THE EFFECT OF EXERCISE ON THE DISTRIBUTION OF GLYCOGEN AND PROTEINS IN THE CARDIAC MUSCLE OF WHITE RATS

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The determination of glycogen in the myocardium of animals during exercise has been carried out almost exclusively by biochemical methods, and results have been contradictory.

Some authors have found that the effect of increased exercise produces no appreciable change in the amount of glycogen. Consequently the idea developed that glycogen formation in the heart was a stable process [1, 6], and that the heart differed in this respect from skeletal muscle in which many authors [4, 8] found a marked decrease of glycogen during muscular work. Other investigators [9, 12] found a considerable drop of myocardial glycogen during exercise, and a marked reduction in the readily extracted labile fraction, whereas the fraction of glycogen bound to protein and resistant to extraction was more stable [9].

Scarcely any histochemical work has been done on the distribution of glycogen in the myocardium of fatigued animals. According to I. A. Tatishvili [5], who determined glycogen by staining with carmine by Best's method, there was no change of the myocardial glycogen.

But little work has been done to determine protein myocardial changes resulting from extreme fatigue. A. A. Darialashvili [3] found some changes of the functional protein groups, and observed that after increased physical work from swimming there was a very small increase in the myocardium of sulfhydryl groups bound to protein.

The object of the present investigation has been to determine histochemically the glycogen, SH-protein groups, and the amounts of histidine, tyrosine, and tryptophane in the myocardium of white rats due to increased physical work.

METHOD

The animals were made to swim for 30 - 60 minutes, and to run in a treadmill for 20 minutes, to complete exhaustion, after which they were immediately killed.

Transverse sections were cut through all the parts of the heart, and were fixed by Shabadash and Carnoy's method and embedded in paraffin. Some of the tissue was fixed in a 10% neutral formol solution, and cut on a freezing microtome. Glycogen was determined by Shabadash's method. Control experiments were carried out by first treating the sections with saliva, or with a 0.1% solution of crystalline α- and β-amylase at 37° for times ranging from 10 min to 2 hr. The sulfhydryl groups were estimated by the method of Yakovlev and Nistratova [8] using nitrobromoacetophenone. Amino acids were determined by Danielli's tetrazone reaction [10], which identified simultaneously the three amino acids: histidine, tryptophane, and tyrosine. The sections were stained in hematoxylin-eosin, in Heidenhain's iron hematoxylin, or in Sudan III.

RESULTS

The control experiments showed that in unfatigued rats there was a large amount of glycogen in the myocardium. It was concentrated in the muscle fibers chiefly in the form of large granules distributed as a half-moon in one part of the fiber, while small granules diffusely filled the whole thickness of the fiber. The glycogen granules were found in the sarcoplasm of the muscle fibers, and some were found in the myofibrils along the cross striations. The large granules were found mostly in the muscle fibers of the external layer of the myocardium of the left and

right ventricles, while the fibers of the internal layers of the ventricles contained small diffusely distributed glycogen grains (Fig. 1). The internal parts of the myocardium were always considerably richer in glycogen than the external.

When the sections were incubated with filtered saliva, or with α- and β-amylase, the large glycogen granules disappeared in the first 10 minutes of incubation, but the small veins required 2 hours of incubation to disintegrate. From a large number of biochemical studies [2, 12] it appears that the small grains which are more resistant to the lysis of the glycogen by amylase belong to the polysaccharide fraction firmly bound to protein, which is difficult to extract. This glycogen is diffusely distributed throughout the muscle fibers of the internal layers of the myocardium.

A study of the sulfhydryl groups of the muscle fibers of the myocardium has shown that the greatest amount of these biologically active functional groups occurs in the myofibrils, and that in the muscle fibers of the internal layers of the myocardium there is a higher concentration of SH-groups. In the sarcoplasm of the muscle fibers the amount of sulfhydryl groups is very small. Similar relationships are observed when determining the amounts of histidine, tryptophane, and tyrosine; the greatest amount was found in the proteins of the myofibrils, whereas in the muscle fibers of the internal layers of the myocardium, the tetrazone reaction was the most intense.

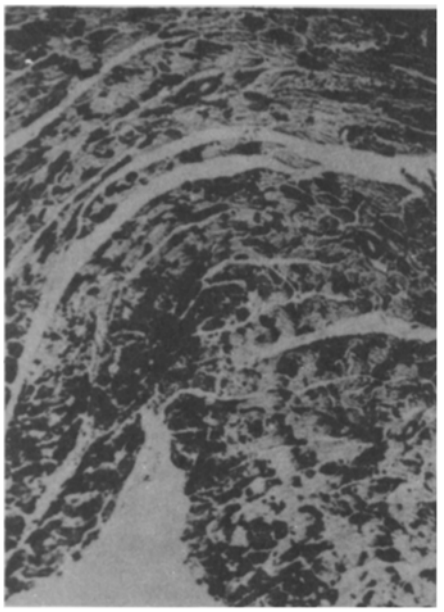

Fig. 1. Large amount of glycogen in the myocardium of the left ventricle of a control rat. PAS reaction. Magnification 80×.

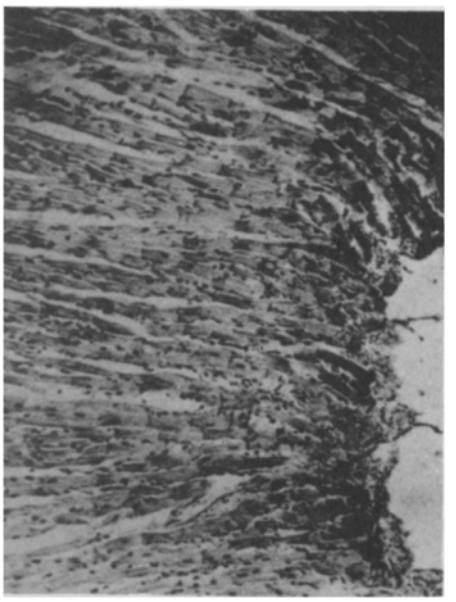

Fig. 2. Almost complete absence of glycogen in the myocardium of the left ventricle of a rat, after fatigue caused by running for 20 minutes in a treadmill. PAS reaction. Magnification 80×.

Staining with hematoxylin-eosin or iron hematoxylin showed a swelling of some of the groups of muscle fibers of the internal myocardial layers and a loss of their cross striation. These fibers stained more intensely with eosin. When comparing the sections stained by different methods, it was found that it was just these fibers which showed a high content of the small glycogen granules, sulfhydryl groups, and amino acids (histidine, tryptophane, and tyrosine).

After a single period of increased muscular work, glycogen content of the myocardium was reduced greatly below the level in the control animals, and it disappeared almost entirely from the muscle fibers into the outer mycocardial layers of both ventricles (Fig. 2). In the internal myocardial layers there was also a considerable reduction, although some glycogen was preserved, particularly in those fibers where it was diffusely distributed and less accessible to enzymatic action.

There is therefore reason to suppose that the glycogen firmly bound to protein and concentrated in the internal myocardial layers is more resistant to glycogenolysis occurring in the myocardium during increased muscular work than are the large grains of glycogen, which are evidently more labile.

In studying the sulfhydryl groups and amino acids in the myocardial proteins of fatigued animals, we found no particular difference in their concentration or distribution from that in the controls. Neither was there any morphological change in the myocardial structure under these circumstances.

Thus, from this work we may claim to have established that physical work taken to the point of fatigue causes the disappearance of glycogen from quite a large area of the cardiac muscle fibers, and that at the same time the concentration of SH-groups of proteins and of the amino acids of proteins, as revealed by the tetrazone method, shows no appreciable change.

SUMMARY

The tetrazone method shows that in normal conditions the internal myocardial layers contain far more glycogen, SH-protein groups and proteins than do the external layers. In the inner layers, the glycogen is diffusely distributed in the form of small granules, whereas in the external layers it is found in the form of large granules. The effect of physical work is to reduce considerably the glycogen content of all parts, especially the external layers. The large glycogen granules are the first to disintegrate. There was no significant change in the protein composition of the cardiac muscle.

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