CHANGES IN PHOSPHOFRUCTOKINASE ACTIVITY IN THE TISSUES OF RABBITS IN VARIOUS STAGES OF FEVER

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Disturbances of carbohydrate metabolism have been described in fevers. Hyperglycemia and glycosuria [8], an increase in the blood lactic acid concentration [11], and a decrease in the glycogen content of the liver [2] have been reported. So far as the changes in the activity of the enzymes of carbohydrate-phosphorus metabolism are concerned, this subject is dealt with inadequately in the literature [1,10].

Particular interest is centered on the investigation of the least "powerful" enzyme links. It is assumed that in various pathological states of the organism, it is these "narrow" enzyme links which are the first to be broken [6]. According to information in the literature, one such enzyme concerned in the processes of glycogenolysis is phosphofructokinase (PFK)* [3].

Data relating to the changes in PFK activity in the skeletal muscles and myocardium of rabbits in various stages of pyrogenal fever are described in this paper.

EXPERIMENTAL METHOD

Experiments were conducted on rabbits weighing 2.5-3.5 kg. Fever was produced by the intravenous injection of pyrogenal, a highly purified lipopolysaccharide, free from toxic impurities (preparation obtained from Kh. Kh. Planel'es, of the Gamaleya Institute of Epidemiology and Microbiology). The pyrogenal was injected in a dose of 10 μg/kg body weight. The temperature was measured in the rectum.

The tissues to be investigated were frozen and ground in liquid oxygen. The PFK activity was determined by a colorimetric method [13], slightly modified by the author in conjunction with A. I. Kulikova.

The tissues were extracted with K-phosphate buffer (0.1 M, pH 7.8) for 1 h. The optimal proportions discovered for the weight of the tissue sample and the weight of the extracting substance were 1:30 for the skeletal muscles and 1:20 for the heart muscle. The extract obtained was added in a volume of 0.1 ml to an incubation medium of the following composition: 0.2 ml fructose-6-phosphate (0.058 M), 0.1 ml ATP (0.04 M), 0.8 ml collidine buffer of pH 7.4-7.6 (0.1 M), 0.1 ml $MgCl_2$ (0.15 M), 0.1 ml KF (0.05 M), and 0.1 ml hydrazine sulfate (0.56 M). The mixture was incubated for 15 min at 37°. The enzyme reaction was stopped by the addition of 1.5 ml of 10% trichloroacetic acid. The phosphotrioses formed were determined in the filtrate by the method of Sibley and Lehninger [16], as modified by Bruns [9]. Standardization of the trioses was carried out by means of dihydroxyacetone [12].

The PFK activity was expressed in units (decrease in fructose-6-phosphate in μmoles/mg protein in the sample per min of incubation). The protein was determined by the biuret method [7].

Altogether, five groups of animals were investigated: group 1 — control rabbits; group 2 — rabbits receiving injections of pyrogenal and their PFK activity investigated in the stage of elevation of the temperature (40-50 min from the time of injection of pyrogenal); group 3 — animals in which the PFK activity was determined at the stage of maximal pyrexia, i.e. 2 h after injection of pyrogenal; group 4 — rabbits in which the PFK activity was investigated in the stage of lowering of the temperature, i.e., 5-6 h after the pyrogenal injection; and group 5 — animals investigated 24 h after the pyrogenal injection.

*The systematic name of the enzyme in accordance with the "Classification and nomenclature of Enzymes" (1962) is ATP: D-fructoso-6-phosphate-1-phosphotransferase.

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Phosphofructokinase Activity (as Loss of Fructose-6-Phosphate in μmoles/mg Protein in Sample per Minute of Incubation) in Skeletal Muscles and Heart at Different Stages of Fever (M ± m)

Tissue	No. of experiments	Group 1 (control)	No. of experiments	Group 2 (45-50 min after injection of pyrogenal)	No. of experiments	Group 3 (2 h after injection of pyrogenal)	No. of experiments	Group 4 (6 h after injection of pyrogenal)	No. of experiments	Group 5 (24 h after injection of pyrogenal)
Muscles	14	1.720±0.110	7	2.040±0.047 P < 0.05	9	1.290±0.063 P < 0.01	6	1.810±0.080 P < 0.6	8	1.710±0.120 P < 0.9
Heart	12	0.420±0.016	7	0.438±0.022 P < 0.6	9	0.323±0.021 P < 0.01	6	0.452±0.043 P < 0.6	8	0.509±0.012 P < 0.01

EXPERIMENTAL RESULTS

Between 45 and 50 min after the injection of pyrogenal (group 2) the temperature was elevated by 1.0-1.2°. The table shows that the PFK activity of the skeletal muscles was increased by 18.6%, while the rate of the phosphofructokinase reaction in the heart muscle remained unchanged.

The next group (3) of rabbits was studied at the stage of the highest rise of temperature. Two h after injection of pyrogenal, the temperature showed its final increase, when it was raised on the average by 2.0-2.1°. The PFK activity in the skeletal and cardiac muscles of these animals was lowered on the average by 23-25%.

In the rabbits of group 4, the temperature 6 h after injection of the pyrogenal remained raised by 0.7°. The velocity of the phosphofructokinase reaction in the muscles and heart was the same as in the control animals.

The animals of group 5 were investigated 24 h after injection of the pyrogenal and their temperature was then normal. It is interesting that the PFK activity in the heart was raised by 21.2%, while in the skeletal muscles it was unchanged.

The results of these investigations thus showed that the PFK activity in the skeletal muscles was increased in the first stage of the febrile reaction (45-50 min after injection of pyrogenal). This increase was evidently associated with an increase in the concentration of inorganic phosphorus in the tissues, as is suggested by data [5] indicating an increased rate of penetration of phosphorus from the blood into the muscles in pyrogenal pyrexia. Phosphorus is known to activate the PFK reaction [15] and to protect it from the depressant action of ATP [14]. At the stage of maximal elevation of the temperature, the inhibition of the activity of the muscle PFK was evidently related to the accumulation of ATP in this tissue on account of an increase in the intensity of oxidative phosphorylation [4]. Probably at this stage of fever, the phosphorus could no longer protect the PFK from the inhibitory action of the excess of ATP.

Depression of the PFK activity in the heart muscle at the stage of the maximal elevation of the temperature may also have been due to the intensification of oxidative phosphorylation and to accumulation of ATP.

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All abbreviations of periodicals in the above bibliography are letter-by-letter transliterations of the abbreviations as given in the original Russian journal. *Some or all of this periodical literature may well be available in English translation.* A complete list of the cover-to-cover English translations appears at the back of the first issue of this year.
