannot pass through the mitochondrial membrane. The NADPH concentration in the cytoplasm is maintained in a similar way [6].

Inhibition of cytoplasmic enzymes on the first day after landing can be explained as a response to the depressed formation of reducing equivalents in the mitochondria. The marked increase in the intensity of activity of these enzymes on the 6th day is evidence of intensification of biosynthesis in the muscle cells [9], for which a rapid inflow of NADH and NADPH from the mitochondria is required.

An experiment involving 20 days of hypokinesia, which reproduces certain of the effects of weightlessness on the muscular system, and subsequent readaptation was next carried out.

It was shown previously that hypokinesia in rats of varied duration leads to considerable changes in dehydrogenase activity in the skeletal muscles [1]. The results of the investigations are illustrated in Fig. 2. Clearly toward the end of the 20th day of hypokinesia there was only a tendency for mitochondrial dehydrogenase activity to fall. On the 1st day of readaptation this tendency was realized only in the case of ICDH, activity of which was significantly depressed, whereas MDH activity increased.

ICDH activity in the cytoplasm was significantly reduced on the 10th day of hypokinesia, and MDH activity on the 6th day of readaptation period. LDH activity, which was reduced by half by the 10th day of hypokinesia, still remained low until the 1st day of readaptation inclusive. After 26 days of readaptation activity of all the enzymes studied was within the control limits. Thus hypokinesia lasting 20 days does not reproduce all the effects of weightlessness in the period of readaptation after space flight of the same duration.

The trend of the changes in rats with hypokinesia and animals of the synchronous group of the in-flight experiment was of the same character as in the control. The main cause of the changes observed in these two groups was restriction of movement. This is an invariable, but not the only component of the weightlessness factor. Under space flight conditions, it is evident that the redistribution of blood, changes in afferentiation, and more marked changes in the water-electrolyte balance and, in particular, in Ca^{++} metabolism [5], have an effect on the test system which is not weaker than that of hypokinesia.

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