α_2-adrenoreceptors and to a decrease in the number of β-adrenoreceptors in brain structures involved in BP regulation.

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MYOCARDIAL ENERGY METABOLISM AND ULTRASTRUCTURE IN AUTOIMMUNE CARDIOMYOPATHY

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Autoimmune myocardial damage plays a leading role in the pathogenesis of cardiomyopathies (CMP), and for that reason we also chose autoimmune CMP as an experimental model [7, 8, 10, 12, 14, 15]. In autoimmune CMP primary specific injury to the myocytes is observed, and not until later are the other vascular elements of the heart involved [1, 3, 4]. Autoimmune CMP can accordingly be used as the nearest approximation to the so-called noncoronary heart disease. In previous clinical and experimental investigations the writers found a disturbance of contractility and relaxation of the myocardium — a process based on disturbances of energy metabolism.

The aim of the present investigation was to study the systems of energy metabolism of the heart, the state of respiratory and phosphorylating activity of the mitochondria, early defects in the sarcolemma of the cardiomyocytes, and the resistance of the heart with CMP to ischemic damage. These parameters can be used to judge the mechanisms of disturbance of energy metabolism in CMP.

EXPERIMENTAL METHOD

Rats weighing 220-250 g with autoimmune CMP, induced by subcutaneous injection of heart muscle homogenate with Freund's complete adjuvant twice a month for 2 months [1], were used in the experiments. After the development of the disease the rats were anesthetized with urethane and the heart quickly removed, placed in cold Krebs–Henseleit solution, which quickly stopped it from beating, and then perfused through the aorta by Langendorf's method with Krebs–Henseleit solution, saturated with carbogen and warmed to 37-38°C. The rate of flow was 10 ml/min/g weight of tissue. The systolic, diastolic, and developed pressures were recorded. Perfusion was accompanied by electrical stimulation of the heart (4 Hz, —4 V) in accordance with the following scheme: group 1) 20 min of perfusion; group 2) 20 min of perfusion + 20 min of ischemia; group 3) 20 min of perfusion + 1, 5, and 15 min of ischemia. At

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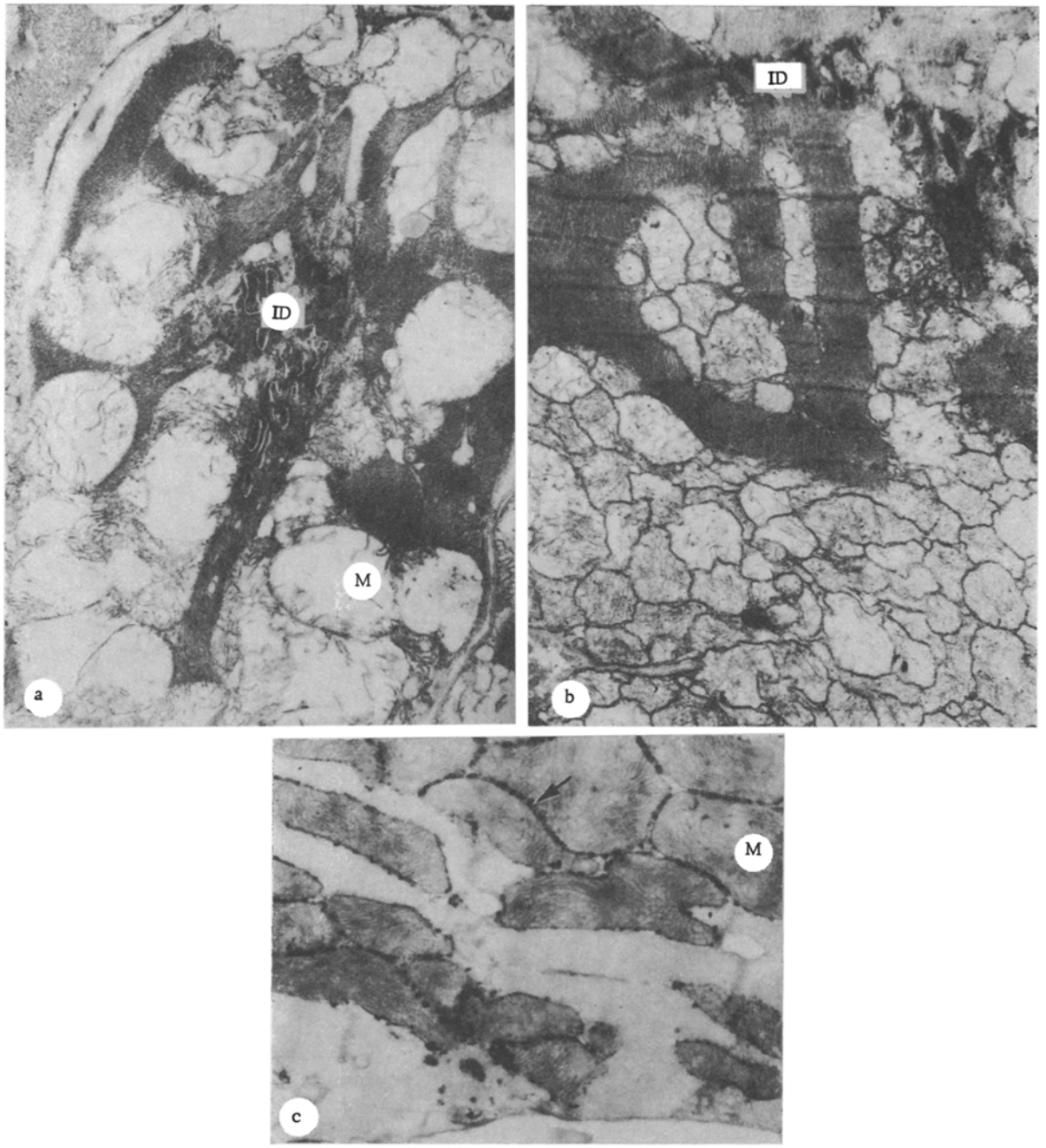

Fig. 1. Cardiomyocytes in autoimmune CNP: a) cardiomyocyte from zone of necrosis; b) cells with reversible changes; c) cardiomyocyte with reversible damage. M) Mitochondria, ID) intercalated disk. a, b) Stained with uranyl acetate and lead citrate; c) fixed with colloidal lanthanum, unstained preparation (colloidal lanthanum residue indicated by arrow). Magnification: a, c) 18,000, b) 10,000×.

the end of the experiments the heart was flattened with cold Wollenberger's forceps and frozen in liquid nitrogen. The frozen hearts were weighed and ground in a mortar with liquid nitrogen to the state of a fine powder, after which 6% $HClO_4$ in 70% methanol was added to the mortar at the rate of 1 ml to 10 mg tissue. The contents of the mortar were thoroughly mixed with a pestle and placed on ice for 40 min for extraction of high-energy phosphates. The homogenate (extract) was poured off into cold centrifuge tubes, and the mortars were rinsed with 6% $HClO_4$ in one-third of the volume used for extraction, and poured into the same tubes. The samples were centrifuged for 15 min at 5000 g and the supernatant decanted and neutralized with 5 N K_2CO_3 to pH 7.4 according to litmus paper. This was followed by further centrifugation for 15 min at 5000 g.

TABLE 1. ATP and CP Concentration (in μmoles/g dry weight) at Different Stages of Ischemia (M ± m)

Experimental conditions	Duration of ischemia, min							
	ATP	CP	ATP	CP	ATP	CP	ATP	CP
Control	8,0±0,15	11,1±1,0	5,9±0,2	4,8±0,4	5,1±0,2	4,1±1,0	4,5±0,2	2,1±0,3
Experiment	5,0±0,1	7,5±0,6	3,57±0,3	4,5±0,8	2,2±0,3	4,16±0,4	1,0±0,3	1,68±0,5
P	<0,001	<0,05	<0,01	>0,05	<0,001	>0,05	<0,001	>0,05

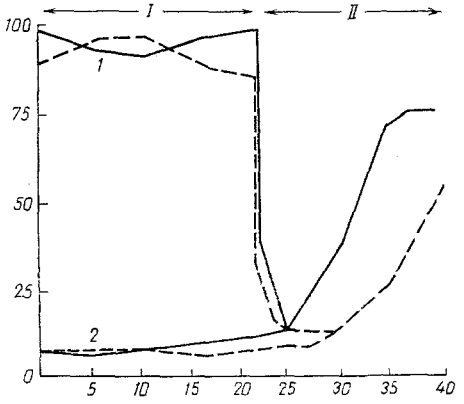

Fig. 2. Time course of changes in contractile function of the heart during perfusion (I) and ischemia (II). Abscissa, time (in min); ordinate, pressure (in mm Hg). 1) Systolic pressure, 2) diastolic pressure. Continuous line — autoimmune CMP; broken line — control.

The ATP concentration in the tissue extracts was determined enzymatically. The AMP concentration was measured in the same system, to which myokinase was added after completion of the reaction with ADP, and the additional decrease in optical density was recorded [6]. To determine creatine phosphate (CP) in the extract, it was hydrolyzed to creatine under the following conditions: 0.2 ml of the sample was added to 1 ml of 0.1 N HCl and incubated for 10 min at 70°C. The tubes were then placed in ice, 1 ml of NaOH was added to each, and the total volume was made up to 3 ml with bidistilled water. The total creatine concentration was then determined by the method in [9]. The CP concentration was calculated as the difference between total and free creatine, obtained without hydrolysis. The concentrations of low-energy phosphates and creatine were calculated per gram dry weight. Parameters of oxidative phosphorylation were determined by a polarometric method. Pieces of tissue from the left ventricle for ultrastructural investigation were fixed with glutaraldehyde and osmic acid, and embedded in epoxide resins in the usual way. After examination of semithin sections, ultrathin sections were cut on a Reichert (Austria) ultramicrotome and examined in a JEM-100B electron microscope (Japan). Early defects in the cardiomyocyte membranes were revealed with the aid of colloidal lanthanum [5].

EXPERIMENTAL RESULTS

Cardiomyopathic damage led to a decrease in the concentrations of ATP by 22%, CP by 45%, and total creatine by 30% compared with the control. To discover the causes of these disturbances of energy metabolism the state of respiratory and phosphorylating activity of the myocardial mitochondria was studied by a polarometric method. However, the results indicated that the energy disturbances which were observed were evidently not due to a disturbance of synthesis of high-energy compounds, in the form of ATP, in the mitochondria but could be caused by other mechanisms. It was accordingly interesting to discover to what extent calcium overloading was possible in such cases, and also to determine the degree of integrity of the intracellular structures (cell membrane, mitochondria).

Investigation of cardiomyocyte ultrastructure showed that irreversibly damaged cells had evidence of lysis of the sarcomeres and marked swelling of mitochondria (Fig. 1a). Atrophy of myofibrils and hyperplasia of small but considerably altered mitochondria were seen in cells

with reversible damage (Fig. 1b). Colloidal lanthanum penetrated into the cytoplasm of the reversibly damaged cells and was distributed around the mitochondria (Fig. 1c), direct evidence of damage to the sarcolemma.

Testing the resistance of the hearts with cardiomyopathy to ischemic loading showed that 20 min of ischemia led to the development of contracture of the heart muscle (Fig. 2). Whereas the development of contracture in the control hearts took place after 9-10 min of ischemia, in the case of CMP similar contracture developed after only 4 min, and the rate of its increase was much greater. To detect correlation between the time of onset of contracture and the level of high-energy compounds, concentrations of ATP and CP were determined in the earlier stages of ischemia — after 1, 5, 15, and 20 min of total ischemia.

Analysis of the results showed (Table 1) that a fall of ATP concentration to 5-4 mmole/g dry weight corresponded to the beginning of contracture of the heart muscle. This fall in hearts with autoimmune CMP took place between the 3rd and 5th minutes of ischemia, but in the control not until the 10th minute. This level can be regarded as the critical intracellular ATP level.

The development of contracture of the heart muscle was probably due to exhaustion of ATP in local pools in myofibrils [11]. Since ATP also is required for relaxation of muscle [2, 13], total hydrolysis to ADP leads to a state of rigor of the cross-linkages and to contracture of the muscles. The rate at which this contracture develops may indicate that metabolic energy for contraction is directly available: The more rapidly the contracture develops, the less efficient the supply of energy for the contractile apparatus. In the writers' view, the disturbances observed in autoimmune CMP may be linked with incompetent myocardial hypertrophy and calicum overloading.

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