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ULTRASTRUCTURAL MANIFESTATION OF EARLY METABOLIC DISTURBANCES IN THE MYOCARDIUM OF DOGS WITH ALLOXAN DIABETES

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Metabolic changes in the myocardium play an important role in the development of cardiovascular lesions accompanying diabetes. The study of the state of the subcellular structures of the myocardium in the initial period of development of the disease is of great importance for clarification of the pathogenesis of these lesions and for the search for methods of rational treatment.

In the investigation described below the ultrastructure of the myocardium was studied in dogs after production of alloxan diabetes.

EXPERIMENTAL METHOD

Altogether 12 experimental dogs (with diabetes) and nine control dogs of both sexes weighing from 12.5 to 32.5 kg were used. Alloxan diabetes was induced by the method described in [1]. Observations began 1 month after the animals had developed diabetes. In blood taken from the aorta and coronary sinus of the control and experimental animals the insulin concentration was determined by a radioimmune method, glucose by the orthotoluidine method, nonesterified fatty acids (NEFA) by Duncombe's method [5], β-lipoproteins after Ledvina [4], and ketone bodies after Natelson [11]; in the myocardial tissue from the control and experimental animals activity of the following enzymes was determined: hexokinase [14] and phosphorylase [9]. Respiration coupled with oxidative phosphorylation was determined polarographically in the mitochondrial fraction isolated from the myocardium by differential centrifugation. Pieces of tissue were taken for electron microscopy from the subendocardial zones of the left ventricles. The material was processed in the usual way. Parallel studies were made of semithin sections from the same blocks, stained by McManus' method for the simultaneous detection of lipid and glycogen inclusions in the heart muscle cells.

EXPERIMENTAL RESULTS

With the model of alloxan diabetes used it was possible to produce diabetes of average and severe degrees in all the experimental dogs, with marked clinical manifestations. The

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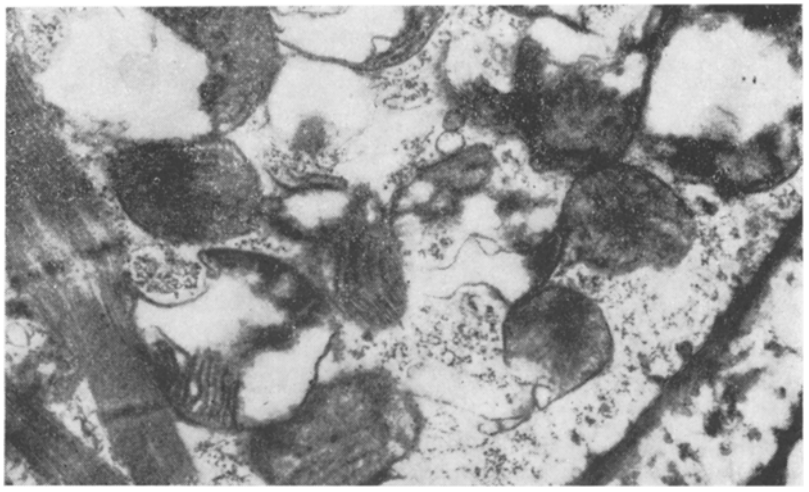

Fig. 1. Destructive changes in mitochondria in a cardiomyocyte of a dog with alloxan diabetes. 30,000 ×.

TABLE 1. Myocardial Extraction of Carbohydrate and Lipid Substrates in Normal and Diabetic Dogs (M ± m)

Biochemical index of blood	Control	Diabetes	P
Glucose, mg/100 ml	5,24±1,75	—15,7±4,9	0,001
NEFA, μeq/100 ml	3,57±1,27	13,87±2,96	0,01
Ketone bodies, mg/100 ml	0,036±0,014	1,625±0,291	0,001
β-lipoproteins, mg/100 ml	10,4±2,6	20,2±3,5	0,05

TABLE 2. Enzyme Activity in Myocardium of Dogs with Diabetes, in Units of Activity/mg Protein

Animals	Hexokinase	Hydrolytic phosphorylase	Total phosphorylase
Control	6,3±0,2	0,4±0,03	2,94±0,15
With diabetes	2,91±0,3	3,6±0,09	0,6±0,04
P	<0,001	<0,001	<0,001

blood insulin level was lowered to 160.8 ± 68.5 microunits/100 ml blood. On the 30th-35th day of the disease, despite a significant rise in the arterial blood glucose level, extraction of glucose by the myocardium was completely suppressed. Meanwhile the assimilation of substrates of the lipid series by the heart muscle from the blood was considerably increased (Table 1), evidence of a switch by the heart to utilization of lipid metabolites.

In the mitochondrial fraction an increase in free oxidation was observed as a result of a disturbance of coupling of the mitochondrial membranes, which was reflected in lowering of the respiratory coefficients in the experimental animals to 1.14 ± 0.02 (normal 2.32 ± 0.23). This may be due to an increase in the concentration of fatty acids in the cardiomyocytes [12]. The decrease in functional activity of the mitochondria was accompanied by changes in their ultrastructural organization. Electron microscopy revealed a decrease in the number of cristae, lysis of the outer and inner membranes, translucency of the matrix, and homogenization and vacuolation of the mitochondria (Fig. 1).

The increased inflow of substrates of the lipid series into the cardiomyocytes and interference with their use as a source of energy on account of the above-mentioned structural and

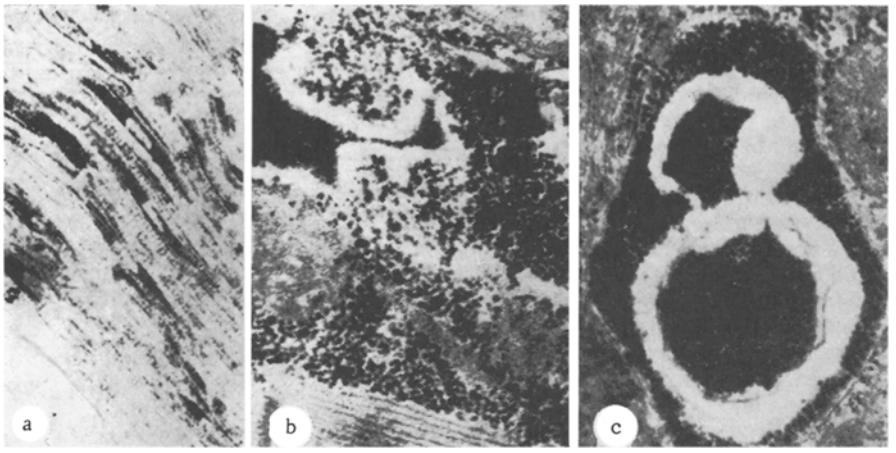

Fig. 2. Accumulation and resorption of glycogen in cardiomyocytes of dog with alloxan diabetes: a) semithin section, PAS reaction for glycogen, 250 ×; b) accumulation of glycogen in peripheral zone of a cardiomyocyte. Ultrathin section, 15,000 ×; c) resorption of glycogen inside a glycogenosome. Ultrathin section 25.000 ×.

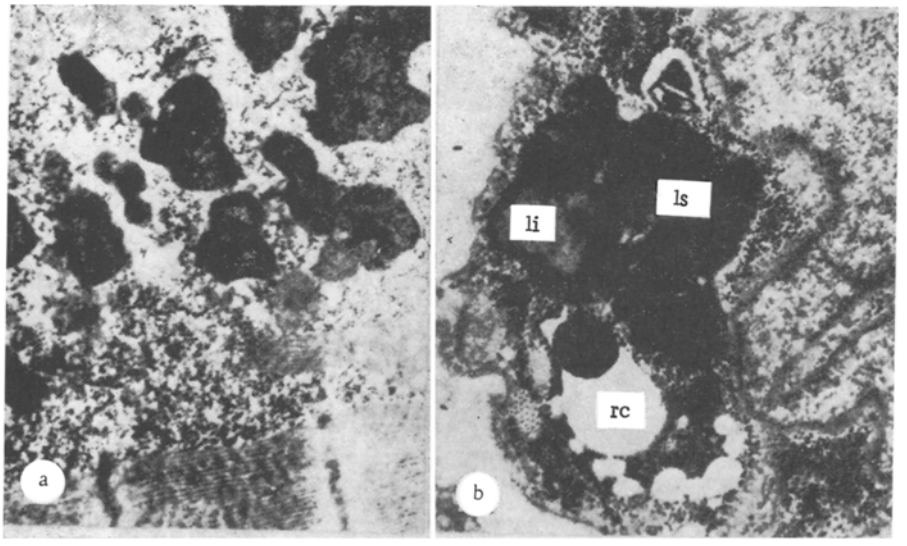

Fig. 3. Lysosomes in a cardiomyocyte: a) accumulation of lysosomes and glycogen in interfibrillary sarcoplasm; b) lysosome (ls), lipid inclusion (li), and resorption cavities (rc) in perinuclear zone of glycogen accumulation.

functional changes in the mitochondria did not, however, lead to increased deposition of lipids in the cytoplasm. Lipid inclusions in the cardiomyocytes of the experimental dogs, just as in the control animals, were solitary and, as a rule, were resorbed. Meanwhile, absence of utilization of glucose, the main precursor of glycogen, by the myocardium led to a much higher content of glycogen in the heart muscle fibers in diabetic animals than in the controls. Clusters of glycogen granules appeared between the myofibrils and myofilaments, beneath the sarcolemma, and around and inside the nuclei. Often they formed "glycogenosomes," bounded by a single membrane (Fig. 2). Glycogen was concentrated not only in muscle cells, but also in others: endothelial cells, pericytes, nerve endings, and even in the intercellular spaces. Other workers [13] have also drawn attention to the increased glycogen content in cells of different organs and tissues, including the heart, in diabetes, but the reason for this phenomenon has not been explained. According to Dagaeva [2], despite an increase in the glycogen content in the myocardium of rabbits with diabetes, the intensity of incorporation of glucose-^{14}C into it is sharply reduced. Interference with glycogen synthesis from glucose in the heart in the presence of insulin deficiency also was reflected in the observed decrease in hexokinase activity (Table 2). Under these conditions the main pathway for glyco-

gen formation in the myocardium in diabetes may be glyconeogenesis on account of an excess of lipid substrates entering the myocardial cells from the blood stream.

The possibility of transformation of lipids into glycogen in diabetes was mentioned as early as 1946 by Laykey [8], who observed direct correlation between the glycogen content in rat heart muscle and the level of ketone bodies in the blood. Conversion of fatty acids into liver glycogen was demonstrated by Lorber, et al. [10] with the aid of radioactive carbon. However, data on glyconeogenesis from lipids are still fragmentary, debatable, and few in number, and the mechanism of this process has not been elucidated.

In recent years information has been obtained to show that hydrolytic enzymes of lysosomes and peroxisomes (microbodies) participate in the oxidation of fatty acids and other substances of the lipid series, and also in the synthesis and breakdown of glycogen [6, 7]. Isolated data on this subject are also available for diabetes [15].

In all the dogs with alloxan diabetes studied in the present investigation a high content of microbodies and, in particular, of lysosomes was observed in the cardiomyocytes (Fig. 3). Judging from the fact that accumulation of lysosomes were found in cells in which the remaining organelles had preserved their structure, they had not performed a proteolytic or destructive role in this case. Lysosomes were located more frequently in the zones of the largest deposits of glycogen and around resorbing lipid structures. The presence of abundant glycogen and lysosomes in heart muscle cells of dogs with diabetes, whereas only solitary lipid inclusions were present, does not seem to be by chance, but rather to be due to metabolic processes connected with the transformation of lipid metabolites, brought by the blood stream into glycogen.

The accumulated glycogen is probably utilized by a hydrolytic mechanism. Evidence of this is given, on the one hand, by the presence of foci of resorption of glycogen accumulations around the lysosomes (Figs. 2 and 3) and, on the other hand, by increased activity of hydrolytic phosphorylase associated with a decrease in activity of phosphorylase (a) (Table 2).

Conversion of the excess of lipids in the myocardium into glycogen in diabetes may play a definite adaptive role. By this means a reserve of intracellular carbohydrates, required for the combustion of fats, is created in the presence of insulin deficiency, and the possibility of development of ketosis also is reduced.

Since only one, relatively early period of diabetes was studied in these dogs, the subsequent dynamics of the phenomena described in dogs is unknown. However, in previous investigations on rats in which diabetes was present for between 6 months and 1 year [3], the writers found no deposition of glycogen in the cardiomyocytes. This suggests that glyconeogeneis from lipids plays a compensatory role mainly in the initial period of development of diabetes, before its triggering mechanisms have been disturbed.

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